IN VIVO INTERACTION OF CARCINOGENIC ACRYLAMIDE WITH CYTOCHROME P450 ISOZYMES AND PHASE II ENZYMES IN RABBIT LIVER, KIDNEY AND LUNG

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ABSTRACT

IN VIVO INTERACTION OF CARCINOGENIC ACRYLAMIDE WITH CYTOCHROME P450 ISOZYMES AND PHASE II ENZYMES IN RABBIT LIVER, KIDNEY AND LUNG

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Acrylamide is an industrially produced chemical with known neurotoxic, reproductive toxin and carcinogenic effects. The carcinogenicity associated with acrylamide is mostly attributed to its metabolism by liver CYP2E1. However, studies investigating the effects of acrylamide on CYP2E1 enzyme are limited. In this study, it was aimed to investigate *in vivo* interaction of carcinogenic acrylamide on microsomal cytochrome P450 enzyme activities, and protein levels, and on cytosolic NQO1 and GST enzyme activities of rabbit liver, kidney and lung of acrylamide-treated rabbits. The *in vivo* protective effect of resveratrol, a phenolic compound, was also investigated on acrylamide toxicity.

New Zealand male rabbits were treated with acrylamide and resveratrol, separately in different doses and conditions. Their combined effects were also investigated. CYP2E1-dependent *p*-Nitrophenol hydroxylase, NDMA N-demethylase and aniline 4-hydroxylase activities were found to be significantly increased in acrylamide-treated rabbit liver (1.80-3.0 fold) and kidney (1.6-fold). Rabbit liver and kidney CYP2E1 protein levels (determined by western blot analyisis) also increased approximately 2-fold due to acrylamide treatment. In rabbit liver, resveratrol was found significantly effective in decreasing both acrylamide-induced CYP2E1-dependent enzyme activities (approximately 1.5-1.80 fold) and CYP2E1 protein levels (approximately 1.5-1.70 fold). Additionally, resveratrol significantly decreased acrylamide-induced CYP2E1 protein level (2-2.5 fold) in rabbit kidney. However, no

significant change was observed in rabbit lung CYP2E1-dependent enzyme activities and CYP2E1 protein levels due to acrylamide, resveratrol or their combined treatments. Furthermore, it was found that acrylamide treatment significantly increased CYP3A6-dependent erythromycin N-demethylase enzyme activity (1.85-fold) and CYP3A6 protein levels in rabbit liver (1.69-fold). No change was observed in CYP2B4-dependent benzphetamine N-demethylase enzyme activities of rabbit liver, kidney and lung by *in vivo* acrylamide, resveratrol or their combined treatments. Moreover, total GST and GST-Mu activities of rabbit kidney (1.5-fold, respectively) and total GST activity of rabbit lung (1.6-fold) were increased significantly only in resveratrol treated group. NQO1 enzyme activity of rabbit kidney was significantly increased by acrylamide treatment (1.6-fold).

The results of the present study have demonstrated for the first time that acrylamide induces rabbit liver and kidney CYP2E1-dependent enzyme activities and CYP2E1 protein levels. The induction of CYP2E1 enzyme activity and protein level by acrylamide treatment can stimulate formation of other toxic compounds and procarcinogens metabolized by CYP2E1 which in turn further potentiates the risk of hepatotoxicity, mutagenicity and carcinogenicity. In the present study, it was also demonstrated for the first time that acrylamide treatment also increases CYP3A6 enzyme activity in rabbit liver which may lead to alterations in drug metabolism. The results of this study have also suggested that resveratrol may have protective effects on acrylamide induced toxicity; however, further *in vivo* studies are required to clarify the effect of resveratrol on both acrylamide-induced toxicity and anti-oxidant enzymes.

Keywords: Acrylamide, CYP2E1, carcinogenicity, resveratrol, Phase I enzymes, Phase II enzymes.

ÖZ

KARSİNOJEN AKRİLAMDİN TAVŞAN KARACİĞER, BÖBREK VE AKCİĞERİNDE SİTOKROM P450 VE FAZ 2 ENZİMLERİ İLE *İN VİVO* ETKİLEŞİMİ

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Akrilamid endüstriyel olarak üretilen, nörotoksik, reprodüktif toksik ve karsinojenik etkileriyle bilinen bir kimyasaldır. Akrilamidin karsinojenisitesi daha çok karaciğer CYP2E1 enzimi ile metabolize olması ile ilişkilendirilmiştir. Ancak, akrilamidin CYP2E1 enzimi üzerine olan etkilerini inceleyen çalışmalar sınırlı sayıdadır. Bu çalışmada, ilk defa, *in vivo* karsinojenik akrilamidin tavşan karaciğer, böbrek ve akciğer mikrozomları sitokrom P450 enzim aktiviteleri ve protein düzeyleri ile sitozolik NQO1 ve GST enzim aktiviteleri üzerine etkilerinin araştırılması amaçlanmıştır. Bununla birlikte, fenolik bir madde olan resveratrolün akrilamid toksisitesi üzerine koruyucu etkileri de araştırılmıştır.

Çalışmada, akrilamid ve resveratrol Yeni Zellanda türü erkek tavşanlara farklı doz ve durumlarda ayrı ayrı uygulanmıştır. Ayrıca, akrilamid ve resveratrolun ortak etkilerini araştırmak için her ikisi birlikte uygulanmıştır. Akrilamidin, CYP2E1'e bağlı tavşan karaciğer (yaklaşık 1.80-3.0 kez) ve böbrek (yaklaşık 1.60 kez) *p*-Nitrofenol hidroksilaz, NDMA N-demetilaz ve anilin 4-hidroksilaz aktivitelerini anlamlı arttırdığı bulunmuştur. Bununla birlikte, akrilamid, tavşan karaciğer ve böbrek CYP2E1 protein düzeylerini (western blot analizi ile belirlenmiştir) yaklaşık 2-kez anlamlı artmıştır. Resveratrolün akrilamid nedenli artmış tavşan karaciğer CYP2E1'e bağlı enzim aktivitelerini (yaklaşık 1.5-1.80 fold) ve CYP2E1 protein düzeylerini (yaklaşık 1.5-1.70 fold) anlamlı düşürdüğü bulunmuştur. Ayrıca, resveratrol akrilamid nedenli artmış böbrek CYP2E1 protein düzeyini de anlamlı

azaltmıştır (2-2.5 fold). Akrilamid, resveratrol ya da her ikisinin verilmesi ile CYP2E1'e bağlı tavşan akciğer enzim aktivitelerinde anlamlı bir değişim gözlenmemiştir. Ayrıca, akrilamid CYP3A6'ya bağlı tavşan karaciğer eritromisin N-demetilaz enzim aktivitesini (1.85-fold) ve karaciğer CYP3A6 protein düzeyini (1.69-fold) anlamlı arttırmıştır. *In vivo* akrilamid, resveratrol ya da her ikisinin uygulanmaları ile CYP2B4'e bağlı tavşan karaciğer, akciğer ya da böbrek benzfetamine N-demetilaz enzim aktivitesinde anlamlı bir değişim gözlenmemiştir. Tavşan böbrek total GST ve GST-Mu aktiviteleri (sırasıyla 1.5-kez) ile akciğer total GST aktiviteleri (1.6-kez) sadece resveratrol uygulanmış grupta anlamlı artmıştır. Tavşan böbrek NQO1 enzim aktivitesi akrilamid verilmesi ile anlamlı artmıştır (1.6 kez).

Bu çalışma, *in vivo* akrilamidin CYP2E1'e bağlı tavşan karaciğer ve böbrek enzim aktivitelerini ve CYP2E1 protein düzeylerini arttırdığını gösteren ilk çalışmadır. Akrilamidin neden olduğu CYP2E1 enzim aktivitesi ve protein düzeyi artışı aynı zamanda CYP2E1 tarafından metabolize edilen diğer toksik ve önkarsinojen maddelerin metabolizmasını arttırarak hepatoksisite ve kanser riskini arttırır. Bu çalışma aynı zamanda ilk defa, *in vivo* akrilamidin CYP3A6 tavşan karaciğer enzim aktivitesini ve CYP3A6 protein düzeyini arttırdığını da göstermiştir ki karaciğer CYP3A6 enzim aktivitesi ve protein düzeyinin artışı karaciğerde ilaç metabolizmasında değişikliklere yol açabilir. Bu çalışma sonuçlarına dayanarak, resevratrolün akrilamid toksisitesinde koruyucu bir etkisi olduğu önerilebilir; ancak, resveratrolün akrilamid toksisitesi ve antioksidan enzim sistemleri üzerine *in vivo* etkisini tanımlamak için ileri çalışmalar yapılmalıdır.

Anahtar kelimeler: Akrilamid, CYP2E1, karsinojenisite, resveratrol, Faz I enzimleri, Faz II enzimleri

To My Family

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APS	Amonium per sulphate
ALT	Alanine amino transferase
AST	Aspartate amino transferase
BCIP	Bromochloroindoylphosphate
BSA	Bovine serum albumin
BIS	N, N'-Methylene bisacrylamide
CDNB	1-chloro-dinitrobenzene
CYP	Cytochrome P450
DCND	1,2-dichloro-4-nitrobenzene
DCPIP	Dichlorphenolindophenol
DNPH	2,4-dinitrophenylhydrazine
ε-ACA	ε-Amino caproic acid
EDTA	Ethylene diamine tetra acetic acid
FAD	Flavin adenine dinucleotide
GSH	Glutathione reduced form
GST	Glutathione S-transferase
HEPES	N-2-hydroxyethyl piperazine-N'-2 ethane sulfonic acid
MFO	Mixed function oxidases
NADH	Nicotinamideadenine dinucleotide, reduced form
$NADP^+$	Nicotinamideadenine dinucleotide phosphate
NADPH	Nicotinamideadenine dinucleotide phosphate, reduced form
NBT	Nitrotetrazolium blue
NDMA	N-nitrosodimethylamine
NQO1	DT-Diaphorase
PAGE	Polyacrylamide gel electrophoresis
pAp	*p*-Aminophenol
PAH	Polycyclic aromatic hydrocarbons
PMSF	Phenylmethylsulfonyl floride
SDS	Sodium dodecyl sulfate

TBA	Thiobarbituric acid
TEMED	N-N-N’-N’-tetramethylenediamine
TRIS	Tris(hydroxymethyl) aminomethane

CHAPTER 1

INTRODUCTION

Acrylamide is an animal neurotoxin, a reproductive toxin and a carcinogen. It has several industrial applications, such as waste water treatment, ore processing, cosmetics, and it is used in biological research laboratories such as biochemistry, molecular biology, genetics and bioengineering. Recently, the detection of acrylamide in heated-processed foods and in foods cooked at higher temperatures especially in carbohydrate rich foods, such as French fries, potato crisps, increased the worldwide concern in acrylamide toxicity to humans (Friedman, 2003; Exon, 2006).

The metabolism of acrylamide is complex and not fully understood yet (Sczerbina *et al.,* 2008). However, several studies carried out in recent years demonstrated that mutagenicity and carcinogenicity associated with acrylamide is mostly attributed to the conversion of acrylamide to its epoxide glycidamide, by liver CYP2E1 (Besaratinia and Pfeifer, 2004; Adler *et al.*, 2000; Manjanatha *et al.*, 2006; Ghanayem *et al.,* 2005b; Rice, 2005).

1.1 Acrylamide

Acrylamide (2-propenamide) is an α-β unsaturated (conjugated) reactive low molecular weight vinyl compound produced industrially and available since mid 1950s (McCollisster, 1964; Wallace and Wallace, 1986; Friedman, 2003; Rice, 2005). In figure 1.1., the structure of acrylamide is given.

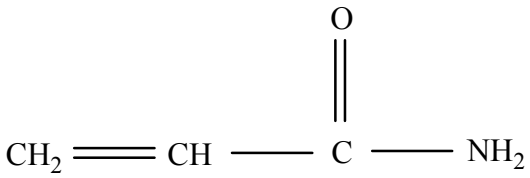

Figure 1.1 The structure of acrylamide

Acrylamide is a colorless, odorless, crystalline, easily polymerizable solid, soluble in water, acetone and ethanol, and has a melting point of 84.5^0 C. The chemical properties of acrylamide are given in Table 1.1.

Table 1.1 Chemical properties of acrylamide[a]

Synonyms	2-Propenamide; ethylene carboxamide; acrylic amide; vinyl amide
CAS No	79-06-1
Molecular weight	71.09
Chemical formula	$CH_2CHCONH_2$
Boiling point	125^0 C
Melting point	87.5^0 C (183^0F)

[a] Taken from Ötles and Ötles (2004)

Acrylamide and its polymers have several industrial applications. It is used as a binding, thickening or flocculating agent in grout, cement, sewage, waste water treatment, pesticide formulations, cosmetics, sugar manufacturing and soil erosion prevention (Friedman, 2003; Exon, 2006). The polymers of acrylamide are used in ore processing, food packaging and plastic products (Exon, 2006). Moreover, acrylamide and its polymers are important in scientific research areas. The polymers of acrylamide are used in biochemistry, molecular biology, genetics and

bioengineering research laboratories for gel electrophoresis as a solid support to separate the proteins. Acrylamide is also found in cigarette-smoke (Smith, 1999).

Since there are several different usage of acrylamide, humans are exposed to acrylamide in various ways including inhalation (smoking), dermal exposure and in drinking water, contaminated from the use of flocculants in water treatment to a lesser extent, and by diet (Friedman, 2003, Exon, 2006)

Human exposure to acrylamide has become a worldwide concern after the publication of International Agency for Research on Cancer (IARC) in 1994 (IARC, 1994) where it is stated that acrylamide is a probable human carcinogen. The probability of carcinogenic and neurotoxic effects of acrylamide to humans has been questioned by the scientists after the observation and studies carried out on the workers involved in a railroad tunnel construction accident through Hallandas, a bedrock bridge in southern Sweden in 1997 (Besaratinia and Pfeifer, 2007; Hagmar *et al.*, 2001). After the accident, the dead fishes in the brook, the paralyzed cows near the brook and the tunnel construction workers with symptoms of neurotoxicity increased the attention to the contents of water of the brook. Ensuing research demonstrated high concentrations of a material called 'Rhoca Gill', an acrylamide-based grouting agent, used for sealing the tunnel leakage, was found in the water. (Hagmar *et al.*, 2001; Besaratinia and Pfeifer, 2007). Ongoing studies were performed on the tunnel workers (Hagmar *et al.*, 2001) and demonstrated increase in hemoglobin-acrylamide adducts in exposed subjects. In the study performed by Hagmar *et al.* (2001) 74 out of 210 individuals of the tested group (tunnel workers) had significantly increased hemoglobin-acrylamide adduct levels compared to non-exposed subjects. Several other investigations were also performed on this issue. In one study conducted with non-smokers (control group), smokers and laboratory workers, although laboratory workers demonstrated the highest hemoglobin adducts due to acrylamide exposure; the elevated levels of hemoglobin adducts even in the control group led to the question whether dietary sources give rise to hemoglobin-acrylamide adducts (Bergmark *et al.*, 1997)

Since then, the presence of acrylamide/adducts in the dietary sources and in blood of animals and humans has been analyzed. Investigations demonstrated that rats fed with fried foods have hemoglobin-acrylamide adducts in their blood (Tareke *et al.*, 2000). In addition, it was also demonstrated that carbohydrate rich-foods (such

as potato chips, French fries etc.) cooked at high temperatures contained acrylamide residues (Tareke *et al.*, 2002). Additional studies were undertaken, and studies performed by Rosen and Helenas (2002) also verified presence of acrylamide in heated carbohydrate-rich foods. Thus, several products were analyzed and different amounts of acrylamide were detected. Processed potato products, bread, breakfast cereals, biscuits, cookies, snacks and coffee are found to contain acrylamide residues (Svensson *et al.*, 2003). In Table 1.2, acrylamide levels in different foods and food products are given.

Mechanism of formation of acrylamide in foods was investigated in several studies (Mottram *et al.*, 2002; Stadler *et al.*, 2002). According to these studies, it was demonstrated that acrylamide formed in foods via a reaction called Maillard reactions in which non-enzymatic browning take place.

The Maillard reaction responsible for food taste utilizes reducing sugars and free amino groups, leading to glycosilamine formation and then Amodori rearrangement products. After degradation, highly reactive compounds like furfural, reductones, acetol or pyruvaldehydes condense with free amino groups forming aldehydes and α-aminoketones. Finally, a set of reactions leads to melanoidins, brown nitrogenous polymers (Martins *et al.,* 2001; Taeymans *et al.,* 2004; Zhang and Zhang, 2007).

Table 1.2 Acrylamide levels in different foods and food products from Norway, Sweden, Switzerland, the United Kingdom and the United States of America[a]

Food/Product Group	**Acrylamide levels (µg/kg)[1]**			
	Mean[2]	Median[2]	Minimum-Maximum	Number of samples
Crisps, potato/sweet potato[3]	1312	34-4161343	170-2287	38
Chips, potato[4]	537	330	<50-3500	39
Batter based products	36	36	<30-42	2
Bakery products	112	<50	<50-450	19
Biscuits, crackers, toast, bread crisps	423	142	<30-3200	58
Breakfast cereals	298	150	<30-1346	29
Crisps, corn	218	167	34-416	7
Bread, soft	50	30	<30-162	41
Fish and seafood products, crumbed, battered	35	35	30-39	4
Poultry or game, crumbed, battered	52	52	39-64	2
Instant malt drinks[5]	50	50	<50-70	3
Chocolate powder[5]	75	75	<50-100	2
Coffee powder[5]	200	200	170-230	3
Beer	<30	<30	<30	1

[a] Taken from European Commission, 2002 (http://ec.europa.eu/food/fs/sc/scf/out131_en.pdf)

[1] The limits of detection and quantification varied among laboratories; values reported as less than a value are below the limit reported by the laboratory.

[2] Mean and median were calculated where individual data were available; sample sizes were extremely small particularly for some food categories; where the mean and median are different it reflects the skewed distribution of the underlying data that were collected in different countries and may represent different food items within the larger category.

[3] Products that are thinly sliced and fried (Includes foods called potato chips in some regions including North America).

[4] Products that are more thickly sliced (Includes foods called French fries in some regions including North America).

[5] The figure relates to the dry powder. The beverage as consumed has a much lower acrylamide concentration.

Mottram *et al.* (2002) have established that $-NH_2$ groups of asparagine in a presence of dicarbonyls from the Maillard reaction are the main substrates for acrylamide synthesis. However, Zyzak *et al.* (2003) have shown that rather carbonyls are required for acrylamide formation from asparagine instead of dicarbonyls. Binding carbonyl source to asparagine forms Schiff base which after decarboxylation process leads to acrylamide and imine molecules. It has also been shown that the presence of enzymes from the group of decarboxylases and temperature could easily degrade asparagine, forming acrylamide intermediate, 3-aminopropionamide (Zyzak *et al.,* 2003) directly leading to acrylamide synthesis in carbohydrates-free environment. It confirmed the previous findings of Stadler *et al.* (2002) that the carbon skeleton of acrylamide molecule derives from asparagines. Stadler *et al.* (2002) have also revealed that water significantly increases acrylamide concentration to almost three folds of its content found in anhydrous conditions. Temperature elevation to 170° C leads further to an increase of acrylamide production.

High content of carbohydrates and asparagine, e.g. in potatoes, wheat and rye grains (cereals), almonds and coffee beans enhances acrylamide formation during frying or baking processes (Tareke *et al.,* 2002; Zhang and Zhang, 2007). The concentration of acrylamide noted during thermal processing of this foodstuff reaches the level of 3 μg/g in French fries and even 7 μg/g in roasting coffee beans. It must be noted, however, that not only pH, processing temperature and moisture influence acrylamide formation but also plant cultivating, food storage, reducing sugar content in prefabricates and processing time (Zhang and Zhang, 2007).

Acrylamide is absorbed through skin, alimentary tract and respiratory system and is distributed in the body with blood and other fluids (Calleman, 1996; Friedman, 2003; LoPachin, 2004). It also passes the blood-brain and placental barriers (Sörgel *et al.,* 2002; Schettgen *et al.,* 2004). The metabolism of acrylamide is complex and not fully understood yet (Sczerbina *et al.,* 2008). However, several studies carried out in recent years demonstrated that mutagenicity and carcinogenicity associated with acrylamide is mostly attributed to the conversion of acrylamide to its epoxide glycidamide, by liver CYP2E1 (Besaratinia and Pfeifer, 2004; Adler *et al.*, 2000; Manjanatha *et al.*, 2006; Ghanayem *et al*., 2005b; Rice, 2005).

1.1.1 The Metabolism and Toxicity of Acrylamide

Two major metabolic pathways participate in the metabolism of acrylamide. The predominant pathway in the metabolism of acrylamide is the epoxidation of acrylamide to its epoxide glycidamide. The other major pathway is through conjugation with glutathione (GSH).

The epoxidation of acrylamide has been demonstrated to be catalyzed by CYP2E1. This metabolic pathway has been demonstrated to occur in rats (Calleman *et al.*, 1990; Sumner *et al.,* 1997), mice (Sumner *et al.,* 1999; Ghanayem *et al.,* 2000) and humans (Bergmark *et al.,* 1993) *in vivo* and *in vitro*. The study carried out by Sumner *et al.* (1999) was the first study that demonstrated a proposed mechanism of acrylamide metabolism in mice. In an *in vitro* study, it was also demonstrated that acrylamide is metabolized to glycidamide in rat hepatocytes (Kruyebashi and Ohno, 2006). In addition to the studies that demonstrated the metabolism of acrylamide by CYP2E1, conjugation of acrylamide with glutathione has been also demonstrated in different studies in rats and mice (Sumner *et al.,* 1997, 1999; Ghanayem *et al.,* 2005a, Kruyebashi and Ohno, 2006). The glutathione has been suggested as the major scavenger of acrylamide *in vivo* (Tong *et al.*, 2004; Kurebayashi and Ohno, 2006). The major metabolism pathways of acrylamide in rats and mice are given in Figure 1.2.

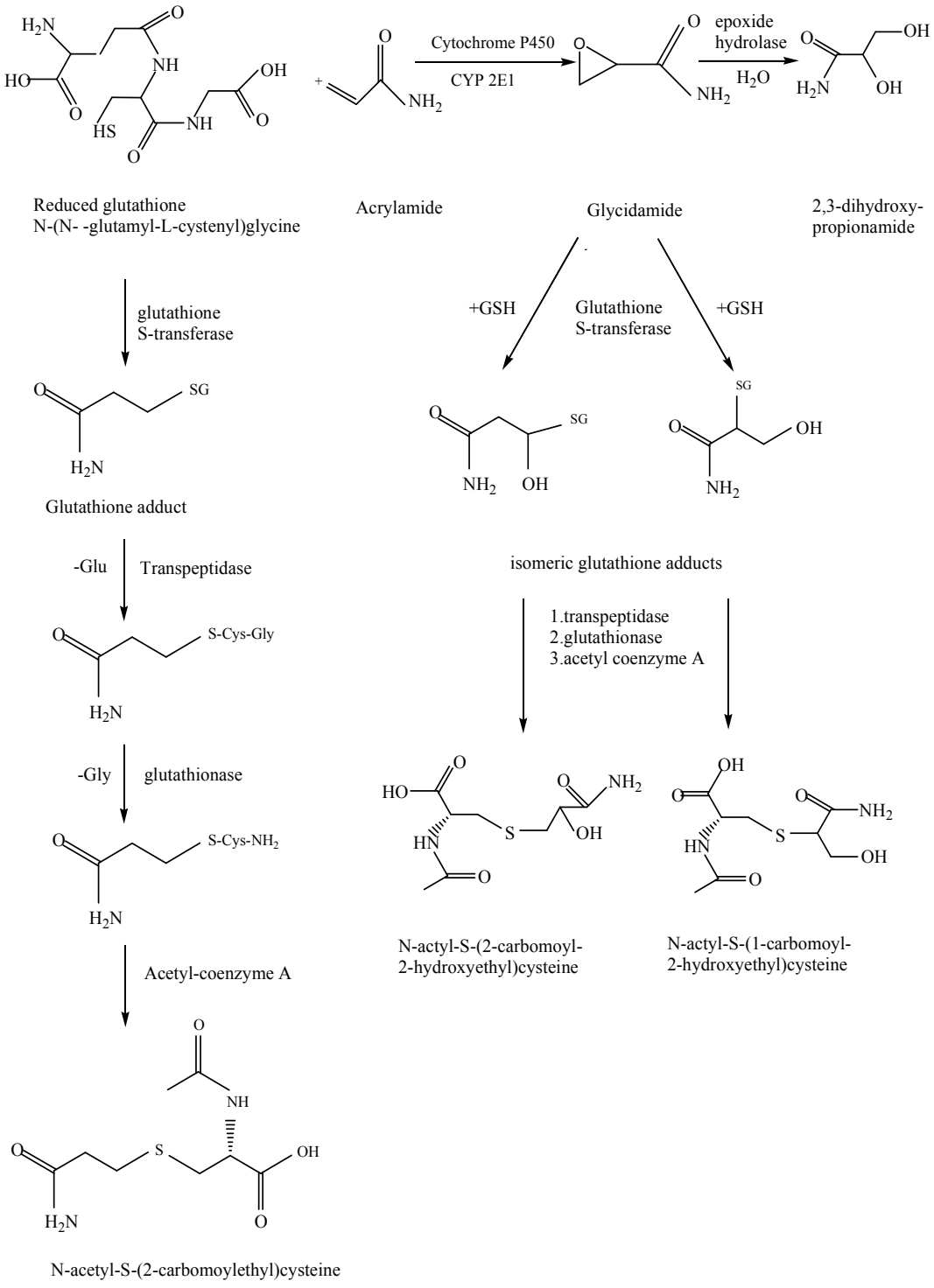

Figure 1.2 The metabolism of acrylamide in rats and mice. It is taken from Friedman, 2003.

As previously mentioned, the metabolism of acrylamide by CYP2E1 resulted in epoxide glycidamide. The structure of glycidamide is given in Figure 1.3. Epoxides are oxygen-containing heterocyclic compounds that are too reactive as alkylating agents. Thus; epoxides can easily react with nucleophillic sites on proteins and DNA *in vivo* (Melnick, 2003). Glycidamide is mutagenic, binds to DNA and can cause genetic damage (Adler *et al.,* 2000; Yousef and El-Demerdash, 2006). Glycidamide is subsequently metabolized through GSH conjugation (given in Figure 1.2) or hydrolysis of its epoxy group is catalyzed by the enzyme epoxy hydrolase (Paulsson *et al.,* 2005).

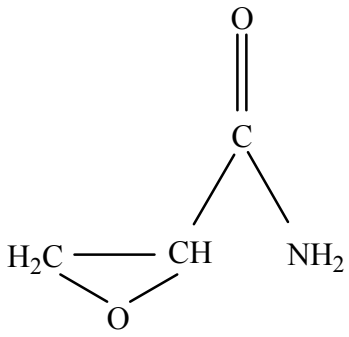

Figure 1.3 The structure of Glycidamide

Both acrylamide and glycidamide forms hemoglobin-adducts in vivo (Calleman *et al.,* 1990). Several studies have been demonstrated acrylamide exposure forms hemoglobin-adducts formation *in vivo* in rats (Bergmark *et al.,* 1991) in humans (Bergmark, 1993, 1997; Hagmar *et al.,* 2001). Although exposure to acrylamide forms hemoglobin adducts in blood, the reactivity of acrylamide with DNA appears to be quite low compared to glycidamide (Solomon et al., 1985, 1999). In addition, Segerbäck *et al.* (1995) suggested in their study DNA adducts only resulted from glycidamide. DNA-adduct formation due to exposure to acrylamide and glycidamide has been demonstrated in rats and mice (Gamboa da Costa *et al.,* 2003; Doerge *et al.*, 2005; Manjanatha *et al.*, 2006).

Acrylamide has been mentioned as a neurotoxin, a reproductive toxin and a carcinogen. The neurotoxic effects of acrylamide have been studied extensively and neurotoxicity is the only toxic effect of acrylamide that have been demonstrated both in humans (He *et al.,* 1989; Calleman, 1993; Costa, 1996; Hagmar, 2001) and in laboratory animals (rodents, rabbits, dogs, cats and Guinea pigs) (Edwards and Parker, 1977; LoPachin *et al.,* 2002; Spencer and Schaumburg, 1974). Acrylamide produces neurotoxicity characterized by ataxia, skeletal muscle weakness and numbness of the hands and feet in both humans and animals (LoPachin, 2004).

The reproductive toxicity of acrylamide has also been extensively studied. The reproductive toxicity of acrylamide has been demonstrated in rats (Field *et al.,* 1990; Wise *et al.*, 1995; Tyl *et al.,* 2000; Tyl and Friedman, 2003) and mice (Shelby *et al.,* 1987; Chapin et al., 1995; Adler *et al.,* 2000; Ghanayem 2005b). Acrylamide induced reproductive toxicity has been characterized by reduction in litter size, abnormal sperm and decreased sperm count, reduction in mating frequency, decrease in the pup body weight at birth (Friedman, 2003).

The carcinogenic effect of acrylamide has been investigated since 1980s (Rice, 2005). Studies have demonstrated that prolonged exposure to acrylamide with various routes lead to increased tumor incidences in mice (Bull *et al.,* 1984a, b). In addition, studies performed in rats demonstrated prolonged exposure to acrylamide resulted in increase in the incidence of thyroid gland tumors and peritoneal mesotheliamas in the region of testis in males and tumors of the mammary gland, central nervous system, thyroid, oral cavity, uterus and clitoral gland in females (Johnson *et al.,* 1986, Friedman, 1995). The carcinogenity of acrylamide has also been carried out in epidemiological human studies (Sobel *et al.,* 1986; Marsh *et al.,* 1999; Mucci *et al.,* 2003; Pelucchi *et al,* 2003; Mucci *et al.,* 2004). In these studies, no tumor incidence has been detected by prolonged exposure to acrylamide in humans. In one study doubling of pancreatic cancer risk have been demonstrated in workers exposed to acrylamide exposure (Marsh *et al.,* 1999).

On the other hand, the genotoxicity and clastogenicity of acrylamide *in vivo* have been demonstrated in studies with rats and mice with the evidences of hemoglobin and DNA adducts formation (Paulsson *et al.,* 2002; 2003; Costa *et al.,* 2003; Besaratinia and Pfeifer, 2004). Thus, metabolism of acrylamide to its epoxide

glycidamide via CYP2E1 has been suggested to be a prerequisite for genotoxicity and mutagenicity of acrylamide.

1.2 Phase I and Phase II Xenobiotic Metabolizing Enzymes

Between absorption and renal excretion of a xenobiotic, two major biochemical mechanisms play important role which are catalyzed by Phase I and Phase II enzymes. The reactions mainly take place in the liver; however, these reactions also occur in lung, kidney, skin and gastrointestinal tracts, adrenal testis, ovary, placenta and brain of mammalian tissues in different amounts and functions. These reactions occur often sequentially. By these reactions, lipophilic compounds entering into the body are biotransformed to more water-soluble compounds and then can be excreted. Therefore, Phase I and Phase II xenobiotic metabolizing enzymes are the major enzymes which are responsible for biochemical metabolic alterations of xenobiotics and drugs.

1.2.1 Phase I Xenobiotic Metabolizing Enzymes

Phase I reactions mainly convert xenobiotics or drugs to a more polar metabolite by introducing or revealing a functional group such as -OH, -NH_2, -SH groups. These Phase I reactions involve oxidation, reduction or hydrolysis reactions. Phase I reactions generally convert lipophillic xenobiotics to hydrophilic compounds and this system usually serves as a route of detoxification and in contrast as a route of metabolic activation to yield metabolites which initiates toxic and carcinogenic events.

Phase I enzymes include hydrolytic enzymes such as amidases, esterases and epoxide hydrolases; reductive enzymes such as azoreductases, disulfide reductases, NADPH-quinone oxidoreductase (NQO1), aldo-keto reductases, nitroreductases; oxidative enzymes such as alcohol and aldehyde dehydrogenases, amine oxidases, flavin-containing monooxygenases (FMOs) and Cytochrome P450 monooxygenases. (Schenkman, 1999)

As given above, cytochrome P450 enzymes dominantly constitutes Phase I enzymes and NQO1 is also one of the Phase I enzymes.

1.2.1.1 Cytochrome P450s

Cytochrome P450s (CYPs) are superfamily of heme proteins which are involved in a variety of metabolic and biosynthetic processes. They play an important role in the metabolism of exogenous substrates including drugs, dietary and environmentally derived toxicants and carcinogens and also in the metabolism of several endogenous compounds, such as fatty acids and steroids (Lu and Levin, 1974; Arınç and Philpot; 1976; Arınç and Adali 1983; Lieber *et al.,* 1997; Gonzalez and Kimura, 2005). CYPs are found to be present in every living species on Earth. As of Febuary 29, 2008; a total of 8128-cytochrome P450 sequences were identified from various organisms, so that 2872 animals, 2867 plants, 1238 fungi, 912 bacteria, 238 other eukaryotic CYPs were counted (http: //drnelson. utmem.edu /CytochromeP450.html). Most of them located in the lipophillic membranes of the endoplasmic reticulum of the liver and other tissues.

These heme proteins have been named as 'Cytochrome P450' according to their spectral properties. The reduced form of these heme proteins (in ferrous form) binds CO and gives a complex that absorbs light maximally 450 nm. Therefore, the name "cytochrome P450" has given to these heme proteins (Omura and Sato, 1964).

Since, so many P450s are found to be present in many organisms, a systemic nomenclature based on structural homology (amino acid sequence) has been constructed by Nebert *et al.* (1987) and become universal. In this systematic nomenclature system, the abbreviation 'CYP' represents the first two letters of CYtochrome and first letter of P450, is used as a preface to designate the gene or protein is a cytochrome P450 dependent monooxygenase. In this nomenclature, the abbreviation CYP followed by an Arabic numeral which expresses the specific family followed by a capital letter represents the subfamily, followed by another Arabic numeral that designate an individual cytochrome P450 form, for example, cytochrome P450 2E1 as CYP2E1 (*CYP2E1* for gene).

According to this system, the members of the same family should have similar sequences in their overlapping portions. At least 40% homology should be shared in the same family members. The members of same subfamily should be share a higher degree of amino acid homology, at least 55%. To represent an

individual number a new P450 sequence should differ by more than 3 % (Nebert *et al.*, 1991; Nelson *et al.*, 1993).

All known cytochrome P450s bind and activate two atoms of oxygen. The general reaction mechanism catalyzed by cytochrome P450 is written as follows:

$$NADPH, H^+ + \mathbf{O_2} + RH \longrightarrow NADP^+ + H_2\mathbf{O} + R\mathbf{O}H \quad (1)$$

where the substrate (R) shows an alkene, aromatic ring or heterocyclic substituent. Substrate (R) represents a site for oxygenation. In the reaction only one of two atom of oxygen is incorporated into substrate while the other reduced to water. Therefore, the reaction also called as 'monooxygenation' and the enzyme as 'monooxygenase'. Cytochrome P450s catalyze several reactions such as oxidation, aromatic hydroxylation, alipathic hydroxylation, epoxidation, oxidative dealkylation including nitrogen, oxygen, sulphur dealkylation reactions, nitrogen oxidation of amines, sulphur oxidation, deamination, desulfration, dechlorination, reductions, hydrolyses reactions.

The Cytochrome P450 monooxygenase system functions as a multicomponent electron transport system, which undergoes a cyclic series of reaction. The liver microsomal cytochrome P450 dependent monooxygenase system contains 2 protein components (Lu and Coon, 1968); cytochrome P450 and cytochrome P450 reductase, and a heat stable factor lipid (Lu *et al.,* 1969); which was later identified as phosphotidylcholine dilauroyl (Lu *et al.,* 1970). Cytochrome P450 catalyzes the monooxygenation reaction; NADPH dependent cytochrome P450 reductase catalyzes the electron transfer from NADPH to cytochrome P450; and finally lipid facilitates the transfer of electrons from NADPH cytochrome P450 reductase to cytochrome P450 (Lu and Levin, 1974). The mechanism postulated by Cytochrome P450s is given in Figure 1.4.

The active site of P450 proteins contains a single iron protoporphyrin IX and the fifth ligand of the heme is bound to the protein through anionic, thiolate sulfur of a cysteine residue. Cytochrome P450s activate the dioxygen for chemical reactions by this heme-thiolate bond. When iron is reduced, the sixth coordination position of the heme may be occupied by exchangeable water molecule, or by O_2.

The reducing equivalents from NADPH are transferred through NADPH cytochrome P450 reductase to cytochrome P450 during hydroxylation of various compounds. The substrate R first combines with Fe^{3+} form. The latter is then oxygenated and a second electron from NADPH converts bound oxygen to O_2 radical. An internal oxidoreduction takes place by the formation of the hydroxylated substrate and H_2O, which contained the oxygen atoms introduced as O_2. Free cytochrome P450 is regenerated in its Fe^{3+} form (Figure 1.4). The mechanism postulated for the hydroxylation of organic substances by microsomal cytochrome P450 dependent monooxygenases is given in Figure 1.4.

As previously mentioned, NADPH dependent cytochrome P450 reductase enzyme catalyze the transfer of electrons from NADPH to cytochrome P450. It is a membrane bound amphipathic protein containing both hydrophobic and hydrophilic peptide. Molecular weight of P450 reductase was determined to be 78 000 Da (Gum and Strobel, 1981; Black and Coon, 1982; İşçan and Arınç, 1986 and 1988, Arınç and Çelik, 2002). Hydrophillic peptide having Mr of 71 000 Da contains 1 mol each of FAD and FMN. Hydrophillic peptide having Mr of 71 000 Da contains 1 mol each of FAD and FMN.

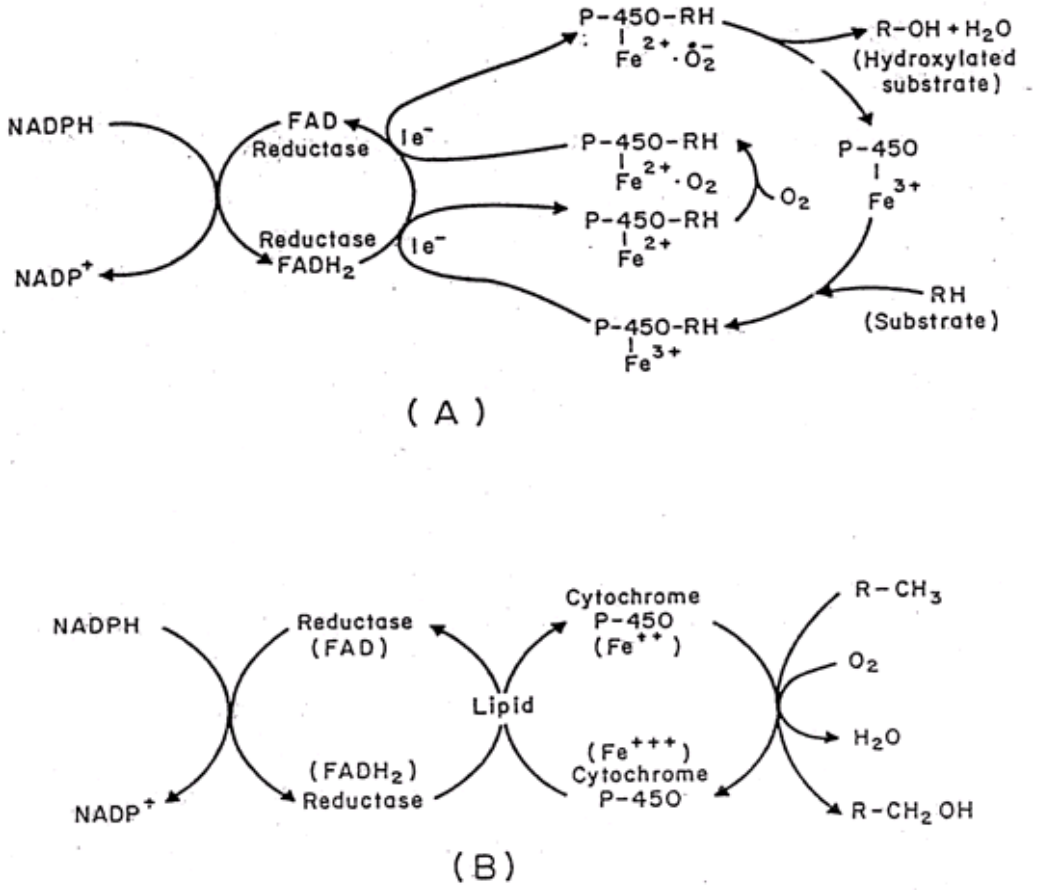

Figure 1.4 (A) Mechanism postulated for the hydroxylation of organic substrates by the liver microsomal P450 system. The substrate "A" first combines with the Fe^{3+}form of P450, which is then produced by one electron from NADPH to the Fe^{3+} form. The latter is then oxygenated, and a second electron from NADPH converts the bound oxygen into the O_2^- radical. An internal oxidoreduction ensues, with the formation of the hydroxylated substrate and H_2O, which contain the oxygen which contain the oxygen atoms introduced as O_2. Free P450 is regenerated in its Fe^{3+} form. In some cells an iron-sulfur protein transfers electrons from $FADH_2$ to P450. (taken from Lehninger, 1975)

(B) The role of lipid in mechanism

Hydrophobic peptide is responsible for proper interaction of reductase with cytochrome P450 and anchoring the reductase to endoplasmic reticulum. All of the three components, cytochrome P450, NADPH cytochrome P450 reductase, and lipid are required to reconstitute the full hydroxylation activity. (Lu *et al.,* 1969, 1970; Lu and Levin, 1974; Arınç and Philphot, 1976; Philphot and Arınç, 1976; Black and Coon, 1986; Adalı and Arınç, 1990; Arınç and Aydoğmuş, 1990; Adalı *et al.,* 1996; Şen and Arınç, 1998, 2000; Bozcaarmutlu and Arınç, 2008).

Among the cytochrome P450 families, there are a few forms that metabolically activate toxicants and carcinogens which are CYP1A1, CYP1A2, CYP1B1, CYP2A6, CYP2E1 and to a limited extent the CYP3A subfamily. (Gonzalez, 2005).

1.2.1.1.1 Cytochrome P4502E1

CYP2E1 is the classical ethanol-inducible cytochrome P450 isozyme, (Lieber, 1997) was formerly referred to as P450LM3a, P450j, and P450ac, P450alc. It was first identified in rabbits (Koop *et al.,* 1982) and later in rats (Ryan *et al.,* 1984; Patten *et al.,* 1986; Yang and Hong, 1993). The human CYP2E1 was subsequently characterized at the gene and protein levels (Song *et al.,* 1986; Yang and Hong, 1993). CYP2E1 is mainly found in liver. In addition, significant amounts of CYP2E1 also found in extrahepatic tissues including lung, kidney, brain, heart, endothelium of large blood vessels, bone marrow and nasopharyngeal tissues (Ding *et al.,* 1986; Ingelman-Sundberg *et al.,* 1993).

CYP2E1 is one of the most conserved forms in the CYP2 family and its orthologues share the same substrate specifity. In contrast to other mammals, CYP2E sub-family of rabbit liver is found to contain two genes which are *CYP2E1* and *CYP2E2*, whose protein products show 97% identical amino acid sequences. The CYP2E2 enzyme is expressed in very young rabbits whereas CYP2E1 is a late developing enzyme. CYP2E2 expression begins at birth and is abundant in one to three-week-old rabbits and its expression diminishes after three weeks. CYP2E1 is not present at birth, appearing during the fourth week and reaching a high level at two months, where it stays until the rabbits are at least six months old (Bonfils *et al.,* 1990; Ding *et al.,* 1991; Rich and Boobis, 1997).

CYP2E1 catalyzes many exogenous compounds; however, most of the substrates of CYP2E1 are exogenous which are industrial solvents, protoxins, procarcinogens and some drugs. In addition, early studies demonstrated that CYP2E1 is not efficient in catalyzing the oxidation of large and charged molecules such as benzo(a)pyrene, testosterone, dimethylamine or benzphetamine (Patten *et al.,* 1986; Yang *et al.,* 1990). On the other hand, CYP2E1 is of critical importance in the metabolic activation of many low-molecular weight carcinogens such as nitrosamines, pyridine, benzene and other toxic agents (Yang and Hong, 1993; Arınç *et al.,* 1991; Yamazaki *et al.,* 1992; Arınç *et al.,* 2000 a, b).

Besides ethanol, CYP2E1 catalyzes endogenous compounds such as acetone, acetoacetate, glycerol and fatty acids (arachidonic acid, lauric acid) (Lieber, 1997). The metabolism of many exogenous compounds by CY2E1 demonstrates the physiological importance of this enzyme.

Most of the substrates of CYP2E1 are exogenous substrates which are industrial solvents, protoxins and procarcinogens. Small molecular weight hydrocarbons such as benzene and styrene; nitrosamines and azocompounds such as NDMA and azoxymethane; heterocyclic compounds like pyridine; halogenated and non-halogenated alkanes and alkenes such as butadiene, chloroform, acrylonitrile, acrylamide, vinyl chloride; aromatic compounds such as acetaminophen, aniline are among various exogenous CYP2E1 substrates (Arınç *et al.*, 1990; Yong and Hong, 1993; Lieber, 1997; Sumner *et al.*, 1999; Arınç *et al.*, 2000 a, b). In addition, human CYP2E1 has been shown to metabolize drugs such as chlorzoxazone, acetaminophen (paracetamol), and the volatile anaesthetics such as sevoflurane, methoxyflurane and isoflurane (Koop *et al.,* 1985; Johansson and Ingelman-Sundberg, 1988; Gorsky and Hollenberg, 1989; Guengerich *et al.,* 1991; Kharasch and Thummel, 1993; Yang and Hong, 1995; Bolt *et al.,* 2003; Bolt and Hengstler; 2008).

Most of the substrates of CYP2E1 are also inducers of this enzyme. Besides ethanol, CYP2E1 is induced by several compounds such as acetone, pyrazole and isoniazid, and oxygen exposure. Some pathophysiological conditions such as starvation, diabetes, and obesity, also increase the level of CYP2E1 (Raucy *et al.,* 1990; Shimojo *et al.,* 1993; Yang and Hong, 1995; Arinç *et al.*, 2005, 2007). It has been observed that CYP2E1 induction due to chemicals or diabetes and obesity potentiates hepatotoxicity of certain compounds such as thioacetamide, chloroform,

carbon tetrachloride and bromobenzene in rats (Hanasono *et al.,* 1975; Wang *et al.,* 2000). On the other hand, inhibitors of CYP2E1 include diallylsulfate (DAS, the ingredient of garlic), disulfram, diallyl sulfoxide, diallyl sulfone, malotilate and disulfiram (Lieber, 1997). Endogenous and exogenous substrates, inducers and inhibitors of CYP2E1 are given in Table 1.3. CYP2E1 also converts these chemicals into more toxic and/or carcinogenic forms (Peter *et al.,* 1990; Guengerich *et al.,* 1991; Kharasch and Thummel 1993; Yang and Hong 1995) and plays a decisive role in the toxicity of CYP2E1 substrates (Bolt et al 2003).

For monitoring the CYP2E1 activity, N-demethylation of NDMA and hydroxylation of *p*-nitrophenol to 4-nitrocathecol are used most effectively. Besides these, hydroxylation of aniline and 6-hydroxylation of chlorzoxazone are also used for monitoring the activity of CYP2E1 (Lieber, 1997; Arınç *et al.*, 2000 a, b; Arınç *et al*., 2005, 2007).

CYP2E1 also plays role in oxidative stress CYP2E1 has been identified as a source of reactive oxygen species in CYP2E1-dependent monooxygenation reactions (Ekstrom and Ingelman-Sundberg, 1989; Persson *et al.,* 1990).

CYP2E1 gene shows several genetic polymorphisms (Lieber 1997; Bolt et al. 2003, Ulusoy *et al.,* 2007a, b). The human CYP2E1 gene is located in 10q24.3-qter region of chromosome 10, and spans 11,413 base pairs with nine exons and a typical TATA box (Umeno *et al.,* 1988). Ulusoy *et al.* (2007) have been studied the genotype and allele frequencies of three CYP2E1 polymorphisms, namely CYP2E1*5B, *6 and *7B in a control group representing Turkish population. In this study, the allele frequencies of the mutated alleles, CYP2E1*5B, CYP2E1*6 CYP2E1*7B were found to be 1.94%, 8.25%, 12.6%, respectively for Turkish popuation (Ulusoy *et al.* 2007a)

Table 1.3 Substrates, inducers and inhibitors of CYP2E1[a]

<table>
<tr><th colspan="2">Substrates</th><th>Inducers</th></tr>
<tr><td colspan="2">Endogenous substrates</td><td rowspan="2">Ethanol
Isopropanol
Acetone
Ketones
Fasting Diabetes
Starving
Benzene
Ether
Pyrozole
Isoniazid
Ether
Pyridine
Dietary lipids
Oxygen exposure</td></tr>
<tr><td colspan="2">Ethanol
Acetone
Acetoacetate
Acetaldehyde
Glycerol
Fatty acids (arachidonic acid, lauric acid)</td></tr>
<tr><td colspan="2">Exogenous substrates</td><td>Inhibitors</td></tr>
<tr><td>Drugs and volatile anasthesics</td><td>Other chemicals and solvents</td><td rowspan="2">Disulfiram
Diallylsulfide
Diallylsulfoxide
Diallylsulfone
Chlormethiazole
Diethyldithiocarbamate
Malotilate
Isothiocyanates
4-methyl-pyrazole</td></tr>
<tr><td>Acetaminophen
Phenacetin
Chlorzoxazone
Isoniazid
Halothane
Enflurane
Isoflurane
sevoflurane</td><td>Benzene
Pyridine
Pyrazole
Acrylonitrile,Methacrylonitrile
Acrylamide
Ethanol
Phenol
Acetaldehydes
Styrene
Diethylether
Acetone
Chloroform
Vinyl chloride,Vinyl bromide
Nitrosamines(such as NDMA)
Carbontetrachloride
Hexane
Butadiene
1,1,1-trichloroethane
1,2-dichlorpropane
Methylchloride
Methylene dichloride</td></tr>
</table>

[a]Adapted from Yang and Hong, 1993; Lieber, 1997; Anzenbacher and Anzenbaherova, 2001.

In addition, the presence of several polymorphisms in CYP2E1 gene have been found to be associated with increased genetic susceptibility to several types of chemical-induced diseases, including several types of cancer (El Zein *et al.,* 1997; Wu *et al.,* 1998; Farker *et al.,* 1998; Liu *et al.,* 2001; Ulusoy *et al.,* 2007 b; Bolt and Hengstler, 2008).

1.2.1.1.2 Cytochrome P4502B4 (CYP2B4)

In mammals, CYP2B subfamily is generally associated with detoxifying metabolism of xenobiotics. It also metabolizes steroid hormones (i.e., testosterone and androstedione) and several important pharmaceutical agents (Lewis and Lake, 1997). In addition, it has also been reported that CYP2B isozymes metabolically activates carcinogens, 6-aminochrysene, and 3-methoxy-4-aminoazobenzene (Lewis and Lake, 1997).

CYP2B family involved in the bioactivation of antitumor agents such as cyclophosphamide and iphosphamide, and also in the metabolism of the pesticide metoxychlor and some promutagens, such as aflatoxin B1 and tobacco specific nitrosamines. Phenobarbital (PB) is considered to be a classical inducer of CYP2B (Waxman and Azaroff, 1992). Benzphetamine, bupropion, chloramphenicol, cocaine, nicotine, phenobarbital, tamoxifen, and testosterone are among the substrates of CYP2B family (Lewis and Lake, 1997; Arınç and Bozcaarmutlu, 2003; Bozcaarmutlu and Arınç, 2008). The inducers of CYP2B family are phenobarbital, phenytoin and rifambin whereas metyrapone, secobarbital, n-Octylamine constitutes the inhibitors of CYP2B family (Danielson, 2002).

CYP2B family has been extensively studied in many different mammalian species and 17 different proteins were identified as of January 2003 (http://drnelson.utmem.edu/Cytochromep450.html). Genes encoding proteins of this subfamily includes (*CYP2B1* and *CYP2B2*, 97% homology) in rats, *cyp2b10 in* mice, (*CYP2B6*) in humans (Pustylnyak, 2007). In rabbits, there are at least two proteins of CYP2B family members which are CYP2B4 and CYP2B5.

In rabbit lung CYP2B4 constitutes the major part of the CYP2B family whereas it is present in small amounts in liver (Serabjit-Singh *et al.,* 1983). It is well known that N-demethylation of benzphetamine is exclusively catalyzed by CYP2B4 in rabbit liver and lung (Philpot and Arinc¸1976; Arınç, 1993) Therefore, for monitoring CYP2B4 activity in rabbit liver and lung N-demethylation of benzphetamine can be used.

Rabbit CYP2B4 mainly metabolizes hydrophobic xenobiotics. Arachidonic acid is metabolized by CYP2B4 in lung (Zeldin *et al.,* 1995). 2-ethynylnaphthalene is an inhibitor of CYP2B4 (Cheng *et al.,* 2007). Phenobarbital treatment increases the level of CYP2B4 in liver whereas its treatment does not change the CYP2B4 levels in lung (Serabjit-Singh *et al.,* 1983; Arınç, 1993).The studies conducted by Arınç *et al.* (2005, 2007) have demonstrated that in diabetic rabbits the level of CYP2B4 and associated enzyme benzphetamine N-demethylase, activity have not been changed in diabetic rabbit liver, (Arınç *et al.,* 2005) kidney and lung microsomes (Arınç *et al.,* 2007).

1.2.1.1.3 Cytochrome P4503A4 (CYP3A4, CYP3A6 in rabbits)

Human CYP3A4 is the most abundant hepatic and intestinal phase I enzyme that metabolizes approximately 50% of the marketed drugs. Four different isozymes were identified which are CYP3A4, CYP3A5, CYP3A43 and CYP3A7 in humans. Among these, CYP3A4 and CYP3A5 are the most abundantly expressed CYPs isozymes in liver and gastrointestinal tract (40% and 80% of total P450 content, respectively) (Cupp and Tracy, 1998; Nebert and Russel, 2002; Lamba *et al.*, 2002; Xie *et al.*, 2004).

CYP3A family participates in the metabolism of certain chemicals (mainly drugs) to their reactive derivatives that cause cell toxicity and cancer (Gonzalez, 2005). The CYP3A4 and CYP3A5 participate in the metabolism of more than 120 prescribed drugs (Liu *et al.*, 2007) and endogenous substrates such as steroids and bile acids.

In rats there are five different CYP3A isoforms that has been identified. In rabbits; however, the only CYP3A isoform identified so far is the CYP3A6 which

has similar P450 predominance and substrate specifity as human isoform of CYP3A4 (Guengerich, 1997; Chirulli *et al.*, 2005; Weber *et al.*, 2001).

CYP3A family has various substrates, inhibitors and inducers which are given in Table 1.4. Since CYP3A4 mainly participates in drug metabolism, altered CYP3A activity is predicted to lead to changes in intestinal metabolism, hepatic biotransformation and renal clearance of affected drugs (Liu *et al.*, 2007). Inhibitors of CYP3A family decrease the metabolism of substrates and generally lead to increased drug effect whereas inducers increase metabolism of substrates and generally lead to decreased drug effect (Cupp and Tracy, 1998). Accordingly, it is not surprising that CYP3A4 (CYP3A6 in rabbits) involved in many clinically drug-drug interactions. CYP3A subfamily does not only involve in drug-drug interactions but also participates in the metabolism of endogenous steroidal compounds such as testosterone, progesterone, androstenedione compounds (Yamazaki and Shimada, 1997; Wang *et al.,* 2000).

Table 1.4 Substrates, inhibitors and inducers of CYP3A family[a]

Substrates	**Inducers**	**Inhibitors**
Midazolam	Sulfinpyrazone	Ketaconozole
Triazolam	Phenytoin	Troleandomycin
Alfentanil	Carbamazepine	Erythromycin
Testosterone	Dexamethasone	Clarithromycin
Cortisol	Phenobarbital	Fluconazole
Alprazolam	Rifampin	1-Aminobenzotriazole
Tacrolimus	Rifampin	Verapamil
Vincristine		
Quinidine		
Erythromycin		

[a] Adapted from review Liu *et al.*, 2007

1.2.1.1.4 NAD(P)H-Quinone Oxidoreductase (NQO1)

NAD(P)H-Quinone Oxidoreductase (NQO1, DT-Diaphorase) is a two-electron reductase that is characterized by its capacity for utilizing either NADH or NADPH as a reducing cofactor and by its inhibition by dicoumarol (Ernster, 1967). It consists of two identical subunits. Each subunit has a molecular weight of 30.000 and contains one flavin adeninedinucleotide (FAD) prosthetic group, noncovalently attached to the protein. It is a cytosolic enzyme (>90%) and widely distributed in animal kingdom most abundantly in liver.

Three different forms of cytosolic NQOs in rats, two isofunctional cytosolic NQOs in mouse has been identified. In humans, four gene loci encoding NQOs has been identified. One of these gene loci is cytosolic NQO1 (DT diaphorase) (Jaislaw, 2000).

NQO1 participates in the reduction of very broad range of substrates including quinones, quinine-imines, glutathionyl-substituted naphtoquinones, dichlorophenolindolphenol, azo and nitro compounds (Ernster, 1967; Lind *et al.,* 1990; Ross, 1997). Both ortho and para quinones are substrates for NQO1 (Segura-Aguliar and Lind, 1989). In addition to quinones, NQO1 functions efficiently as a nitro-reductase utilizing substrates such as dinitropyrenes, nitrophenylaziridines and nitrobenzamides (Hajos and Winston, 1991; Knox *et al.,* 1988). NQO1 is also capable of performing 4-electron reduction of azo-dyes and nitro-compounds (Boland *et al.,* 1991; Huang *et al.,* 1979).

NQO1 generally categorized as a detoxification enzyme and an anti-oxidant enzyme. However, NQO1 also participate in the bioactivation of chemically reactive metabolites. They reduce quinones to hydroquinones bypassing the potentially toxic semiquinone radical intermediates (Lind *et al.,* 1982). Since all hydroquinones are not redox-stable; redox-labile hydroquinones can react with molecular oxygen to form semiquinones and generate reactive oxygen species (Cadenas, 1995).

NQO1 can be induced by some pro-carcinogens such as 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and polycyclic aromatic hydrocarbons, azo dyes and phenolic anti-oxidants (Li and Jaiswal, 1992) Studies demonstrated that lung, liver, breast and colon tumors have elevated levels of NQO1 expression

(Schlager and Powis, 1990; Malkinson, 1992; Cresteil and Jaiswal, 1991, Jarrett *et al.,* 1998). In cancer teatment quinine-containing alkylating agents have been used and their qoinone moiety needs to be reduced for activation of their alkylating substituents. Thus, NQO1 is important in cancer treatment that high amounts of this enzyme found in tumor tissues and it has the ability to reduce these quinone-containing alkylating agents. Among this quinone-containing alkylating agents indolequinones, mitosenes (Mitomycin C), aziridinylbenzoquinones (Diaziquone, AZQ), pyrolobenzimidazolequinones quinolinequinones, benzoquinone mustards have been considered as potential agents for NQO1 directed approaches to chemotheraphy.

1.2.2 Phase II Xenobiotic metabolizing Enzymes

The Phase II xenobiotic metabolizing enzymes mainly participate in the conjugation (addition) reactions. A characteristic of most conjugation reactions is the replacement of a hydrogen atom present in a –OH, -NH_2 or –SH group, by the addition of conjugating subgroups. This functional group such as –OH, -NH_2 or –SH groups may either be present on the parent xenobiotic or drug, or they may have resulted from a Phase I reaction of oxidation, reduction or hydrolysis. The conjugating subgroups include glucuronate, acetate, glutathione, glycine, sulfate and methyl groups. Thus, in these conjugation reactions which are mediated by Phase II xenobiotic metabolizing enzymes; the xenobiotic or drug becomes linked to an endogenous moiety through one or more functional groups (Schenkman, 1999).

These conjugation reactions, catalyzed by Phase II xenobiotic metabolizing enzymes, mainly involve glucuronidation, acetylation, glutathione conjugation, glycine conjugation, sulfate conjugation, methylation. These enzymes may be located in cytosols or microsomes. The majority of Phase II xenobiotic metabolizing enzymes are specific transfer enzymes which are UDP-glucuronyl acid transferase, N-Acetyl transferase, ACYL-Co-A glycine transferase, and sulfotransferases. In addition, transmethylase and epoxide hydrolase are other Phase II xenobiotic metabolizing enzymes. With the exception of acetylation and methylation, Phase II xenobiotic metabolizing enzymes result in a high increase in xenobiotic/drug hydrophillicity and promote the excretion of foreign chemicals.

1.2.2.1 Glutathione-S-Transferases (GSTs)

The Glutathione S-transferases (GSTs) are supergene family of dimeric enzymes that catalyze the conjugation of reduced glutathione (GSH) with a variety of nonpolar compounds that contain an electrophillic carbon, nitrogen or sulphur atom. (Strange *et al.*, 2000; Hayes *et al.*, 2005). CDNB (1,2-dichloro-4-nitrobenzene) is the general substrate for GSTs in cytosolic extracts of liver (Armstrong, 1997). GSTs are probably present in all life forms, in microbes, flies, plants and mammals.

There are three main families of GSTs which are cytosolic, mitochondrial and microsomal GSTs. The cytosolic and mitochondrial GSTs comprise the soluble enzymes that are only distantly related. The three families share the feature that they catalyze conjugation of GSH with 1,2-dichloro-4-nitrobenzene (CDNB) (Lander *et al.,* 2004; Robinsson *et al.,* 2004; Jakobbson *et al.,* 1999).

GSTs catalyze GSH-dependent detoxification of reactive electrophile xenobiotics such as genotoxic chemicals carcinogens, cytotoxic chemotherapeutic agents, environmental and chemical carcinogens such as pollutants, herbicides, insecticides which are exogenous substrates of GSTs. In Table 1.5 the GST-catalyzed exogenous and endogenous compounds are given. GSTs detoxifies epoxides formed from aflatoxin B1, 1-nitropyrene, 4-nitroquinoline, polycyclic aromatic hydrocarbons (PAHs), styrene those derived from environmental carcinogens and heterocyclic amines, produced by cooking protein rich foods.

Table 1.5 Exogenous and endogenous substrates of GSTs[a]

Exogenous substrates	
Chemotheraupetic agents	Adriamycin, busulfan, carmustine chlorambucil, *cis*-platin, crotonyloxymethyl-2-cyclohexenone cyclophosphamide, ethacrynic acid, melphalan, mitozantrone, thiotepa
Enviromental and Chemical carcinogens	Acrolein, atrazine, DDT, inorganic arsenic, lindane, malathion, methyl parathion, muconaldehyde, tridiphane
Endogenous compounds (by products of oxidative stress)	α-β-unsaturated aldehydes, quinones, epoxides, hydroperoxides

[a]Adapted from Hayes *et al.*, 2005; Coles and Kaldbular, 2003

GSTs also catalyze several endogenous compounds (Table 1.5). They are involved in the metabolism of by products of oxidative stress resulted from degradation of aromatic amino acids (phenylalanine and tyrosine), synthesis of steroid hormones, synthesis of important metabolites of arachidonic acid, modulation of signaling pathways.

Some environmental and dietary carcinogens are genotoxic only after activation *in vivo*. Since Cytochrome P450s are the most efficient enzymes of carcinogen activation, this CYP-catalyzed oxidation are also frequently followed by conjugation with GSH via GST, and other detoxifying enzymes.

The general mechanism catalyzed by all GSTs is given below:

$$GSH + R\text{-}X \longrightarrow GSR + HX \quad (2)$$

GSTs detoxify the xenobiotics through the mercapturic acid pathway (Keen, 1978). They catalyzes the first four step required for the synthesis of mercapturic acid. The subsequent reactions are the removal of the γ-glutamyl moiety and glycine

from the glutathione conjugate, followed finally by N-acetylation of the resulting cysteine conjugate. Therefore, it is also noteworthy to mention that, the effectiveness depends on also glutamate cysteine ligase and glutathione synthase to supply GSH and the actions of transporters to remove glutathione conjugates from the cell.

In the present study, the soluble cytosolic GSTs are in concern. Cytosolic GSTs represent the largest family of such transferases. The majority of the cytosolic GSTs are found in the cytoplasm of the cell.

Mammalian cytosolic GSTs are all dimeric with subunits of 199-244 amino acids in length. In mammalian species based on their amino acid sequence similarities seven classes of cytosolic GSTs are named which are called Alpha, Mu, Pi, Sigma, Theta, Omega and Zeta. In non-mammalian species other classes are designated as Beta, Delta, Epsilon, Lambda, Tau and 'U' class. In human and rodents, cytosolic GST isoenzymes within a class typically share >40% identity, and those between classes share <25 % amino acid sequence identity. (Sheehan *et al.,* 2001; Edwards and Dixon, 2004).

Cytosolic GSTs catalyze several reactions including conjugation, reduction, isomerization. They also bind covalently and non-covalently to hydrophobic nonsubstrate ligands which contributes to intracellular transport, disposition of xenobiotics and hormones. These compounds include azo-dyes, bilirubin, heme, PAHs, steroid and thyroid hormones.

GSTs have been found to be expressed in all organs studied. However, their levels of expression vary widely both between organs and between individuals (Coles and Kaldbular, 2003). Cytosolic GSTs exhibit genetic polymorphisms and this phenomenon has been associated with susceptibility to various diseases such as cancer and inflammatory diseases (Strange *et al.*, 2000).

1.3 Phenolic Compounds and Beneficial Effects

Flavonoids are part of a family of naturally occurring phenolic compounds. They are found in vegetables, fruits, nuts, and beverages such as coffee, tea, red wine and as well as herbal remedies (Hollmann and Katan, 1997). The large number of compounds arises from the various combinations of multiple hydroxyl and methoxyl group substituents in their flavonoid skeleton (Hodek *et al.,* 2002).

The flavonoids have several biochemical and pharmacological properties. One of the most important and widely studied effects of flavonoids is their cancer preventive activities. Various different mechanisms involve in the cancer protective effects of flavonoids. One of these mechanisms is the alterations of enzyme expression and/or activities by these flavonoids.

Since, Phase I and Phase II enzymes participate in the metabolism, bioactivation and detoxification of toxic compounds and drugs, modulation of these enzymes by flavonoids is important in cancer chemoprevention. Studies have demonstrated that flavonoids modulate activities and expression of specific CYP isozymes (Wood *et al.,* 1986). In addition, the inductions of some of the Phase II enzymes by flavonoids are also effective in cancer chemoprevention (Moon *et al.,* 2006). Among these flavonoids, a recently important flavonoid with well-known cancer chemoprevention effect is resveratrol.

1.3.1 Resveratrol

Resveratrol (trans-3,4',5-trihydroxystilbene) is a plant originated polyphenolic compound found in grapes, peanuts, mulberries and red wine (Soleas *et al.*, 1997; Sanders *et al.*, 2000; Piver *et al.,* 2003). Resveratrol is synthesized by grapes in response to fungal infections. It is found in *cis* and *trans* form of which *trans* form is the naturally occurring form (Soleas *et al.*, 1997). The structure of resveratrol was given in Figure 1.5.

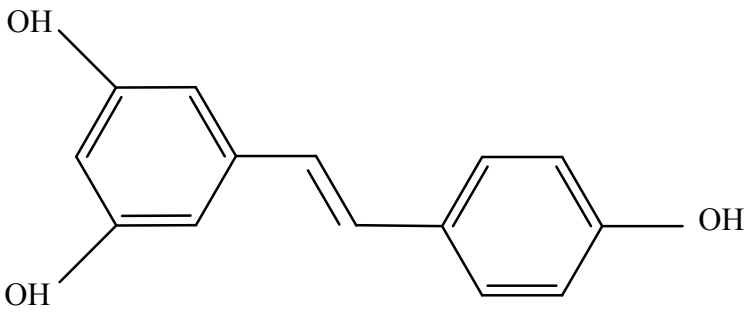

Figure 1.4 Structure of resveratrol (trans-3,4',5-trihydroxystilbene)

Several beneficial effects of resveratrol have been suggested in the studies. First of all, resveratrol has protective effect against cardiovascular diseases (Goldberg, 1996; Soleas *et al.*, 1997; Constant, 1997; Szmitko and Verma, 2005; Penumathsa *et al.*, 2006)). The beneficial effects of resveratrol in cardiovascular diseases have also been shown in rats (Ray et al., 1999; Miatello *et al.,* 2005). The effects of resveratrol have been attributed to its antioxidant and anticoagulant properties (Fauconneau et al., 1997).

Resveratrol has also anticoagulant properties; it is an inhibitor of platelet aggregation and eicosonoid production in human platelets and neutrophiles (Bertelli *et al.,* 1996). It participates in the modulation of the synthesis of hepatic apolipoproteins and lipids (Frankel *et al.,* 1993).

The beneficial effects of resveratrol are not limited to cardiovascular disease prevention and its anti-coagulant property. Resveratrol is also an anti-cancer agent. Resveratrol plays role in the inhibition of tumor initiation, promotion and progression (Jang *et al.*, 1997; Dong, 2003). It has been demonstrated that initiation of colonic tumor development was inhibited by resveratrol in rats. In addition, anti-proliferative effects of resveratrol on the growth of human colon cancer cells have been demonstrated *in vitro* (Sengottuvelan *et al.*, 2006; Schneider *et al.*, 2000). The inhibitory effects of resveratrol have also demonstrated on human breast cancer cells *in vitro* and prostate cancer (Tang *et al.*, 2006; Stewart, 2003).

Most of the chemical carcinogens require metabolic activation through cytochrome P450 systems. One of the possible mechanism by which phenolic compounds may exert their anti-carcinogenic effects is through an interaction by cytochrome P450 system, either by the inhibition or activation of certain types of these enzymes (Guengerich and Shimada, 1998; Hursting *et al.,* 1999; Sporn and Suh, 2000).

It has been demonstrated that resveratrol inhibits the CYP1A1 expression and activity which are important in tumor progression (Ciolina *et al.,* 1998; Ciolina and Yeh, 1999) and also CYP1B1 and CYP1A2 (Chun *et al.,* 1999; Chang *et al.,* 2000).

1.4 Aim of the present study

Acrylamide is known as an animal neurotoxin, a reproductive toxin and a carcinogen. Specifically, the carcinogenic effects of acrylamide has received great attention in recent years due to detection of acrylamide and its residues in human diet and also detection of acrylamide adducts (hemoglobin and DNA) in blood of both animals and humans (Tareke *et al.,* 2000; 2002; Rosen and Helenas; Bergmark 1997; Hagmar, 2001).

Although acrylamide metabolism is complex and not fully understood yet (Sczerbina *et al.,* 2008), several recent studies in rodents have demonstrated that acrylamide is metabolized to its epoxide glycidamide by one of the CYP enzyme which is CYP2E1. Furthermore, it was also suggested that this metabolic pathway, i.e. the conversion of acrylamide to its more toxic epoxide by liver CYP2E1, is the major reason for acrylamide carcinogenicity. (Besaratinia and Pfeifer, 2004; Adler *et al.*, 2000; Manjanatha *et al.*, 2006; Ghanayem *et al.,* 2005b; Rice, 2005).

CYP2E1 is one of the cytochrome P450s that metabolically activates procarcinogens and carcinogens to their more carcinogenic forms. Substrates of this enzyme may also serve as its inducers. The induction of this enzyme leads to further bioactivation of toxic metabolites to their more toxic derivatives, and also leads to formation of reactive oxygen species which are cancer suspects. Furthermore, CYP2E1 enzyme has several polymorphisms in its gene and for this reason it has been associated with increased genetic susceptibility to several types of chemical-induced diseases, including several types of cancer. Although, it was demonstrated that acrylamide is metabolized mainly by liver CYP2E1 the *in vivo* effects of acrylamide on CYP2E1 activities and protein levels has not been clarified yet. In addition to CYP2E1, among cytochrome P450 enzymes CYP1A1, CYP1A2, and CYP1B1isozymes are also associated with an increased risk of certain type of cancers or toxic effects. However, there is also no available data regarding *in vivo* acrylamide effects on other cytochrome P450s involved in the toxication process. In addition to CYP2E1, studies have also been suggested that the major acrylamide scavenger in the body is GSH, thereby GST enzymes. Thus, the effects of *in vivo* acrylamide treatment on GST enzymes were also studied.

Besides acrylamide, another compound investigated in the present study was resveratrol. Resveratrol is a recently important flavonoid with its well-known chemoprevention effects on different types of cancer (Jang *et al.*, 1997; Dong, 2003). Resveratrol is found in grapes, red wine, mulberries and peanuts that it can be easily consumed by humans in their diets like other phenolic compounds. Among several mechanisms, these flavonoids implement their chemoprevention effect by modulation of enzyme expression and/or activities such as inhibition of CYPs (mainly metabolize procarcinogens to more toxic compounds) or activation of anti-oxidant enzymes such as GSTs. In the present study, resveratrol was chosen to investigate whether it is protective against carcinogenic acrylamide, by modulating enzyme activities such as CYPs or anti-oxidant enzymes (GSTs).

In this regards, the aims of the present study were:

1. to determine the *in vivo* effects of carcinogenic acrylamide on liver, kidney and lung microsomal cytochrome P450 enzyme activities in acrylamide treated and control rabbits

2. to determine the changes in protein levels of cytochrome P450s of rabbit liver and kidney microsomes by *in vivo* effects of carcinogenic acrylamide

3. to determine the *in vivo* effects of acrylamide on GST enzyme activities on rabbit liver, kidney and lung cytosols, accordingly to analyze changes in anti-oxidant status of the rabbits due to acrylamide administration

4. to investigate the *in vivo* protective effects of resveratrol against effects of carcinogenic acrylamide on rabbit liver, lung and kidney microsomal CYP enzyme activities

5. to examine the *in vivo* protective effects of resveratrol on the protein levels of cytochrome P450s of rabbit liver and kidney microsomes changed by *in vivo* effects of acrylamide

6. to determine the *in vivo* protective effects of resveratrol on GST enzyme activities changed by *in vivo* effects of acrylamide on rabbit liver, kidney and lung cytosols, accordingly to analyze changes in anti-oxidant status of the rabbits due to resveratrol and acrylamide administration

In order to achieve these goals, rabbits were treated with acrylamide, resveratrol and their combined solutions in two different doses and conditions

(before and after acrylamide treatment) *in vivo*. Cytochrome P450 dependent enzyme activities and protein levels were investigated. In addition to cytochrome P450 dependent enzyme activities, another Phase I enzyme which is NQO1 activities were also determined. Furthermore, to investigate the change in anti-oxidant parameters, total GST and GST-Mu activities were determined in all groups.

CHAPTER 2

MATERIALS AND METHODS

2.1 Materials

Resveratrol was kindly provided by Mikrogen Pharma, İstanbul, Turkey.

Acrylamide (A8887), ε-aminocaproic acid (ε-ACA; A2504), aspartic acid (A9006), ammonium persulfate (APS; A 3678), α-ketoglutarate (K3752), l-aspartate (A9256), l-alanine (A7469) bovine serum albumin (BSA; A7511), bromochloroindoylphosphate (BCIP; B8503), 1-chloro-dinitrobenzene (CDNB; C6396), dichlorphenolindophenol (DCPIP; D1878), 3,3'-methylene–bis (4-hydroxycoumarin) (M1390), erythromycin (E-0774), glucose-6-phosphate dehydrogenase (G8878), glutathione reduced form (G4251), N-2-hydroxyethylpiperazine-N-2, ethane sulfonic acid (HEPES; H3375), β-mercaptoethanol (M6250), N'-N'-methylene bisacrylamide (BIS; A2504), N-nitrosodimethylamine (NDMA; N3632), nitrotetrazolium blue (NBT; N6876), N'-N'-dimethylformamide (F7508), pyruvate (P2256), phenazine methosulfate (P9625), phenylmethane sulfonyl fluoride (PMSF; P7626), 2-amino-2(hydroxymethyl)-1,3-propandiol (Tris; T1378), sodium potassium tartrate (S2377) were purchased from Sigma Chemical Company, Saint Louis, Missouri, USA.

Copper (II) sulfate (Cu_2SO_4; 02790), 1,2-dichloro-4-nitrobenzene (DCNB; D85662), glycerol (4093), potassium dihydrogen phosphate (5101), dipotassium hydrogen phosphate (4871), magnesium chloride (5833), sodium hydroxide (06462), p-nitrophenol (106798), zinc chloride (8815), trichloroacetic acid (TCA; 00256) were the products of E. Merck, Darmstadt, Germany.

Aniline (A0759) was purchased from Fisher Scientific Company, Chemical Manufacturing Division, Fair Lawn, New Jersey, USA.

Glucose-6-phosphate dehydrogenase (G3789), glycine (A4554), β-nicotinamide adenine dinucleotide phosphate ($NADP^+$; A-1394), β-nicotinamide adenine dinucletide phosphate tetrasodium salt (NADPH; A1395), β-nicotinamide

adenine dinucleotide reduced form (NADH; N8129) were purchased from Applichem Biochemica, Chemica Synthesis Services, Darmstadt, Germany.

Ammonium acetate (25006), acetic acid (glacial) (27225), acetyl acetone (33005), ethanol (32221), sodium chloride (13423), potassium chloride (13424) were obtained from Riedel de Haen, Sigma Aldrich Company.

Benzphetamine-HCL was kindly provided by Dr. J. F. Stiver of UpJohn Co., USA.

All the other chemicals used in this study were of analytical grade and were from commercial sources at the highest grade of purity.

2.2 Animals and Treatments

2.5 months old adult male New Zealand white rabbits (weighing 2.0-2.9 kg) were purchased from Lameli Animal Husbandry, Ankara, Turkey. The rabbits were caged separately and housed for 7 days before the beginning of the treatments at 23-25^0 C on a 12 h light/12 h dark cycle with free access to water and food (commercial chow). The procedure involving animals and their care were carried out in accordance with the declaration of Helsinki.

After one week period, the rabbits were randomly selected and divided into 5 groups consisted of 4-5 animals each. The animals were treated with acrylamide and resveratrol in two different doses in two different doses and conditions: 1) acrylamide dissolved in distilled water was injected subcutaneously to the animals 2) resveratrol dissolved in carboxymethyl cellulose was administered to the animals, intragastrically. First group consisted of control animals administered by physiological saline. Second group, acrylamide group (“AA” group), was injected 3 dose of 100 mg/kg body weight (b.w.) acrylamide at day 1, day 5 and day 8. Third group, resveratrol group (“RESV” group), was administered 3 dose of 25 mg/kg b.w. resveratrol at day 1, day 5 and day 8. In the fourth group (“RESV+AA” group), the animals were treated with 25 mg/kg b.w resveratrol at day 1, day 5 and day 8; 6 hours following resveratrol treatment, animals were injected by 100 mg/kg body weight acrylamide at day 1, day 5 and day 8. Finally, in the fifth group (“AA+RESV” group), the animals were injected with100 mg/kg b.w. acrylamide at day 1, day 5 and day 8; then, at day 8, 6 hours following the last acrylamide

injection, a single dose of 100 mg/kg b.w. resveratrol was administered to the animals. Schematic representation of the treatments was shown in Figure 2.1. All of the animals ware sacrificed 20 h after last treatment by decapitation.

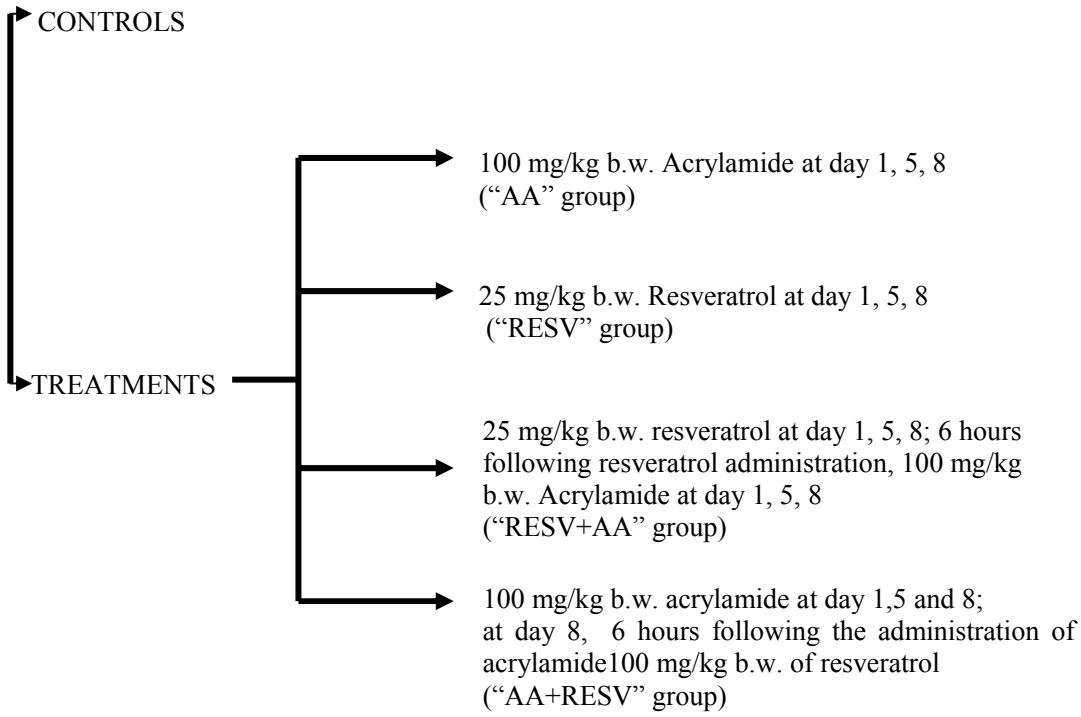

Figure 2.1 Schematic representation of acrylamide and resveratrol treatments of rabbits

2.3 Methods

2.3.1 Preparation of Rabbit Liver Microsomes

Rabbit liver microsomes were prepared according to the method of Arınç and Adalı (1990) with slight modifications. Adult male New Zealand rabbits purchased from Lameli Animal Husbandry, Ankara, Turkey, were killed by decapitation. The livers each weighing about 40-80 g was removed immediately after killing the animals. Gall bladders were removed from the livers to prevent the inhibitory effect of the gall bladders' content on monooxygenase activity. Then, the livers were placed on crushed-ice. After the removal of connective and fatty tissues, the livers were washed several times with cold distilled water and then with 1.15 % KCl to remove the excess blood. All subsequent steps were carried out at 0-4 ^{0} C. After draining and blotting on a filter paper, the livers were weighed for each rabbit tissue and minced by scissors. Then, the minced liver tissues were homogenized in 1.15% KCl solution containing 2 mM EDTA, 0.25 mM ε – ACA and 0.1 mM PMSF at a volume equal to 3 times the weight of liver by using Potter-Elvehjem glass homogenizer coupled with a motor (Black and Decker, V850, multispeed drill)-driven teflon pestle at 2 400 rpm. Fifteen passes were made for the homogenization of liver tissue.

The resulting homogenate was centrifuged at 10 031 rpm (10 800 xg) (Sigma 3K30 Centrifuge, Osterode am Harz, Germany) with 12156 rotor for 25 minutes to remove cell debris, nuclei and mitochondria. Then, the supernatant fraction containing the endoplasmic reticulum and soluble fraction of the cells was filtered through double layers of cheese-cloth by the help of a Buchner funnel. The microsomes were sedimented from supernatant by centrifugation at 45 000 rpm (145 215 xg) for 50 minutes using a T 1270 type rotor in Sorvall Combi Plus Ultracentrifuge (Sorvall, Du Pont Company, Newton, Connecticut, USA). The supernatant fraction (cytosol) were taken and shocked with liquid nitrogen and then stored at – 80 ^{0} C in order to use in other studies related with cytosolic enzymes. In order to remove the excess hemoglobin, the sedimented firmly packed microsomal pellet was suspended in 1.15 % KCl solution containing 2 mM EDTA and centrifuged again at 45 000 rpm (145215 xg) for 50 minutes using a T1270 type rotor

in Sorvall Combi Plus Ultracentrifuge (Sorvall, Du Pont Company, Newton, Connecticut, USA). The supernatant fraction was discarded and then, the washed microsomal pellet was resuspended in 25% glycerol containing 1mM EDTA, at a volume of 0.5 ml for each gram of liver tissue. The resuspended microsomes were homogenized manually by using the teflon-glass homogenizer in order to get a homogenous microsomal suspension. Finally, the homogenized microsomal suspensions were gassed with nitrogen in eppendorf tubes and stored in -80^0 C for enzymatic assays. The homogenized microsomal suspensions contained approximately 15-40 mg of protein per ml.

2.3.2 Preparation of Rabbit Kidney and Lung Microsomes

Rabbit kidney and lung microsomes were prepared essentially with the same procedure used for the preparation of rabbit liver microsomes with some modifications. (Arınç *et. al.,* 2000a, b)

After decapitation of the rabbits, the kidney and lungs were removed and immediately placed on crushed ice. All subsequent steps were carried out at $0 - 4\ ^0$ C. After removal of fatty and connective tissues, organs were washed several times by using cold distilled water and then with 1.15% KCl solution. After draining and blotting on a filter paper, the lung and kidney tissues were weighed for each rabbit. Then, the minced kidney and lung tissues were homogenized in 1.15% KCl solution containing 2 mM EDTA, 0.25 mM ε – ACA and 0.1 mM PMSF by using Potter-Elvehjem glass homogenizer coupled with a motor (Black and Decker, V850, multispeed drill)-driven teflon pestle at 2 400 rpm in order to get homogenous lung and kidney tissues. The volume of the 1.15% KCl solution containing 2 mM EDTA, 0.25 mM ε – ACA and 0.1 mM PMSF was equal to 2.2 times the weight of kidney and 2.5 times the weight of lung tissues. Fifteen passes were used for homogenization of the minced kidney and lung.

The resulting kidney or lung homogenate was centrifuged at 10 031 rpm (10800 xg) (Sigma 3K30 Centrifuge, Osterode am Harz, Germany) with 12156 rotor for 25 minutes to remove cell debris, nuclei and mitochondria. Then, the supernatant fraction containing the endoplasmic reticulum and soluble fraction of the cells was filtered through double layers of cheese-cloth by the help of a Buchner funnel. The microsomes were sedimented by centrifugation at 45 000 rpm (145 215xg) for 50 minutes using a T 1270 type rotor in Sorvall Combi Plus Ultracentrifuge (Sorvall, Du Pont Company, Newton, Connecticut, USA). The supernatant fraction (cytosol) were taken and shocked with liquid nitrogen and then stored at – 80^0 C in order to use other studies related with cytosolic enzymes. In order to remove the excess hemoglobin, the sedimented firmly packed microsomal pellet was suspended in 1.15% KCl solution containing 2 mM EDTA and centrifuged again at 45 000 rpm (145 215 xg) for 50 minutes using a T1270 type rotor in Sorvall Combi Plus Ultracentrifuge (Sorvall, Du Pont Company, Newton, Connecticut, USA). The supernatant fraction was discarded and the washed microsomal pellets were resuspended in 25% glycerol containing 1mM EDTA, at a volume of 0.3 ml for each gram of kidney and lung tissues. The resuspended microsomes were homogenized manually by using the teflon-glass homogenizer in order to get a homogenous microsomal suspension. Finally, the homogenized microsomal suspensions were gassed with nitrogen in eppendorf tubes and stored in – 80^0 C for enzymatic assays. The homogenized microsomal suspensions were contained approximately 20-50 mg of protein per ml for kidney and 15-35 mg of protein per ml for lung tissues.

2.3.3 Determination of Transaminases Activity in Blood Serum

The transaminases constitute a group of enzymes which catalyze the interconversion of amino acids and α-ketoacids by transfer of amino groups. The α-ketoglutarate/L-glutamate couple serves as the amino group acceptor and donor in pair in all amino transfer reactions. These enzymes are referred to as aminotransferases; AST (Aspartate Aminotransferase) and ALT (Alanine Aminotransferase.

2.3.3.1 Determination of Aspartate Aminotransferase (AST) Activity

Animal cells contain a variety of amino transferases. AST is found in practically every tissue of the body, including red blood cells, also it is particularly high in concentration in cardiac muscle and liver, intermediate in skeletal muscle and in much lower concentrations in others.

The enzyme aspartate aminotransferase (AST) reversibly transfer an amino group from aspartate to the α-ketoglutarate and forms oxaloacetate. In this study, the AST enzyme activity in blood serum was determined according to the method of Reitman and Frankel (1957). The method involves the direct combination of formed oxaloacetate with 2,4-dinitrophenylhydrazine (DNPH) and the measurement of the color in alkaline solution. The AST catalyzed reaction and measurement of AST activity are given in Figure 2.2 and Figure 2.3. Although the ultraviolet (UV) method is the reference method, the colorimetric methods eliminates the need of specific instruments and lends itself more readily to multiple analysis, while giving results which compare favorably with the ultraviolet technique.

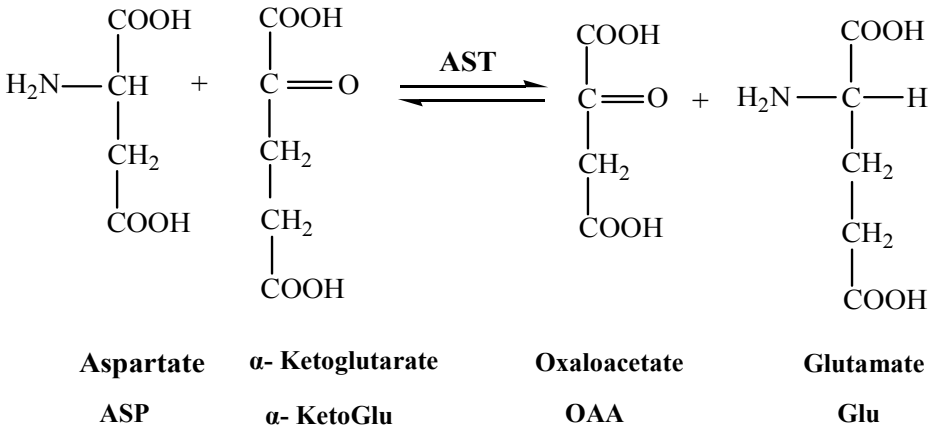

Figure 2.2 The reaction catalyzed by AST

OAA + 2,4-dinitrophenylhydrazine ⟶ Phenylhydrozone of OAA

Figure 2.3 The reaction used for the determination of AST activity

The incubation mixture contained 1 ml aspartic acid and α-ketoglutarate as substrate and 0.2 ml serum. Substrate solution was prepared by the addition of 40 ml of 1 N NaOH to the mixture of 0.0584 g of α-ketoglutarate and 5.32 g D-L-aspartate). The pH of the solution was adjusted to 7.4±0.1 by adding 1 N NaOH dropwise, with stirring. Then, the solution was diluted to 200 ml with 100 mM phosphate buffer, pH 7.4. Serum is obtained by centrifuging the rabbit blood at 5000 rpm for 10 minutes by using NF 1215 centrifuge, (Nuve Instruments Ltd., 06640 Ankara, Turkey).

All the tubes were mixed and only the test tubes were incubated in a water bath at 37^0 C for 1 hour. After the incubation, the test tubes were removed from the water bath and 1 ml of color reagent which was prepared as dissolving 0.039 g DNPH in 200 ml of 1 N HCl was added to all tubes including blanks and standards. Then all tubes were mixed at room temperature for 20 minutes.

Colorimetric method assigns transaminase unit values to several concentration of the pyruvate, an intermediate standard. Pyruvate standard was prepared freshly by dissolving 20 mg pure Na-pyruvate in 100 ml phosphate buffer, pH 7.4. Four different concentrations of pyruvate standards (0.02 mg/ml, 0.04 mg/ml, 0.06 mg/ml, 0.08 mg/ml) and pyruvate standard solution were added to tubes and all tubes were completed to 1 ml with substrate solution) which refer to 24, 61, 114, 190 AST units were used for the construction of standard curve. The enzyme activities of samples were calculated using this linear standard curve.

Finally, 10 ml of 0.4 N NaOH was added to all tubes and they were allowed to stand for at least 5 minutes. Then the blank was set to 0.250 absorbance in spectrophotometer at 505 nm. The absorbances of the tube contents developing brown color were read. A standard curve was constructed by using the absorbances and AST units of the standards. Blank was plotted at zero concentration and 0.25 absorbance directly. The AST units of the samples were calculated by using this calibration curve. One transaminase unit is defined, in terms of the UV method, is the

amount of enzyme activity in 1 ml of serum that will lower the absorbance by 0.001 in 1 minute under the described conditions of that method.

2.3.3.2 Determination of Alanine Aminotransferase (ALT) Activity

Alanine aminotransferase (ALT) is similar to AST and was determined by the same method (Reitmann and Frankel, 1957) except that substrate contained D-L-Alanine instead of aspartic acid and the product measured was pyruvic acid rather than oxalacetic acid. The ALT catalyzed reaction and measurement of ALT activity are given in Figure 2.4. and Figure 2.5, respectively.

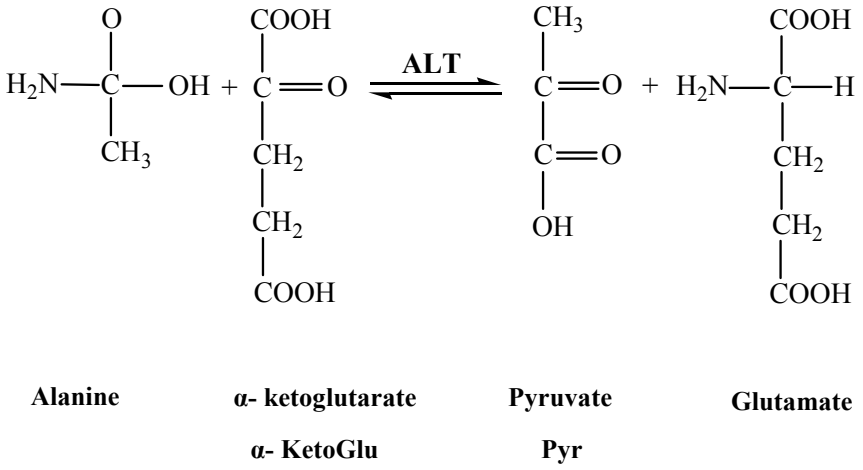

Figure 2.4 The reaction catalyzed by ALT

Figure 2.5 The reaction used for the determination of ALT activity

The incubation mixture contained 1 ml alanine and α-ketoglutarate as a substrate and 0.2 ml serum. Substrate solution was prepared by the addition of 20 ml of dH_2O to the mixture of 0.0584 g of and 3.56 g D-L-alanine. The pH of the solution was adjusted to 7.4±0.1 by adding 1 N NaOH dropwise, with stirring. Then, the solution was diluted to 200 ml with phosphate buffer, pH 7.4.

Pyruvate standard was prepared as described in AST activity. Three different concentrations of pyruvate standard solution (0.02 mg/ml, 0.04 mg/ml, 0.06 mg/ml) was added to tubes and tubes was completed to 1 ml with substrate solution which refer to 28, 57, 97 ALT units were used for the construction of standard curve. The enzyme activities of samples were calculated using this linear standard curve.

All the tubes were mixed and only the test tubes were incubated at 37^0 C. After the incubation, the test tubes removed from water bath and 1 ml of color reagent (prepared as dissolving 0.039 g DNPH in 200 ml of 1 N HCl was added to all tubes including blanks and standards. Then all tubes were incubated at room temperature for 20 minutes. Finally, 10 ml of 0.4 N NaOH was added to all tubes and they were allowed to stand for at least 5 minutes. Then the blank was set to 0.250 absorbance in spectrophotometer at 505 nm. The absorbances of the tube contents developing brown color were read. A standard curve was constructed by using absorbances and ALT units of the standards. Blank was plotted at zero concentration and 0.25 absorbance directly. The ALT units of the samples were calculated by using this calibration curve.

2.3.4 Protein Determination

The protein concentration of microsomes and cytosols were measured according to the method of Lowry *et. al.* (1951). As a standard, crystalline bovine serum albumin was used. Initial dilution (1:200) was performed for microsomes and cytosols before the preparation of the reaction tubes. Moreover, dilution within tube was carried out by taking 0.10, 0.25, 0.50 ml of initially diluted samples into reaction tubes and completed it to final volume of 0.5 ml with distilled water. Then, 2.5 ml of Lowry alkaline copper reagent (prepared as 2 % copper sulphate, 2 % sodium potassium tartrate and 0.1 N NaOH containing 20 % sodium carbonate in a ratio of 1:1:100 in the written order) was added to each tube. All tube contents were mixed

and let stand for 10 minutes at room temperature for copper reaction in alkaline medium. After that, 2 N folin reagent was diluted 1:1 ratio by distilled water and 0.25 ml of diluted reagent was added to each tube and mixed within 8 seconds by vortex. The tubes were incubated for 30 minutes at room temperature. The intensity of resulting color was measured at 660 nm. Standard tubes with five different protein concentrations (0.02, 0.05, 0.10, 0.15 and 0.20 mg/ml of BSA) were prepared from crystalline bovine serum albumin, no dilutions were performed and same steps were carried out for standard tubes. A standard curve was plotted according to the readings of intensity of standards and by using the slope of the standard curve the protein amounts of the samples were calculated.

2.3.5 Determination of Cytochrome P450 Enzyme Activities

2.3.5.1 Determination of Aniline 4-Hydroxylase Activity

Aniline 4-hydroxylase activity was determined according to the method of Imai *et al.* (1966), by measuring the quantity of *p*-aminophenol (pAp) formed for rabbit liver, kidney and lung microsomes. In figure 2.6, aniline 4-hydroxylase reaction is shown:

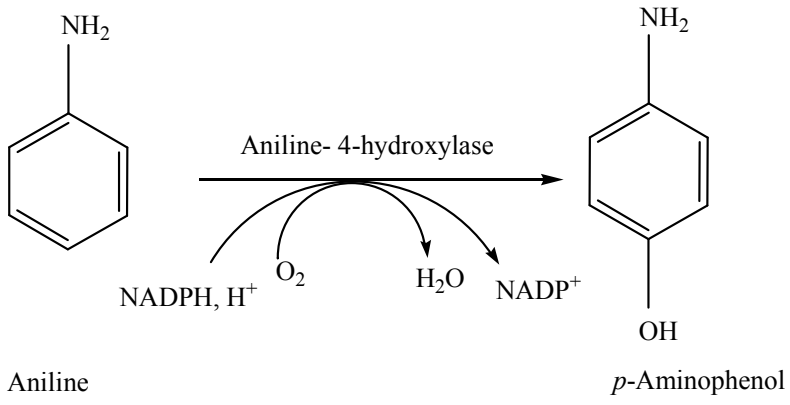

Figure 2.6 Aniline 4-hydroxylation reaction

The assay conditions optimized for microsomal aniline 4-hydroxylase by Arınç and İşcan (1983) were also used for the determination of rabbit liver, kidney and lung microsomal aniline 4-hydroxylase with some modifications.

A typical assay medium contained 100 mM Hepes buffer, pH 7.6, 10 mM aniline, 1.5 mg microsomal protein for liver, 4 mg microsomal protein for kidney and lung, and 0.5 mM NADPH generating system in a final volume of 1ml. A typical assay mixture used for the determination of aniline 4-hydroxylase activity is given in Table 2.1.

NADPH generating system was prepared by adding 0.5 units of glucose-6 phosphate dehydrogenase into a test tube containing 2.5 mM glucose-6-phosphate, 2.5 mM $MgCl_2$, 14.6 mM HEPES buffer pH 7.8 and 0.5 mM $NADP^+$. Then the test tube containing NADPH generating system was incubated at 37^0 C for 5 minutes and finally kept in crushed ice until used. 1 unit of glucose 6-phosphate dehydrogenase is equal to the amount of enzyme reducing 1 μmole of $NADP^+$ in one minute at 25^0 C.

The reaction was started by the addition of 0.15 ml NADPH generating system to the incubation mixture and to zero time blanks to which 0.5 ml 20% TCA was added before the addition of NADPH generating system. The reaction was carried out at 37^0 C for 25 minutes with constant moderate shaking in a shaking water bath (Nüve Instruments Ltd., 06640 Ankara, Turkey). Then, the reaction was stopped by the addition of 0.5 ml 20% TCA at the end of the incubation time. The denatured proteins were transferred to the eppendorf centrifuge tubes, and centrifuged at 13 100 rpm (16 000 xg) at microcentrifuge Thermo Scienific Microlite RF refrigerated microcentrifuge (Thermo scientific, Milford, Massachusetts, USA) for 25 minutes at 4^0 C. After the centrifugation, 1 ml of aliquots containing p-aminophenol was removed and was mixed with 0.5 ml of 20 % Na_2CO_3 and with 0.5 ml of 0.4 N NaOH solution containing 4 % phenol. Then, the mixture was incubated at 37^0 C for 30 minutes in a water bath. The intensity of blue color developed was measured at 630 nm using (Schimadzu UV-1240 spectrophotometer, Schimadzu Co., Analytical Instruments Division, Kyoto, Japan).

p-Aminophenol solution was used as a standard. Since, it is light sensitive; 0.5 mM *p*-aminophenol solution was prepared freshly and kept in the dark. Standards at four different *p*-aminophenol concentration (2.5, 5, 12.5, 25 nmoles) containing aniline and other incubation constituents were run under the same conditions as for

reaction mixture. A standard curve of *p*-aminophenol was constructed and used for the calculation of enzyme activity.

Table 2.1 The constituents of the incubation mixture for determination of aniline 4-hydroxylase activity in rabbit liver, kidney and lung microsomes

Constituents	Stock solutions	Volume to be added (ml)	Final concentration in 1 ml incubation mixture
HEPES buffer, pH 7.6	400 mM	0.250	100 mM
Aniline	100 mM	0.100	10 mM
Microsomes	---------		1.5 mg for liver 4 mg for kidney 4 mg for lung
NADPH Generating System			
Glucose-6-phosphate	100 mM	0.025	2.5 mM
$MgCl_2$	100 mM	0.025	2.5 mM
HEPES buffer pH 7.8	200 mM	0.073	14.6 mM
$NADP^+$	20 mM	0.025	0.5 mM
Glucose-6-phosphate dehydrogenase	1980 U	0.00025	0.5 U
Distilled water		To 1 ml	

2.3.5.2 Determination of p-Nitrophenol Hydroxylase Activity

The *p*-Nitrophenol hydroxylation to 4-nitrocathecol (1,2-dihydroxy-4-nirobenzene) was measured according to the method of Reinke and Moyer (1985). The assay conditions that were optimized for lung microsomal *p*-nitrophenol hydroxylase by Arınç and Aydoğmuş (1990) were also used for the determination of *p*-nitrophenol hydroxylase activity of rabbit liver, kidney microsomes. The p-nitrophenol hydroxylation reaction is associated with CYP2E1 isozyme of cytochrome P450 enzyme family. In Figure 2.7., the *p*-nitrophenol hydroxylation reaction is given.

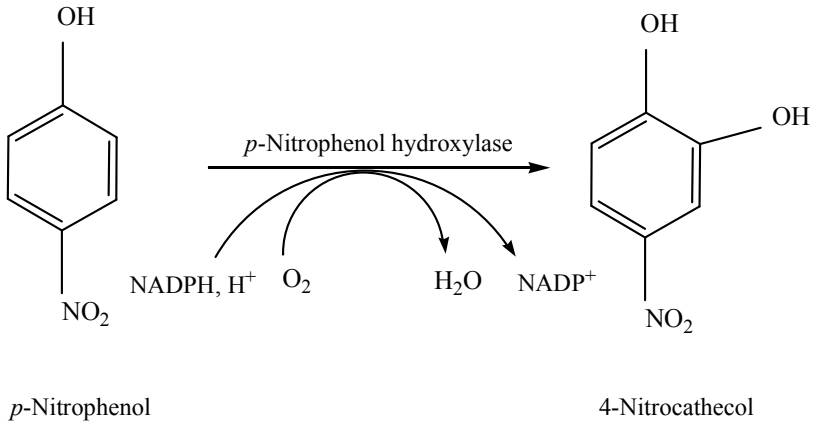

Figure 2.7 *p*-Nitrophenol Hydroxylation Reaction

A typical assay mixture used for the determination of *p*-nitrophenol hydroxylase activity is given in Table 2.2. The optimized assay mixture contained 0.25 mM *p*-nitrophenol, 100 mM Tris-HCl buffer, pH 6.8, 1.5 mg microsomal protein for liver, 4 mg microsomal protein for kidney and 2 mg microsomal protein for lung and 0.5 mM NADPH generating system in a final volume of 1.0 ml.

The reaction was started by the addition of 0.15 ml NADPH generating system and carried out at 37^0 C with moderate shaking in a water bath (Nüve Instruments Ltd., 06640 Ankara Turkey) for 10 minutes. Just before the addition of NADPH generating system, 0.5 ml 0.6 N perchloric acid was added to zero time blank tubes. The reaction was terminated by the addition of 0.5 ml of 0.6 N perchloric acid. Then the denatured proteins were transferred to eppendorf tubes and removed by centrifugation at 13 100 rpm (16000 xg) for 20 minutes at 4^0 C using Thermo Scientific Microlite RF refrigerated microcentrifuge (Thermo scientific, Milford, Massachusetts, USA).

After centrifugation, 1.0 ml aliquot of the supernatant was mixed with 0.1 ml of 10 N NaOH in eppendorf tubes for complete ionization of 4-nitrocatechol. Then, the mixture again centrifuged at 13 100 rpm (16000 xg) for 5 minutes at 4^0 C, using Thermo Scientific Microlite RF refrigerated microcentrifuge, (Thermo Scientific, Milford, Massachusetts, USA) immediately. After second centrifugation, again 1.0

ml aliquot was taken and the 4-nitrocatechol formed was determined spectrally at 546 nm (Schimadzu UV-1240 spectrophotometer, Schimadzu Co., Analytical Instruments Division, Kyoto, Japan) by using an extinction coefficient of 9.53 mM^{-1} cm^{-1} (Koop *et al.*, 1986).

Table 2.2 The constituents of incubation mixture for the determination of *p*-nitrophenol hydroxylase activity of rabbit liver, kidney and lung microsomes.

Constituents	**Stock solutions**	**Volume to be added (ml)**	**Final Concentration in 1.0 ml incubation mixture**
Tris buffer, pH 6.8	400 mM	0.25	100 mM
p-nitrophenol	2.5 mM	0.1	0.25 mM
Microsomes	---------		1.5 mg for liver 4 mg for kidney 2 mg for lung
NADPH Generating System			
Glucose-6-phosphate	100 mM	0.025	2.5 mM
$MgCl_2$	100 mM	0.025	2.5 mM
HEPES buffer pH 7.8	200 mM	0.073	14.6 mM
$NADP^+$	20 mM	0.025	0.5 mM
Glucose-6-phosphate dehydrogenase	1980	0.00025	0.5 U
Distilled water		To 1 ml	

2.3.5.3 Determination of N-Nitrosodimethylamine (NDMA) N-Demethylase Activity

NDMA N-demethylase activity was measured according to the method of Gorsky and Hollenberg (1989) and formaldehyde formed was measured by the method of Nash (1953) as modified Cochin and Axelrod (1959) for rabbit liver, kidney and lung microsomes. The assay mixture given in Table 2.3 contained 100 mM HEPES buffer, pH 7.7, 2.5 mM NDMA, and 0.75 mg, 1 mg, 2 mg microsomal protein for liver, kidney and lung, respectively, and finally 0.5 mM NADPH generating system in final concentration in 0.5 ml final volume. The NDMA N-demethylation reaction is associated with cytochrome 2E isozymes. In figure 2.8, NDMA N-demethylation reaction is given:

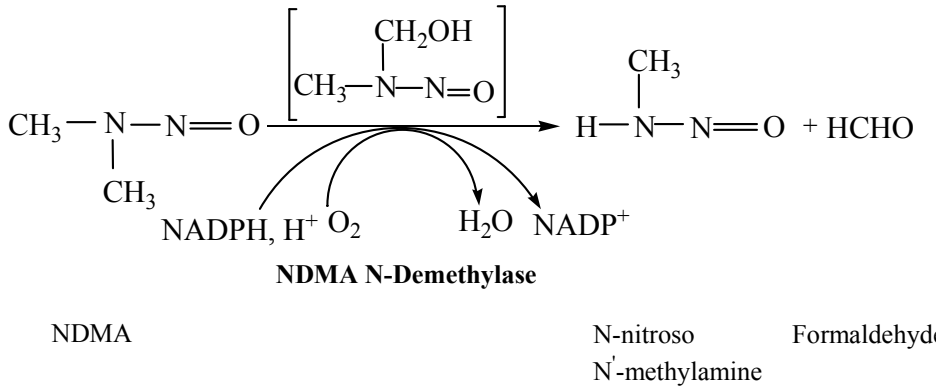

Figure 2.8 NDMA N-demethylation reaction

NDMA N-demethylation reaction was initiated by the addition of 0.075 ml of NADPH generating system to incubation mixture and to zero time blanks to which 0.5 ml of 0.75 N perchloric acid was added just before the addition of the cofactor. Then, the reaction was carried out at 37 ^{0} C for 20 minutes with constant moderate shaking in a shaking water bath (Nüve Instruments Ltd., 06640 Ankara Turkey). After 20 minutes, the enzyme reaction was finalized by the addition of 0.5 ml 0.75 N perchloric acid solution. Then, the denatured proteins were transferred to the

eppendorf centrifuge tubes, and centrifuged at 13 100 rpm (16 000 xg) at Thermo Scientific Microlite RF refrigerated microcentrifuge Thermo Scientific Microlite RF refrigerated microcentrifuge (Thermo scientific, Milford, Massachusetts, USA) for 20 minutes at 4 ^{0} C.

After centrifugation, 0.7 ml aliquots were mixed with 0.525 ml Nash reagent (prepared by the addition of 0.4 ml acetyl acetone, just before use, to 100 ml solution containing 30.8 g ammonium acetate and 0.6 ml of glacial acetic acid) again in eppendorf tubes and the mixture was incubated for 10 minutes at 50 ^{0} C in a water bath. After this step, the mixture were centrifuged immediately at 13 100 rpm (16 000 xg) at microcentrifuge Thermo Scientific Microlite RF refrigerated microcentrifuge (Thermo scientific, Milford, Massachusetts, USA) for 5 minutes at 4 ^{0} C. Then, the formaldehyde amount was determined by measuring the absorbance at 412 nm using (Schimadzu UV-1240 spectrophotometer, Schimadzu Co., Analytical Instruments Division, Kyoto, Japan) spectrophotometer.

As a standard, 0.5 mM freshly prepared formaldehyde solution was used. . The tubes containing standards at four concentrations (12.5, 25, 50, 100 nmoles) with other incubation constituents were run under the same conditions as for reaction tubes. A standard formaldehyde calibration curve was constructed and the slope of this curve was used for calculation of enzyme activity.

Table 2.3 The constituents of the incubation mixture for the determination of NDMA N-demethylase activity of rabbit liver, kidney and lung microsomes

Constituents	Stock solutions	Volume to be added (ml)	Final Concentration in 0.5 ml incubation mixture
HEPES buffer, pH 7.7	400 mM	0,125	100 mM
NDMA	25 mM	0,050	2.5 mM
Microsomes	---------		0.75 mg for liver 2 mg for kidney 1 mg for lung
NADPH Generating System			
Glucose-6-phosphate	100 mM	0.0125	2.5 mM
$MgCl_2$	100 mM	0.0125	2.5 mM
HEPES buffer pH 7.8	200 mM	0.036	14.6 mM
$NADP^+$	20 mM	0.0125	0.5 mM
Glucose-6-phosphate dehydrogenase	1980 U	0.00025	0.5 U
Distilled water		To 0.5 ml	

2.3.5.4 Determination of Benzphetamine N-demethylase Activity

Benzphetamine N-demethylation reaction is found to be associated with cytochrome P450 2B isozymes (Adalı and Arınç, 1990). Benzphetamine is a specific substrate for the phenobarbital inducible forms of CYP isozymes. Benzphetamine is N-demethylated by mixed function oxidases in the presence of molecular oxygen and NADPH. At the end of the reaction norbenzphetamine, $NADP^+$, water and formaldehyde are produced. The benzphetamine N-demethylation reaction is given in figure 2.9.

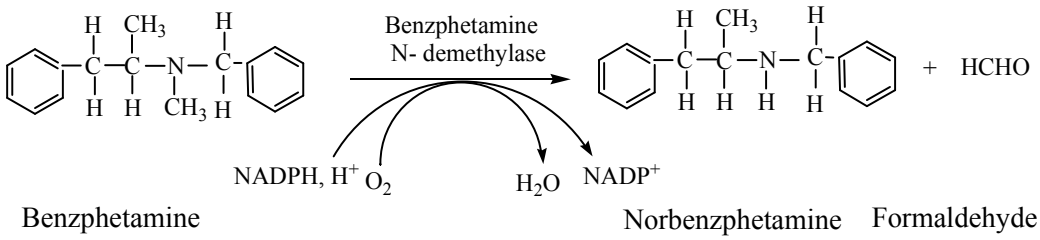

Figure 2.9 Benzphetamine N-demethylation

Benzphetamine N-demethylase activity of rabbit liver microsomes was determined colorimetrically by measuring the amount of formaldehyde formed according to the method of Nash (1953) as modified by Cochin and Axelrod (1959).

A typical assay mixture used for the determination of benzphetamine N-demethylase activity is given in Table 2.4, contained 100 mM HEPES buffer pH 7.7, 1.5 mM benzphetamine, 0.5 mg microsomal protein for rabbit liver, and finally 0.5 mM NADPH generating system in 0.5 ml final volume.

Benzphetamine N-demethylation reaction was initiated by the addition of 0.075 ml of NADPH generating system to incubation mixture and to zero time blanks to which 0.5 ml of 0.75 N perchloric acid was added just before the addition of the cofactor. Then, the reaction was carried out at 37 ^{0} C for 10 minutes with constant moderate shaking in a shaking water bath. After 10 minutes, the enzyme reaction was finalized by the addition of 0.5 ml 0.75 N perchloric acid solution. Then, the denatured proteins were transferred to the eppendorf centrifuge tubes, and centrifuged at 13 100 rpm (16000 xg) at microcentrifuge (Thermo scientific, Milford, Massachusetts, USA) for 20 minutes at 4 ^{0} C.

After centrifugation, 0.7 ml aliquots were mixed with 0.525 ml Nash reagent (prepared by the addition of 0.4 ml acetyl acetone, just before use, to 100 ml solution containing 30.8 g ammonium acetate and 0.6 ml of glacial acetic acid) again in eppendorf tubes and the mixture was incubated for 10 minutes at 50 ^{0} C in a water bath. After this step, the mixture were centrifuged immediately at 13 100 rpm (16 000 xg) at microcentrifuge (Thermo scientific, Milford, Massachusetts, USA) for 5 minutes at 4 ^{0} C. Then, the formaldehyde amount was determined by measuring the

absorbance at 412 nm using (Schimadzu UV-1240 spectrophotometer, Schimadzu Co., Analytical Instruments Division, Kyoto, Japan) spectrophotometer.

As a standard, 0.5 mM freshly prepared formaldehyde solution was used. . The tubes containing standards at four concentrations (12.5, 25, 50, 100 nmoles) with other incubation constituents were run under the same conditions as for reaction tubes. A standard formaldehyde calibration curve was constructed and the slope of this curve was used for calculation of enzyme activity.

Table 2.4 The constituents of the incubation mixture for the determination of Benzphetamine N-demethylase activity of rabbit liver, kidney and lung microsomes

Constituents	Stock solutions	Volume to be added (ml)	Final Concentration in 0.5 ml incubation mixture
HEPES buffer, pH 7.7	400 mM	0.125	100 mM
Benzphetamine	7.5 mM	0.1	1.5 mM
Microsomes	---------		0.5 mg for liver 0.5 mg for lung 1.5 mg for kidney
NADPH Generating System			
Glucose-6-phosphate	100 mM	0.0125	2.5 mM
$MgCl_2$	100 mM	0.0125	2.5 mM
HEPES buffer pH 7.8	200 mM	0.036	14.6 mM
$NADP^+$	20 mM	0.0125	0.5 mM
Glucose-6-phosphate dehydrogenase	1980 U	0.00025	0.5 U
Distilled water		To 0.5 ml	

2.3.5.5 Determination of Erythromycin N-demethylase activity

Erythromycin N-demethylation is associated by cytochrome P4503A isozymes in mammals (Combelbart *et al.*, 1989; Wang *et al.*, 1997). Erythromycin is N-demethylated by P450 mixed function oxidases in the presence of molecular oxygen and formaldehyde is produced. In Figure 2.10, the structure of erythromycin is given.

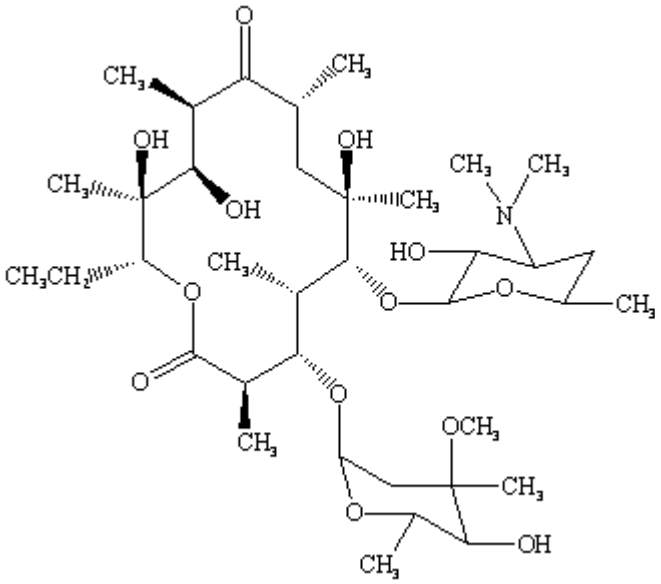

Figure 2.10 Erythromycin Structure

Erythromycin N-demethylase activity of rabbit liver microsomes were determined colorimetrically by measuring the amount of formaldehyde formed according to the method of Nash (1953) as modified by Cochin and Axelrod (1959). A typical assay mixture used for the determination of erythromycin N-demethylase activity is given in Table 2.5, contained 100 mM HEPES buffer pH 7.8, 1.0 mM erythromycin, 1.5 mg microsomal protein for liver and 0.5 mM NADPH generating system in a final volume of 0.5 ml.

Erythromycin N-demethylation reaction was initiated by the addition of 0.075 ml of NADPH generating system to incubation mixture and to zero time blanks to which 0.5 ml of 0.75 N perchloric acid was added just before the addition of the cofactor. Then, the reaction was carried out at 37 ^{0} C for 15 minutes with constant moderate shaking in a shaking water bath (Nüve Instruments Ltd., 06640 Ankara Turkey). At the end of the incubation time, the enzyme reaction was finalized by the addition of 0.5 ml 0.75 N perchloric acid solution. Then, the denatured proteins were transferred to the eppendorf centrifuge tubes, and centrifuged at 13 100 rpm (16 000

xg) at microcentrifuge (Thermo scientific, Milford, Massachusetts, USA) for 20 minutes at 4 ^{0} C.

After centrifugation, 0.7 ml aliquots were mixed with 0.525 ml Nash reagent (prepared by the addition of 0.4 ml acetyl acetone, just before use, to 100 ml solution containing 30.8 g ammonium acetate and 0.6 ml of glacial acetic acid) again in eppendorf tubes and the mixture was incubated for 10 minutes at 50 ^{0} C in a water bath. After this step, the mixture were centrifuged immediately at 13 100 rpm (16 000 xg) at microcentrifuge (Thermo scientific, Milford, Massachusetts, USA) for 5 minutes at 4 ^{0} C. Then, the formaldehyde amount was determined by measuring the

Table 2.5 The constituents of the incubation mixture for the determination of Benzphetamine N-demethylase activity of rabbit liver microsomes

Constituents	Stock solutions	Volume to be added (ml)	Final Concentration in 0.5 ml incubation mixture
HEPES buffer, pH 7.7	400 mM	0.125	100 mM
Erythromycin*	7.5 mM	0.010	1 mM
Microsomes	---------		1.5 mg for liver
NADPH Generating System			
Glucose-6-phosphate	100 mM	0.0125	2.5 mM
$MgCl_2$	100 mM	0.0125	2.5 mM
HEPES buffer pH 7.8	200 mM	0.036	14.6 mM
$NADP^+$	20 mM	0.0125	0.5 mM
Glucose-6-phosphate dehydrogenase	1980 U	0.00025	0.5 U
Distilled water		To 0.5 ml	

* Erythromycin was prepared by dissolving 73.4 mg of erythromycin in 50 % ethanol. The final concentration of ethanol was 1% reaction mixture

absorbance .Then, the formaldehyde amount was determined by measuring the absorbance at 412 nm using (Schimadzu UV-1240 spectrophotometer, Schimadzu Co., Analytical Instruments Division, Kyoto, Japan) spectrophotometer.

As a standard, 0.5 mM freshly prepared formaldehyde solution was used. The tubes containing standards at four concentrations (12.5, 25, 50, 100 nmoles) with other incubation constituents were run under the same conditions as for reaction tubes. A standard formaldehyde calibration curve was constructed and the slope of this curve was used for calculation of enzyme activity.

2.3.6 Western Blot Analysis – Protein Blotting

Rabbit liver and kidney CYP2E1 protein level and rabbit liver CYP3A6 protein level were determined according to the method of Towbin et. al. (1979) with some modifications. Polyclonal anti-rabbit CYP2E1 (Oxford Biomedical Research, MI, USA), polyclonal anti-human CYP3A4 (BD Biosciences, San Jose, CA, USA) were used as primary antibody for CYP2E1 and CYP3A6, respectively.

First microsomal proteins were separated by polyacrylamide gel electrophoresis in the presence of SDS, in a discontinuous buffer system as described by Laemmli (1970) by using 4% stacking gel and 8.5% separating gel. The separating and stacking gel solutions were prepared just before use as given in Table 2.6 in a given order.

Vertical slab gel electrophoresis was carried out by using Scie-plasv10-CDC vertical electrophoresis unit (Southam England). By using the gel sandwich, polyacrylamide slab gels were prepared. The gel sandwich was prepared between two glass plates leaving 1 mm space between two glass plates and central gel running unit was assembled. The unit was placed in melted agar. In order to prevent leakage

Table 2.6 Components of separating and stacking gel solutions

Constituents	**Separating gel (8.5%) (0,375 M Tris, pH 8.8)**	**Stacking gel (4%) (0.125 M Tris, pH 6.8)**
Gel solution (ml)	8.5	1.3
Distilled water (ml)	13.55	6.1
Separating gel buffer (ml)	7.5	-
Stacking gel buffer (ml)	-	2.5
10 % SDS (ml)	0.3	0.1
Ammonium persulfate (ml)	0.15	0.05
TEMED (ml)	0.015	0.01
Total volume (ml)	30	10

of stacking and separating gel solutions, both sides of the unit were sealed with agar. Then, firstly separating gel solution was prepared and poured to the center of the gel sandwich until the desired height of the solution in the sandwich was obtained. In order to obtain a smooth gel surface the top of the gel polymerizing solution was covered with a layer of isobutanol. After polymerization of separating gel, the alcohol layer was poured off totally, and the stacking gel solution was prepared and poured to the center of the separating gel sandwich over the gel until the gel sandwich was filled completely. Then, immediately 1 mm thick teflon comb with 12 wells was inserted into the stacking gel solution. After polymerization of the stacking gel, the teflon comb was carefully removed without destroying the wells. Then, the wells were filled with electrode running buffer (25 mM Tris, 192 mM glycine and 0.1% SDS) using a syringe with a fine needle to remove any air bubbles are unpolymerized chain particles. Then the gel unit was completed with electrode running buffer to the necessary volume. After the preparation of the gel unit, the proteins were prepared. The protein samples were diluted 1:3 (3 part sample and 1 part buffer) with 4x sample dilution buffer (0.25 M Tris-HCl pH 6.8, 8% SDS, 40% glycerol 20% β-mercaptoethanol, and 0.01 % bromphenol blue) and were boiled in a

boiling water bath for 2 minutes. Each sample was applied to different wells by Hamilton syringe.

After application of the samples, gel running unit were placed in the main buffer tank filled with an appropriate amount of the electrode running buffer. Then the electrophoresis unit was connected to the power supply Bio-Rad model 2 (Bio-Rad Laboratories, Richmond, California, USA). The electrophoresis was run at 10 mA 100V for stacking gel and 20 mA and 200V for separating gel.

After electrophoresis was completed, gel was removed for western blot. Firstly, the gels were equilibrated in transfer buffer (25 mM Tris, 192 mM glycine and 20% methanol) for 15 minutes in order to adjust the final size of the gel and remove the buffer salts and SDS which were used in the SDS-polyacrylamide gel electrophoresis. Nitrocellulose membrane was cut 1 cm larger than the dimension of the gel and two pieces of filter paper (Whatman # 1) were cut to a dimension of a little bit larger than the membrane. Nitrocellulose membrane, two filter paper and fiber pads of the transfer sandwich were placed in transfer buffer (25 mM Tris, 192 mM glycine and 20% v/v methanol) and saturated with this solution. Western blot sandwich was prepared as seen in Figure 2.11. A test tube was rolled gently over the sandwich. This was a critical step in order to remove air bubbles between the layers due to the fact that any air bubbles between gel and membrane will block the transfer of the proteins form gel to membrane. Later, the sandwich is placed into the Bio-Rad Trans –Blot Cell and the cell was filled with cold transfer buffer. The transfer was carried out at cold room (4^0 C) for 90 minutes at 90 V and 400 mA.

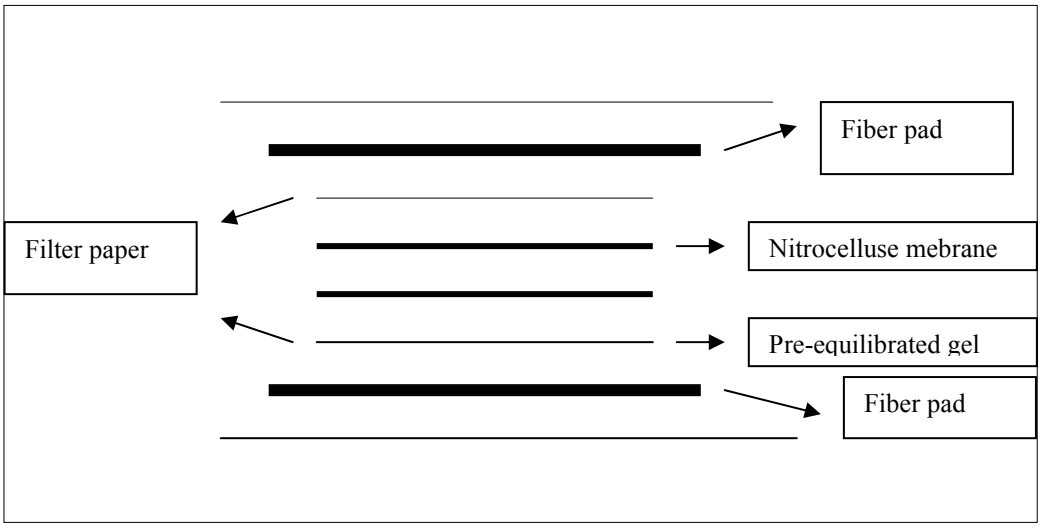

Figure 2.11 Cross section of the Western blot sandwich

At the end of the blotting period, blot; that is, the membrane having the transferred protein on it obtained and taken from the cell and placed on a plastic dish, such a way that the protein side facing up. Then, the blot was washed with TBST (Tris Buffered Saline and Tween 20: 20 mM Tris-HCl pH 7.4, 0.5 M NaCl, and 0.05% Tween 20) for 10 minutes under constant shaking in order to remove the salts and buffers of transfer medium. After that, the blot was incubated with blocking solution (5% non-fat dry-Milk in TBST) for 40 minutes in order to fill the empty spaces between bound proteins so the non-specific binding of antibodies on the membrane will be prevented.

The blot was incubated with primary antibody for 2 hours under constant shaking. As primary antibodies, polyclonal anti-rabbit CYP2E1 (1:1000 ratio), and polyclonal anti-human CYP3A4 (1:5000 ratio) were used. Then, the blot was washed 3 times with 50 ml TBST for 5 minutes each. The washing steps are necessary to remove excess antibody from the membrane. The blot was then incubated with secondary antibody conjugated to marker enzyme-alkaline phosphatase (anti-rabbit IgG-ALP conjugate, 1:5000 ratio) for 1 hour. The blot was washed 3 times with TBST for 5 minutes each to remove excess antibody. Since the excess antibody will give reaction with substrate solution nonspecifically the complete removal of the excess antibody between each steps are extremely important. Finally, blot was

incubated with substrate solution given in Table 2.7 as described by Ey and Ashman (1986) to visualize the specifically bound antibodies.

Table 2.7 Preparation of substrate solution for immunodetection

Solution A (pH of the solution was adjusted to 9.55 with saturated Tris before completing the final volume.)	2.67 ml of 1.5 M Tris-HCl, pH 8.8 4.0 ml of 1 M NaCl 0.82 ml of 100 mM $MgCl_2$ 0.04 ml of 100 mM $ZnCl_2$ 0.086 ml of DEA 12.2 mg NBT Distilled water to 40 ml
Solution B	2 mg/ml Phenazine methosulfate in distilled water
Solution C	5.44 mg/0.136 ml N,N-dimethyl formamide
Finally, NBT/BCIP substrate solution was prepared by mixing solution A with solution C and 0.268 ml of solution B.	

The final images were photographed by using computer based gel imaging instrument (Infinity 3000-CN-3000 darkroom) (Vilber Lourmat, Marne-la-Vallee Cedex1, France) by using Infinity-Capt Version 12.9 software. Proteins bans were quantified using Scion Image version Beta 4.0.2 software.

2.3.7 Determination of NQO1 (DT-Diaphorase Enzyme Activity)

DT-Diaphorase is an FAD-containing flavoprotein, which catalyzes the oxidation of NADH and NADPH by various dyes and quinones. DT-diaphorase is widely distributed in animal kingdom most abundantly in liver, and also relatively high activities are found in kidney and brain.

DT-Diaphorase activity is determined according to the method of Ernster and Navazio (1958). DT-Diaphorase (NQO1) reaction is given in Figure 2.12.

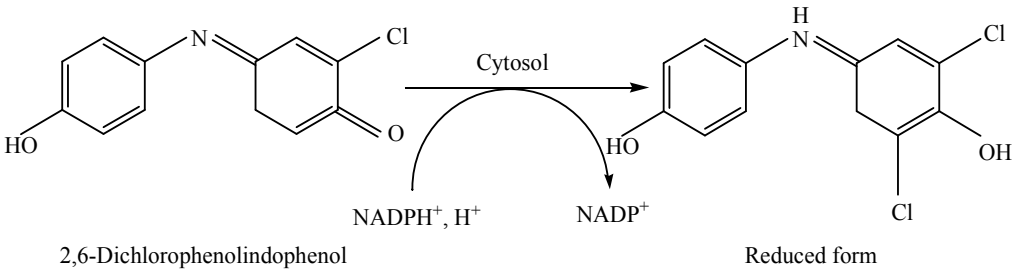

Figure 2.12 DT-Diaphorase (NQO1) reaction

The typical assay mixture used for the determination of DT-Diaphorase (NQO1) activity is given in Table 2.8. Into 1 ml glass cuvette 250 µl 0.1 M potassium phosphate buffer, pH 7.4, 605 µl dH_2O, 25 µl 28 mg/ml bovine serum albumin (BSA, activator for DT-diaphorase), 80 µl 0.5 mM 2,6-dichlorophenolindophenol (DCPIP, electron acceptor), and 20 µl 10 mM NADPH were added and the reaction was started by the addition of the 20 µl cytosol (1/10 diluted for liver, 1/25 diluted for kidney cytosols, 1/7.5 diluted for lung cytosols) into the cuvette. Then, the reaction was followed by recording the reduction of the electron acceptor, (DCPIP) for 2 minutes at 600 nm at Hitachi U-2800 Spectrophotometer (Hitachi High Technologies Corporation, Tokyo, Japan) against blank containing 1 ml 0.1 M potassium phosphate buffer pH: 7.4. Each time, there were dicoumarol-inhibited readings to measure the non-enzymatic activity by adding 40 µl dicoumarol into the assay mixture. Finally, the activity of DT- diaphorase was calculated by using 0.021 $mM^{-1}.cm^{-1}$ extinction coefficient for DCPIP.

Table 2.8 The constituents of assay mixture for DT-Diaphorase

Constituents	Stock solutions	Volume to be added (µl) (Reaction)	Volume to be added (µl) (Dicoumarol-inhibited)	Final concentration in 1 ml glass cuvette
Potassium phosphate buffer pH:7.4	0.1 M	250	250	0.025 M
dH_2O	-	605	565	-
BSA	28 mg/ml	25	25	0.7 mg/ml
DCPIP	0.5 mM	80	80	0.04 mM
Dicoumarol	0.5 mM	-	40	0.02
NADPH	10 mM	20	20	0.2 mM
Cytosols	-	20	20	-

2.3.8 Glutathione S-Transferase Activity Determination

2.3.8.1 Determination of Total Glutathione S-Transferase (GST) Activity

Total GST activity was measured according to the method of Habig *et. al.* (1974) with some modifications. 1-chloro-dinitrobenzene (CDNB) is a common substrate for all isozymes of GST which can be used as a substrate for the determination of total GST catalyzed GSH (reduced glutathione) oxidation which is monitored by the increase in the absorbance at 340 nm due to the 1-glutathione 2,4-dinitrobenzene-(DNB-SG) formation. The GST reaction is given in Figure 2.13.

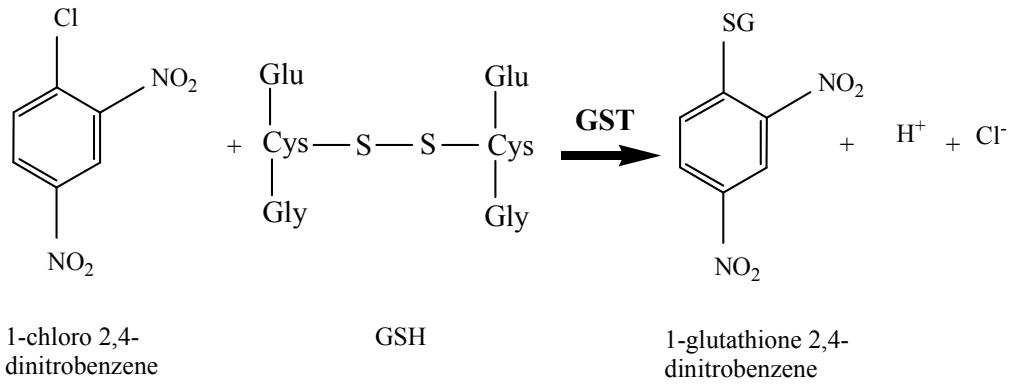

Figure 2.13 Total Glutathione S-transferase reaction

The typical assay mixture used for the determination of total GST activity is given in Table 2.9. Before GST assay each cytosols sample was centrifuged 1 time at 13 100 rpm (16 000 xg) at microcentrifuge Thermo Scientific Microlite RF refrigerated microcentrifuge (Thermo scientific, Milford, Massachusetts, USA). The assay was performed in a 1 ml quartz cuvette. Into 1 ml quartz cuvette, 835 µl 50 mM Potassium phosphate buffer, pH 7.0, 65 µl 20 mM GSH and 50 µl 20 mM CDNB were added, and the reaction was started by the addition of 50 µl of enzyme (1/1000 diluted for liver cytosols, 1/100 diluted for kidney cytosols, 1/250 diluted for lung cytosols). Then, thioether formation was followed for 2 minutes at 340 nm at Hitachi U-2800 Spectrophotometer (Hitachi High Technologies Corporation, Tokyo, Japan). Each time, there should be blank readings (reaction with no enzyme) in order to subtract non-enzymatic product formation from the GST assay.

Then, the enzyme activity was calculated as the amount of thioether (µmol) formed by 1 mg total protein containing cytosol in one minute by using 0.0096 $\mu M^{-1}.cm^{-1}$ as an extinction coefficient of thioether formed by GST.

Table 2.9 The constituents of the incubation mixture for the determination of Glutathione S-transferase activity of rabbit liver, kidney and lung cytosols

Constituents	Stock solutions	Volume to be added (ml) to 1 ml quartz cuvette	Final concentration in 1 ml quartz cuvette
Potassium phosphate buffer pH 7.0	50 mM	835 µl	41.75 mM
CDNB* (in ethanol:dH_2O, 3:2)	20 mM	50 µl	1 mM
GSH	20 mM	65 µl	1.3 mM

*CDNB should be freshly prepared and after weighing the proper amount of CDNB it should be dissolved first in ethanol then the proper amount of dH_2O should be added (ethanol/dH_2O: 3/2). Stored in dark.

2.3.8.2 Determination of GST-Mu Activity

GST-Mu activity was measured according to the method of Habig *et al.* (1974) with some modifications. 1,2-dichloro-4-nitrobenzene (DCNB) is a substrate for GST-Mu activity determination. DCNB was used as substrate for the determination of GST-Mu activity in the presence of the cofactor reduced glutathione (GSH) by monitoring the thioether formation at 345 nm.

The typical assay mixture used for the determination of total GST activity is given in Table 2.10. Before GST assay each cytosols sample was centrifuged 1 time at 13 100 rpm (16 000 xg) at microcentrifuge Thermo Scienific Microlite RF refrigerated microcentrifuge (Thermo scientific, Milford, Massachusetts, USA). The assay was performed in a 1 ml quartz cuvette. Into 1 ml quartz cuvette, 835 µl 120 mM Potassium phosphate buffer, pH 7.0, 65 µl 20 mM GSH and 50 µl 20 mM DCNB were added, and the reaction was started by the addition of 50 µl of enzyme (no dilutions were performed for both liver, kidney and lung cytosols). Then, the activity increase was followed for 2 minutes at 345 nm at Hitachi U-2800 Spectrophotometer (Hitachi High Technologies Corporation, Tokyo, Japan). Each time, there were blank readings (reaction with no enzyme) in order to subtract non-

enzymatic product formation from the GST assay. Then, the enzyme activity was calculated as the amount of thioether (nmol) formed by 1 mg total protein containing cytosol in one minute by using 0.0085 $\mu M^{-1}.cm^{-1}$ as an extinction coefficient of thioether formed by GST.

Table 2.10 The constituents of the incubation mixture for the determination of GST-Mu activity of rabbit liver, kidney and lung cytosols

Constituents	Stock solutions	Volume to be added (ml) to 1 ml quartz cuvette	Final concentration in 1 ml quartz cuvette
Potassium phosphate buffer pH 7.0	120 mM	835 µl	100 mM
DCNB* (in ethanol:dH_2O, 3:2)	20 mM	50 µl	1 mM
GSH	20 mM	65 µl	1.3

*DCNB should be freshly prepared and after weighing the proper amount of DCNB, it should be dissolved first in ethanol then the proper amount of dH_2O should be added (ethanol/dH_2O: 3/2). Stored in dark.

2.3.9 Statistical Analysis

Statistical analysis was performed by using Minitab statistical software package for Windows. All results were expressed as means with their Standard Error of Means (SEM). Student's *t*-test and $p < 0.05$ was chosen as the level for significance.

CHAPTER 3

RESULTS

In vivo effects of acrylamide and resveratrol treatment on hepatic, renal and pulmonary Mixed Function Oxidase (MFO) enzyme activities were studied by measuring aniline 4-hydroxylase, *p*-nitrophenol hydroxylase, NDMA N-demethylase, benzphetamine N-demethylase and erythromycin N-demethylase activities. *In vivo* effects of acrylamide and resveratrol treatment on CYP2E1 and CYP3A6 protein level were also studied by western blot analysis. In addition to MFO enzyme activities, *in vivo* effects of acrylamide and resveratrol treatment on rabbit hepatic, renal and pulmonary cytosolic GST and NQO1 enzyme activities were also studied. Moreover, AST and ALT activities were also determined in blood serum for both control and treated rabbits. Values given in tables for enzyme activities are the average of duplicate determinations.

3.1 Effects of Acrylamide and Resveratrol Treatment on Transaminases (AST and ALT) of Rabbit Blood Serum

Determination of transaminases of rabbit blood serum was carried out described as under the 'Materials and Method' section. All measured AST activities of each rabbit serum values for both control and treated rabbits were shown in Table 3.1. According to the results, no statistically significant change was observed in rabbits administered with 100 mg/kg b.w. acrylamide subcutaneously on days 1, 5, 8 (presented as "AA" group) and in rabbits administered with 25 mg/kg b.w. resveratrol intragastrically on days 1, 5 and 8 (represented as "RESV" group) compared to controls. In addition, no statistically significant differences were observed in rabbits administered with 25 mg/kg b.w. resveratrol and with 100 mg/b.w. acrylamide subcutaneously 6 hours following administration of resveratrol on days 1, 5 and 8 a.m. (presented as "RESV+AA" group). Furthermore, no

statistically significant change was observed in rabbits administered with 100 mg/kg b.w. acrylamide subcutaneously on days 1, 5 and 8 and with a single dose resveratrol intragastrically 6 hours following last administration of acrylamide on day 8 (presented as “AA+RESV” group). All measured AST activities of each rabbit serum values for both control and treated rabbits are given in Table 3.1.

As in the case of AST activities, rabbit blood serum ALT activities resulted in no statistically significant change in “AA” and “RESV” groups with respect to controls. In addition, in “RESV+AA” and “AA+RESV” groups there was no statistically significant change compared to “AA” group in ALT enzyme activity. In Figure 3.1, the effects of acrylamide and resveratrol in ALT and AST activities of rabbit blood serum are given.

Table 3.1 AST and ALT activities of blood serum

	AST Activities (unit/mg/protein)					
Treatments	**Rabbits**					
	1	2	3	4	5	Average (Mean±SEM)
Control[1]	0.71	0.98	0.88	0.86		0.84±0.06 (N=4)
AA[2]	0.81	0.99	0.54	0.61	0.75	0.74±0.09 (N=5) [a]
RESV[3]	0.46	0.87	0.74	0.85		0.73±0.09 (N=4) [b]
RESV+AA[4]	0.56	1.19	0.87	0.54		0.79±0.15 (N=4) [c]
AA+RESV[5]	0.97	0.73	1.39	0.80		0.97±0.15 (N=4) [c]
	ALT Activities(unit/mg/protein)					
Treatments	**Rabbits**					
	1	2	3	4	5	Average (Mean±SEM)
Control[1]	0.76	0.53	0.52	0.66		0.62±0.06 (N=4)
AA[2]	0.67	0.63	0.7	0.41	0.49	0.58±0.06 (N=5) [a]
RESV[3]	0.37	0.49	0.57	0.55		0.50±0.05 (N=4) [b]
RESV+AA[4]	0.24	0.61	0.81	0.26		0.48±0.14 (N=4) [c]
AA+RESV[5]	0.46	0.72	0.81	0.46		0.62±0.09 (N=4) [c]

a: no change respective to controls

b: no change respective to controls

c: no change respective to "AA" group

[1] Control rabbits were injected with saline

[2] "AA": Rabbits were administered with acrylamide subcutaneously (s.c.) at a dose of 100 mg/kg b.w on days 1, 5 and 8

[3] "RESV": Rabbits were administered intragastrically (i.g) with resveratrol at a dose of 25 mg/kg b.w on days 1, 5 and 8

[4] "RESV+AA": Rabbits were intragastrically administered with resveratrol at a dose of 25 mg/kg b.w on days 1, 5 and 8 a.m.; 6 hours following administration of resveratrol, rabbits were administered with acrylamide subcutaneously at a dose of 100 mg/kg b.w on days 1, 5 and 8, p.m.

[5] "AA+RESV": Rabbits were subcutaneously administered with acrylamide at a dose of 100 mg/kg b.w on days 1, 5 and 8 a.m.; 6 hours following last administration of acrylamide rabbits were administered with single dose 100 mg/kg b.w. resveratrol intragastrically on day 8, p.m.

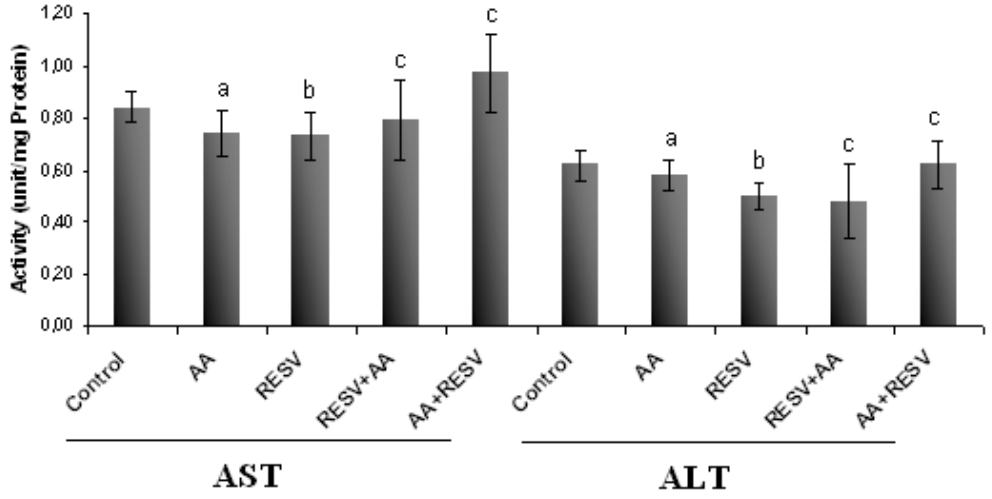

Figure 3.1 AST and ALT activities of rabbit blood serum

3.2 Effects of Acrylamide and Resveratrol Treatment on Mixed Function Oxidase Enzyme Activities

3.2.1 Effects of Acrylamide and Resveratrol Treatment on Mixed Function Oxidase Enzyme Activities of Rabbit Liver Microsomes

3.2.1.1 Aniline 4-hydroxylase Activity

Aniline 4-hydroxylase activity was determined by using 1.5 mg/ml hepatic microsomal protein for both control and treated rabbits. All measured aniline 4-hydroxylase activities of each rabbit liver values are given in Table 3.2 for both control and treated rabbits. These results demonstrated that aniline-4-hydroxylase activity of rabbit liver microsomes resulted in statistically significant 1.80-fold (p<0.05) increase in “AA” group with respect to controls. No statistically significant change was observed in the “RESV” group with respect to controls. These results indicated that in the “RESV+AA” group the enzyme activity significantly decreased, 1.51-fold (p<0.05), over the “AA” group. In “AA+RESV” group 1.48 fold (p<0.05) significant decrease was observed with respect to “AA” group. The effects of *in vivo*

acrylamide and resveratrol treatment on liver aniline 4-hydroxylase activity are given In Figure 3.2.

Table 3.2 Aniline 4-hydroxylase activities of rabbit liver microsomes

	Aniline 4-hydroxylase activity nmol pAp/min/mg protein				
Treatment	**Rabbits**				
	1	**2**	**3**	**4**	**Average (Mean±SEM)**
Control	0.73	0.57	0.76	0.63	0.67±0.04 (N=4)
AA	1.15	1.50	0.97	1.19	1.20±0.11[a*] (N=4)
RESV	0,86	0.61	0.76	0.78	0.75±0.05[b] (N=4)
RESV+AA	0.86	0.71	0.65	0.94	0.79±0.07[c*] (N=4)
AA+RESV	0.64	0.70	0.94	0.99	0.82±0.08[c*](N=4)

a*: $p<0.05$, with respect to controls

b: no change with respect to controls

c*: $p<0.05$, with respect to "AA" group

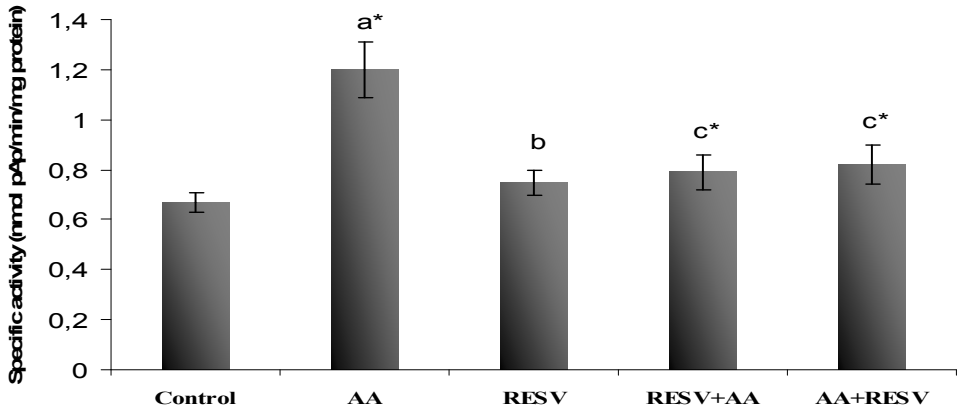

Figure 3.2 Aniline 4-hydroxylase activities of rabbit liver microsomes

3.2.1.2 *p*-Nitrophenol Hydroxylase Activity

The hydroxylation of *p*-Nitrophenol to 4-nitrocathecol of rabbit liver microsomes was determined by using 1.5 mg/ml hepatic microsomal protein for both control and treated rabbits. All measured *p*-Nitrophenol hydroxylase activities of each rabbit liver values are given in Table 3.3 for both control and treated rabbits. Thus, liver microsomal *p*-nitrophenol hydroxylase activity in "AA" group were found to be increased 3.16-fold ($p<0.001$) with respect to control rabbits. According to the results, no statistically significant change was observed in "RESV" group with respect to controls. In "RESV+AA" group 1.43-fold decrease ($p<0.005$) was observed in liver microsomal *p*-nitrophenol hydroxylase activity compared to "AA" group. In addition, *p*-nitrophenol hydroxylase activity of rabbit liver microsomes also decreased 1.66 fold ($p<0.05$) in "AA+RESV" group compared to "AA" group. The effects of *in vivo* acrylamide and resveratrol treatments on liver *p*-nitrophenol hydroxylase activity are given In Figure 3.3.

Table 3.3 *p*-Nitrophenol hydroxylase activities of rabbit liver microsomes

	***p*-Nitrophenol hydroxylase activity (nmol product/min/mg protein)**								
Treatment	**Rabbits**								
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	Average (Mean±SEM)
Control	0.20	0.18	0.23	0.22	0.24	0.18	0.12	0.18	0.19±0.01 (N=8)
AA	0.61	0.73	0.57	0.51	0.59	-	-	-	0.60±0.01^{a***}(N=5)
RESV	0.38	0.24	0.38	0.21	-	-	-	-	0.30±0.05^{b} (N=4)
RESV+AA	0.44	0.44	0.38	0.41	-	-	-	-	0.42±0.01^{c**} (N=4)
AA+RESV	0.27	0.25	0.50	0.43	-	-	-	-	0.36±0.06^{c*} (N=4)

a***: $p<0.001$, with respect to controls

b: no change with respect to controls

c*: $p<0.05$, with respect to "AA" group

c**: $p<0.01$, with respect to "AA" group

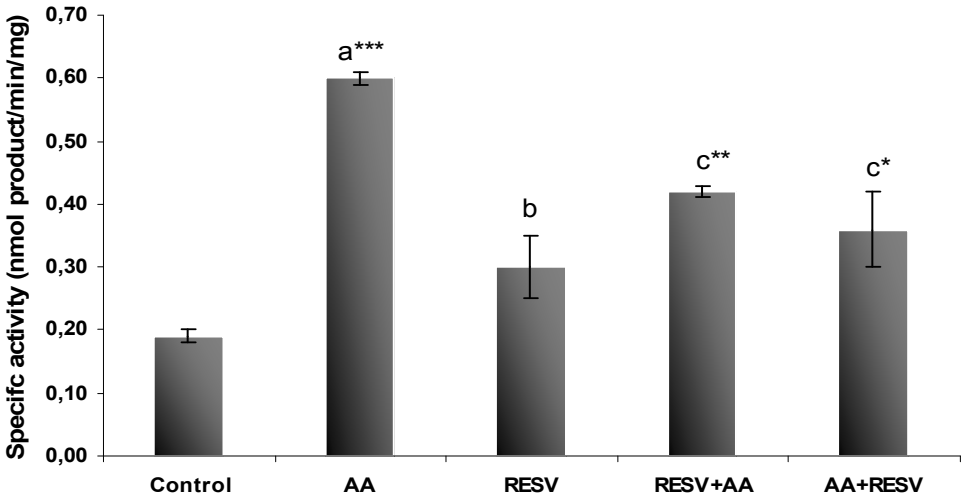

Figure 3.3 *p*-Nitrophenol hydroxylase activities of rabbit liver microsomes

3.2.1.3 NDMA N-demethylase Activity

The NDMA N-demethylase activity was determined using 0.75 mg hepatic microsomal protein in 0.5 ml reaction mixture for both control and treated rabbits. All measured NDMA N-demethylase activities of each rabbit liver values for both control and treated rabbits are given in Table 3.4. According to these results, the NDMA N-demethylase activity of "AA" group increased 2.6-fold ($p<0.001$) compared to control group. There was no statistically significant change in "RESV" group with respect to control. In addition, NDMA N-demethylase activities were decreased 1.8-fold ($p<0.05$) in "RESV+AA" group and 1.6-fold ($p<0.05$) in "AA+RESV" group. The effects of *in vivo* acrylamide and resveratrol treatments on liver NDMA N-demethylase activity of rabbit liver microsomes are given in Figure 3.4.

Table 3.4 NDMA N-demethylase activities of rabbit liver microsomes

	NDMA N-demethylase activity (nmol HCHO/min/mg protein)								
Treatments	**Rabbits**								
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	Average (Mean±SEM)
Control	0.22	0.17	0.39	0.26	0.35	0.13	0.12	0.14	0.22±0.04 (N=8)
AA	0.61	0.64	0.45	0.56					0.57±0.04^{a***} (N=4)
RESV	0.33	0.29	0.28	0.29					0.30±0.01^{b} (N=4)
RESV+AA	0.38	0.30	0.45	0.43					0.39±0.03^{c*} (N=4)
AA+RESV	0.32	0.35	0.49	0.32					0.37±0.04^{c*}(N=4)

a***: p<0.001, with respect to controls

b: no change with respect to controls

c*: p<0.05, with respect to "AA"group

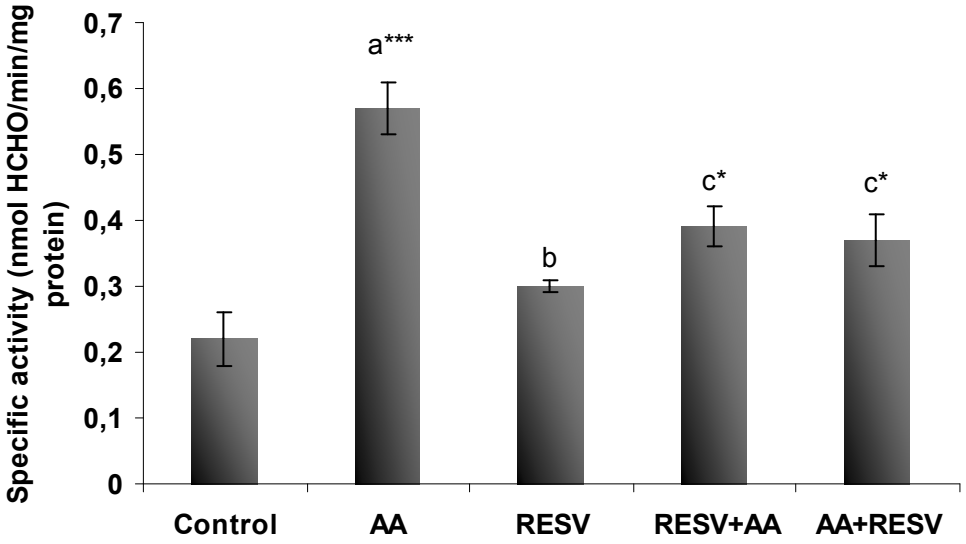

Figure 3.4 NDMA N-demethylase activities of rabbit liver microsomes

3.2.1.4 Benzphetamine N-demethylase Activity

Benzphetamine N-demethylase activity was determined using 0.5 mg hepatic microsomal protein in 0.5 ml reaction mixture for both treated and control rabbits. All measured Benzphetamine N-demethylase activity of each rabbit liver values are given in Table 3.5 for both control and treated rabbits. According to the results, the benzphetamine N-demethylase activity increased 1.19-fold (p<0.05) in "AA" group with respect to controls. However, there is no statistically significant change in enzyme activity of RESV group over controls. In addition, no statistically significant change in enzyme activity of "RESV+AA" or "AA+RESV" group with respect to "AA" group. The effects of acrylamide and resveratrol treatments on benzphetamine N-demethylase activity of rabbit liver microsomes are given in Figure 3.5.

Table 3.5 Benzphetamine N-demethylase activities of rabbit liver microsomes

	Benzphetamine N-demethylase activity (nmol HCHO/min/mg protein)						
Treatments	**Rabbits**						
	1	**2**	**3**	**4**	**5**	**6**	**Average (Mean±SEM)**
Control	6.9	5.4	7.3	5.4	6.3	5.8	6.2±0.32 (N=6)
AA	7.3	7.9	6.9	7.6	7.2		7.4±0.17[a*] (N=5)
RESV	7.8	6.0	6.3	5.2			6.3±0.54[b] (N=4)
RESV+AA	6.3	8.4	5.6	6.9			6.8±0.60[c] (N=4)
AA+RESV	6.1	6.2	6.7	7.8			6.7±0.39[c] (N=4)

a* p<0.05, with respect to controls

b: no change with respect to controls

c: no change with respect to "AA"group

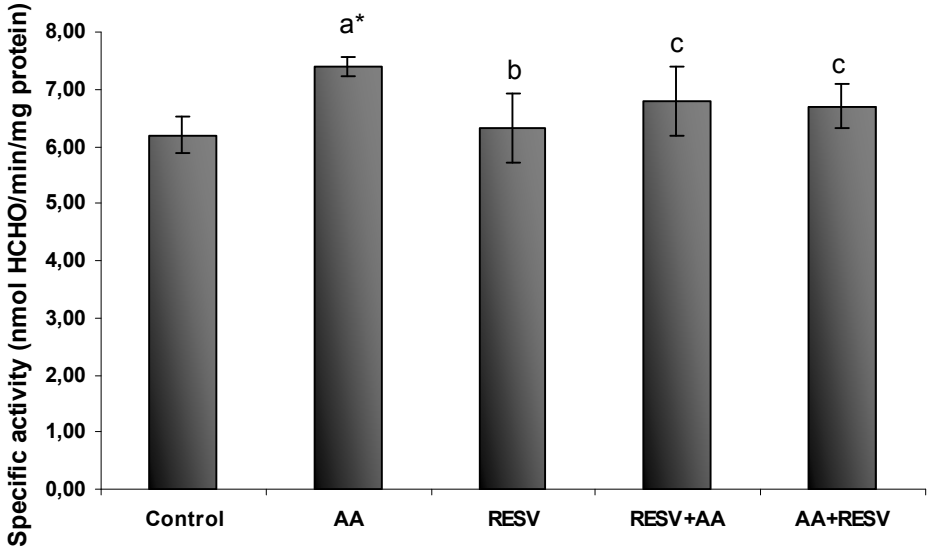

Figure 3.5 Benzphetamine N-demethylase activities of rabbit liver microsomes

3.2.1.5. Erythromycin N-demethylase Activity

Erythromycin N-demethylase activity was determined by using 1.5 mg hepatic microsomal protein in 0.5 ml reaction mixture for both control and treated rabbits. All measured erythromycin N-demethylase activity of each rabbit liver values are given in Table 3.6 for both control and treated rabbits. According to the results, activity of erythromycin N-demethylase activity increased 1.85-fold (p<0.01) significantly in "AA" group with respect to controls. No statistically significant change was observed in the "RESV" group compared to controls. The enzyme activity significantly lowered 2.17-fold (p<0.01), and 1.85-fold (p<0.01) in the groups "RESV+AA" and "AA+RESV", respectively. The effects of *in vivo* acrylamide and resveratrol treatments on liver erythromycin N-demethylase activity are given in Figure 3.6.

Table 3.6 Erythromycin N-demethylase activities of rabbit liver microsomes

	Erythromycin N-demethylase activity (nmol HCHO/min/mg protein)						
Treatments	**Rabbits**						
	1	**2**	**3**	**4**	**5**	**6**	**Average (Mean±SEM)**
Control	0.21	0.17	0.25	0.29	0.11	0.16	0.20±0.03 (N=6)
AA	0.36	0.52	0.30	0.32	0.37	-	0.37±0.04^{a**} (N=5)
RESV	0.30	0.15	0.24	0.18	-	-	0.22±0.03^{b} (N=4)
RESV+AA	0.19	0.17	0.11	0.22	-	-	0.17±0.02^{c**} (N=4)
AA+RESV	0.19	0.25	0.16	0.21	-	-	0.20±0.02^{c**} (N=4)

a** p<0.01, with respect to controls

b: no change with respect to controls

c**: p<0.01, with respect to "AA" group

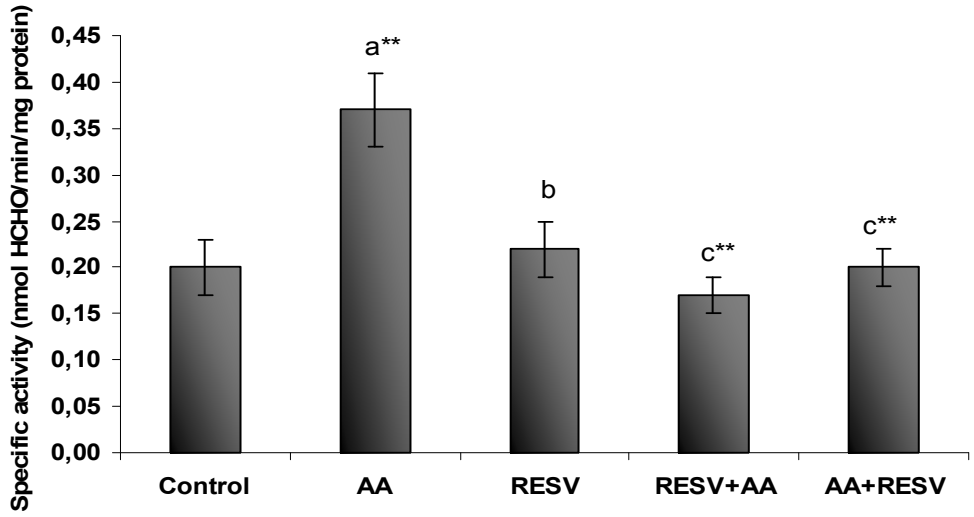

Figure 3.6 Erythromycin N-demethylase activities of rabbit liver microsomes

3.2.2 Effects of Acrylamide and Resveratrol Treatment on Mixed Function Oxidase Enzyme Activities of Rabbit Kidney Microsomes

3.2.2.1 Aniline 4-hydroxylase Activity

Aniline 4-hydroxylase activity was determined using 4 mg renal microsomal protein in 1 ml reaction mixture for both treated and control rabbits. All measured aniline 4-hydroxylase activity of each rabbit kidney values are given in Table 3.7 for both control and treated rabbits. Aniline 4-hydroxylase activity of rabbit kidney microsomes was increased 1.60-fold (p<0.001) significantly with respect to controls. No significant change was observed in "RESV" group compared to controls in aniline 4-hydroxylase activity of rabbit kidney microsomes. In addition, there was no statistically significant change in the aniline 4-hydroxylase activities of "RESV+AA" or "AA+RESV" groups compared to "AA" groups. The effects of acrylamide and resveratrol treatments on aniline 4-hydroxylase activities are given in Figure 3.7.

Table 3.7 Aniline 4-hydroxylase activities of rabbit kidney microsomes

	Aniline 4-hydroxylase activity (nmol pAp/min/mg protein)					
Treatments	**Rabbits**					
	1	**2**	**3**	**4**	**5**	**Average (Mean±SEM)**
Control	0.022	0.028	0.025	0.025	-	0.025±0.001 (N=4)
AA	0.044	0.039	0.041	0.034	0.044	0.040±0.002[a***] (N=5)
RESV	0.036	0.021	0.019	0.027	-	0.026±0.004[b] (N=4)
RESV+AA	0.025	0.049	0.048	0.033	-	0.039±0.005[c] (N=4)
AA+RESV	0.042	0.026	0.044	0.038	-	0.038±0.004[c] (N=4)

a***: p<0.001, with respect to controls

b: no change with respect to controls

c: no change with respect to "AA" group

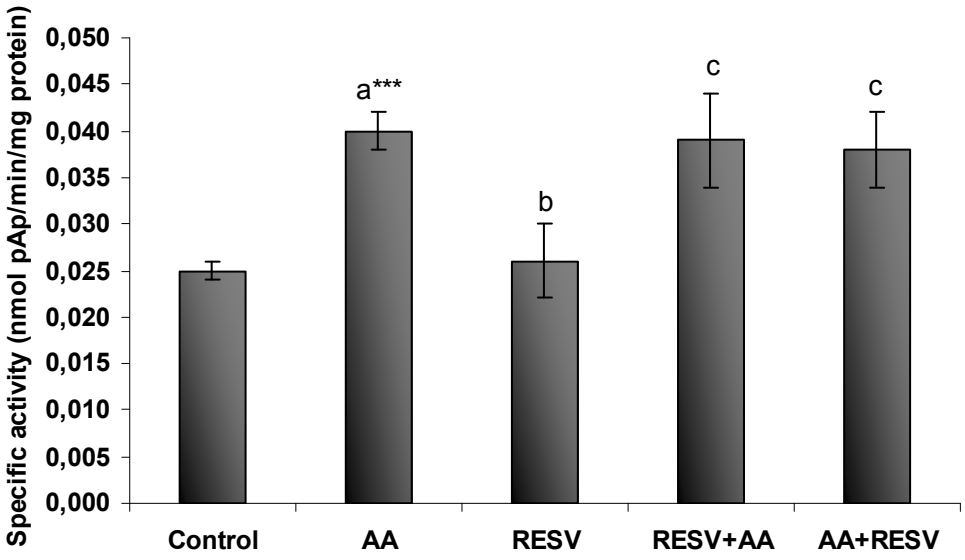

Figure 3.7 Aniline 4-hydroxylase activities of rabbit kidney microsomes

3.2.2.2 *p*-Nitrophenol hydroxylase Activity

The hydroxylation of *p*-Nitrophenol to 4-nitrocathecol was determined using 4 mg renal microsomal protein in 1 ml reaction mixture for both treated and control rabbits. All measured *p*-Nitrophenol hydroxylase activity of each rabbit kidney values are given in Table 3.8 for both control and treated rabbits. Acrylamide treatment in "AA" group resulted in 1.56-fold increase ($p<0.05$) in the *p*-Nitrophenol hydroxylase enzyme activities of rabbit kidney microsomes with respect to controls. In "RESV" group no statistically significant difference was observed compared to controls. Moreover, the enzyme activities of "RESV+AA" and "AA+RESV" groups did not change significantly compared to "AA" group. The effects of acrylamide and resveratrol treatments on kidney microsomal *p*-Nitrophenol hydroxylase activities are given in Figure 3.8.

Table 3.8 *p*-Nitrophenol hydroxylase activities of rabbit kidney microsomes

	***p*-Nitrophenol hydroxylase activity (nmol product/min/mg protein)**						
Treatments	**Rabbits**						
	1	**2**	**3**	**4**	**5**	**6**	**Average (Mean±SEM)**
Control	0.016	0.017	0.016	0.014	0.018	0.013	0.016±0.001 (N=6)
AA	0.025	0.023	0.014	0.031	0.031	-	0.025±0.003[a*](N=5)
RESV	0.018	0.016	0.020	0.021	-	-	0.018±0.001[b](N=4)
RESV+AA	0.022	0.030	0.021	0.025	-	-	0.025±0.002[c](N=4)
AA+RESV	0.020	0.017	0.028	0.020	-	-	0.021±0.002[c](N=4)

a*: $p<0.05$, with respect to controls

b: no change with respect to controls

c: no change with respect to "AA"group

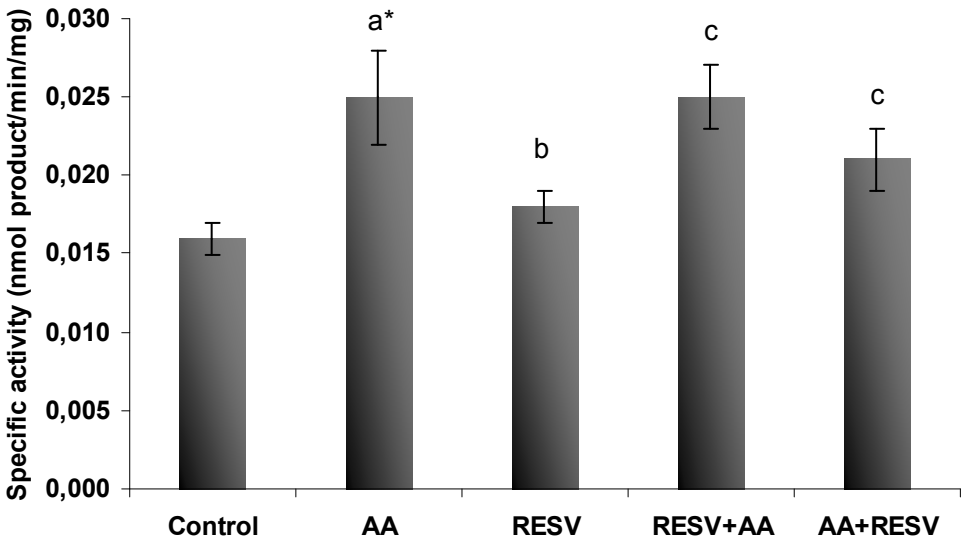

Figure 3.8 *p*-Nitrophenol hydroxylase activities of rabbit kidney microsomes

3.2.2.3 Benzphetamine N-Demethylase Activity

Benzphetamine N-demethylase activity was determined using 1.5 mg renal microsomal protein in 0.5 ml reaction mixture for both control and treated rabbits. All measured benzphetamine N-demethylase activity of each rabbit kidney values are given in Table 3.9 for both control and treated rabbits. According to these results, in "AA" group, there was no statistically significant change with respect to controls in benzphetamine N-demethylase activity. However, in "RESV" group a statistically significant change was observed as 1.52-fold increase ($p<0.01$) in benzphetamine N-demethylase activity of rabbit kidney microsomes with respect to controls. On the other hand, there was no statistically significant change in "RESV+AA" or "AA+RESV" group with respect to "AA" group. The effects of acrylamide and resveratrol treatments on kidney microsomal benzphetamine N-demethylase activities are given in Figure 3.9.

Table 3.9 Benzphetamine N-demethylase activities of rabbit kidney microsomes

	Benzphetamine N-demethylase activity (nmol HCHO/min/mg protein)					
Treatments	**Rabbits**					
	1	**2**	**3**	**4**	**5**	**Average (Mean±SEM)**
Control	0.22	0.23	0.25	0.30		0.25±0.02 (N=4)
AA	0.37	0.25	0.27	0.63	0.37	0.38±0.07[a] (N=5)
RESV	0.35	0.39	0.33	0.44		0.38±0.02[b**] (N=4)
RESV+AA	0.28	0.33	0.44	0.19		0.31±0.05[c] (N=4)
AA+RESV	0.33	0.19	0.60	0.28		0.35±0.09[c] (N=4)

a: no change with respect to controls

b**: $p<0.01$, with respect to controls

c: no change with respect to "AA" group

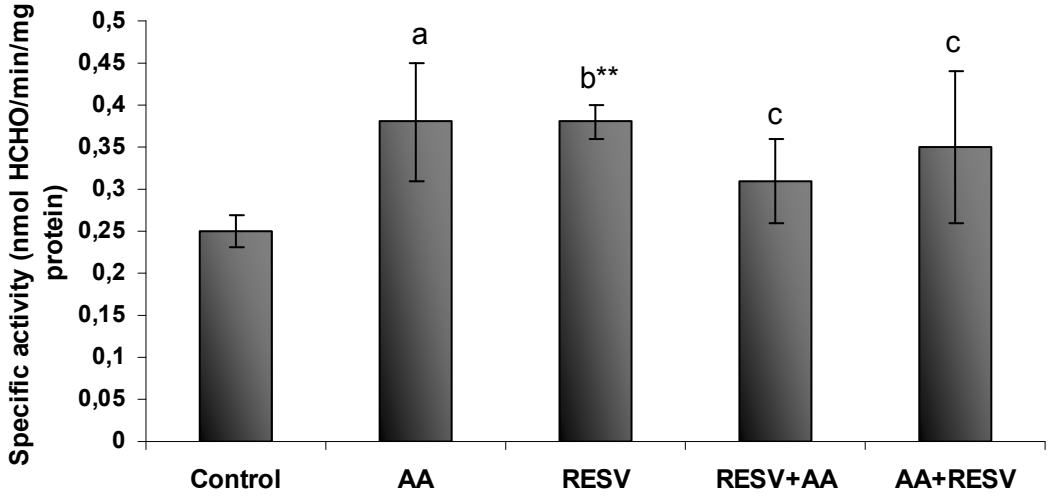

Figure 3.9 Benzphetamine N-demethylase activities of rabbit kidney microsomes

3.2.3 Effects of Acrylamide and Resveratrol Treatment on Mixed Function Oxidase Enzyme Activities of Rabbit Lung Microsomes

3.2.3.1 *p*-nitrophenol Hydroxylase Activity

p-Nitrophenol hydroxylase activity was determined by using 2 mg pulmonary microsomal protein in 1 ml reaction mixture for both control and treated rabbits. All measured *p*-nitrophenol hydroxylase activity of each rabbit lung values are given in Table 3.10 for both control and treated rabbits. According to these results, there was no statistically significant change in *p*-nitrophenol hydroxylase activities of "AA" and "RESV" groups with respect to controls. In addition, the *p*-nitrophenol hydroxylase activities of "RESV+AA" and "AA+RESV" groups did not change compared to "AA" group. The effects of acrylamide and resveratrol treatments on lung microsomal *p*-nitrophenol hydroxylase activities are given in Figure 3.10.

Table 3.10 *p*-Nitrophenol hydroxylase activities of rabbit lung microsomes

	***p*-Nitrophenol hydroxylase activity (nmol product/min/mg protein)**							
Treatments	**Rabbits**							
	1	**2**	**3**	**4**	**5**	**6**	**7**	**Average (Mean±SEM)**
Control	0.25	0.25	0.16	0.25	0.12	0.13	0.12	0.18±0.01(N=7)
AA	0.24	0.15	0.23	0.19	0.19	-	-	0.20±0.02[a](N=5)
RESV	0.16	0.13	0.14	0.22	-	-	-	0.16±0.02[b](N=4)
RESV+AA	0.22	0.17	0.20	0.16	-	-	-	0.19±0.01[c](N=4)
AA+RESV	0.19	0.24	0.13	0.14	-	-	-	0.18±0.01[c](N=4)

a: no change with respect to controls

b: no change with respect to controls

c: no change with respect to "AA" group

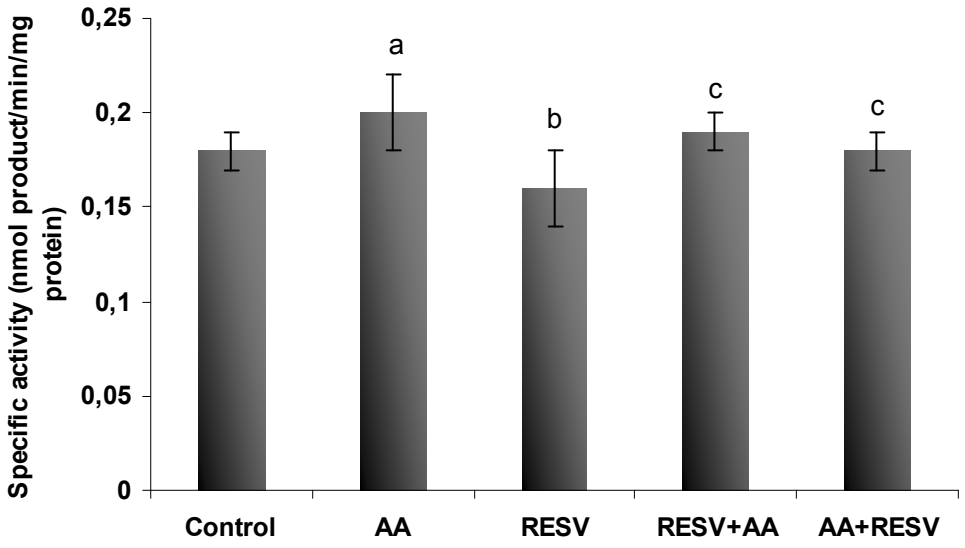

Figure 3.10 *p*-Nitrophenol hydroxylase activities of rabbit lung microsomes

3.2.3.2 NDMA N-demethylase Activity

NDMA N-demethylase activity was determined by using 1 mg pulmonary microsomal protein in 0.5 ml reaction mixture for both control and treated rabbits. All measured NDMA N-demethylase activity of each rabbit lung values are given in Table 3.11 for both control and treated rabbits. The obtained results demonstrated that there was no change in the activity of NDMA N-demethylase activity of rabbit lung microsomes in "AA" or "RESV" groups compared to control group. Moreover, there was no statistically significant change in "RESV+AA" and "AA+RESV" groups with respect to "AA" group. The effects of acrylamide and resveratrol treatments on lung microsomal NDMA N-demethylase activities are given in Figure 3.11.

Table 3.11 NDMA N-demethylase activities of rabbit lung microsomes

	NDMA N-demethylase activity (nmol HCHO/min/mg protein)								
Treatments	**Rabbits**								
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**Average (Mean±SEM)**
Control	0.20	0.18	0.23	0.22	0.24	0.18	0.12	0.18	0.07±0.02(N=8)
AA	0.06	0.14	0.11	0.12	0.12	-	-	-	0.11±0.01[a](N=5)
RESV	0.12	0.09	0.12	0.08	-	-	-	-	0.10±0.01[b](N=4)
RESV+AA	0.07	0.13	0.09	0.10	-	-	-	-	0.09±0.01[c](N=4)
AA+RESV	0.05	0.10	0.22	0.05	-	-	-	-	0.10±0.04[c](N=4)

a: no change with respect to controls

b: no change with respect to controls

c: no change with respect to "AA"group

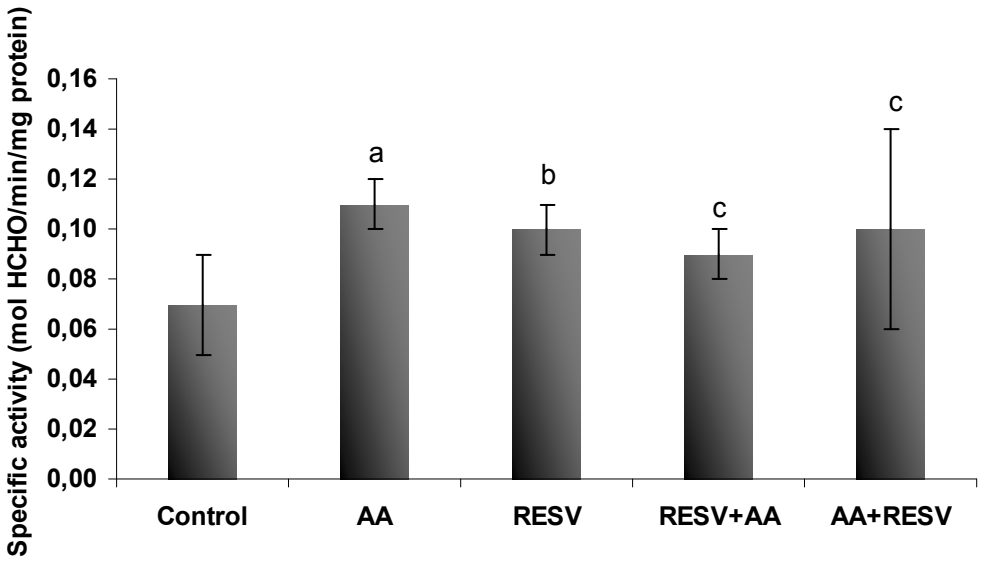

Figure 3.11 NDMA N-demethylase activities of rabbit lung microsomes

3.2.3.3 Benzphetamine N-Demethylase Activity

Benzphetamine N-demethylase activity was determined by using 0.5 mg pulmonary microsomal protein in 1ml reaction mixture for both control and treated rabbits. All measured benzphetamine N-demethylase activity of each rabbit lung values are given in Table 3.12 for both control and treated rabbits. According to these results, no significant change was observed in "AA" and "RESV" groups with respect to control group in lung microsomal benzphetamine N-demethylase activity. In addition, there was also no significant change in the benzphetamine N-demethylase activity for "RESV+AA" and "AA+RESV" groups compared to "AA" group. The effects of acrylamide and resveratrol treatments on lung microsomal benzphetamine N-demethylase activities are given in Figure 3.12.

Table 3.12 Benzphetamine N-demethylase activities of rabbit lung microsomes

	Benzphetamine N-demethylase activity (nmol HCHO/min/mg protein)					
Treatments	**Rabbits**					
	1	**2**	**3**	**4**	**5**	**Average (Mean±SEM)**
Control	8.8	7.2	8.4	10.2	-	8.7±0.62
AA	9.7	6.9	9.4	8.4	8.1	8.5±0.50[a]
RESV	9.4	8.1	7.9	7.8	-	8.3±0.40[b]
RESV+AA	9.7	6.5	9.5	9.60	-	8.8±0.80[c]
AA+RESV	6.7	10.4	4.7	4.9	-	6.7±1.3[c]

a: no change with respect to controls

b: no change with respect to controls

c: no change with respect to "AA"group

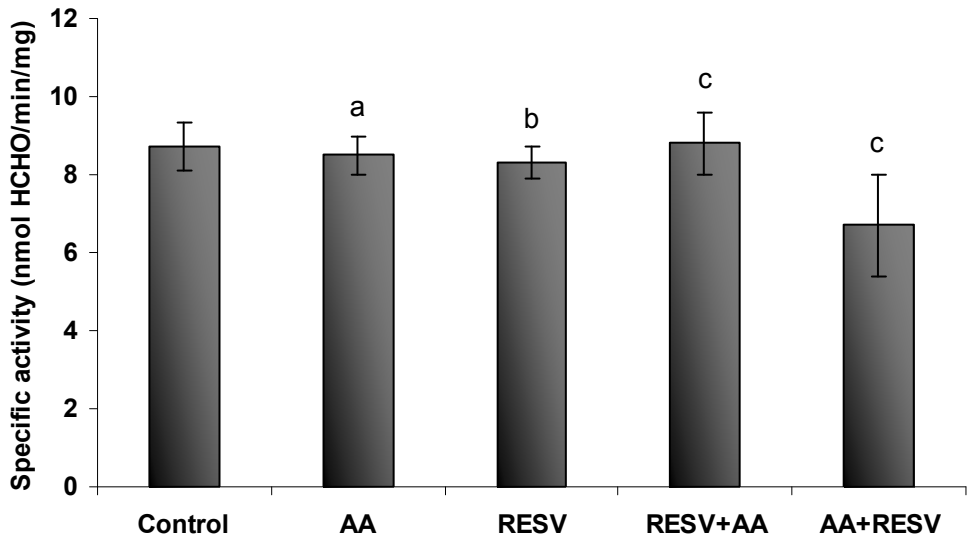

Figure 3.12 Benzphetamine N-demethylase activity of rabbit lung microsomes

3.3 Changes in Cytochrome P4502E1 and CYP3A6 Protein levels as Determined by Western Blot Analysis

Changes in Cytochrome P4502E1 protein levels in hepatic and renal microsomes and changes in Cytochrome P4503A6 protein levels in hepatic microsomes of the samples due to acrylamide and resveratrol treatment were determined by western blotting coupled with immunodetection. For the analysis of CYP2E1, a polyclonal anti-rabbit CYP2E1 and anti-rabbit IgG-ALP conjugate were used as primary and secondary antibody, respectively. For the analysis of CYP3A6, a polyclonal anti-human CYP3A4 and anti-rabbit IgG-ALP conjugate were used as primary and secondary antibody, respectively.

3.3.1 Changes in Cytochrome P4502E1 Protein Levels in Rabbit Liver Microsomes

3.3.1.1 Changes in Cytochrome P4502E1 Protein Levels in Acrylamide Treated Rabbit Liver Microsomes

The western blot analysis of liver microsomes of "AA" group rabbits injected subcutaneously with 100 mg/kg b.w. acrylamide at day 1, 5, 8, and controls are given in Figure 3.13. Intensity of each band was quantified as an arbitrary unit, relative peak area (R.P.A.) per mg microsomal protein by Scion Image software program. Band intensities of each lane and average values (Mean±SEM) are given in Table 3.13. According to these results, CYP2E1 protein level in "AA" group resulted in statistically significant increase, 2.06-fold ($p<0.01$), compared to controls. The effect of acrylamide treatment on rabbit liver microsomal CYP2E1 protein level is given in Figure 3.14.

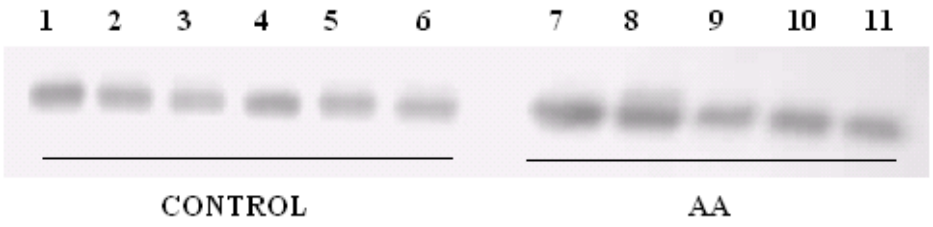

Figure 3.13 Immunochemical detection of liver microsomal cytochrome P4502E1 of control rabbits (Lane 1-6) and "AA" group rabbits (100 mg/kg b.w. s.c at day 1, 5, 8; Lane 7-11).

Table 3.13 Band intensities (represented as arbitrary unit of R.P.A/mg microsomal protein) of each sample in the western analysis of rabbit liver microsomal cytochrome P4502E1 (controls and "AA" group)

Lane No	**1**	**2**	**3**	**4**	**5**	**6**	**Average (Mean±SEM)**
Control R.P.A/mg protein	2031	1658	1196	1674	1443	1564	1594±113 (N=6)
Lane No	**7**	**8**	**9**	**10**	**11**	**-**	
AA R.P.A/mg protein	3645	4081	2516	3157	3032		3286±268[a**] (N=5)

a**: $p<0.01$ with respect to controls

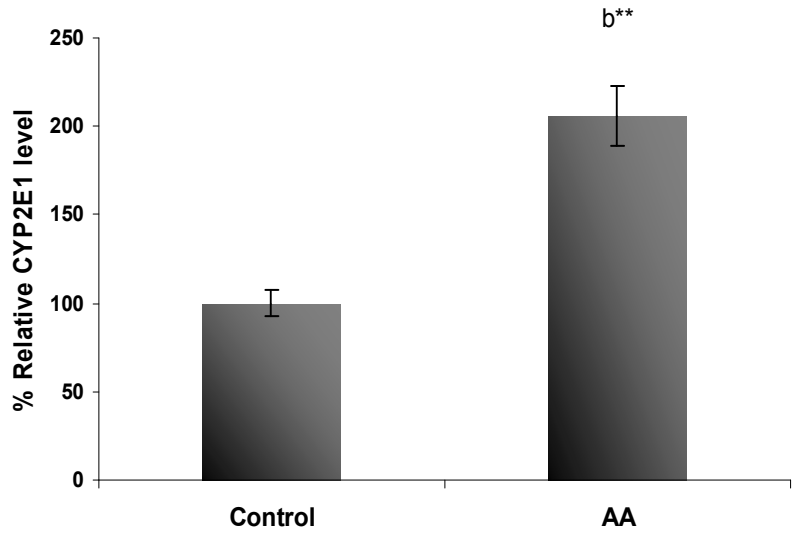

Figure 3.14 Effect of acrylamide treatment on CYP2E1 protein level of rabbit liver microsomes as determined by western blot analysis. Liver microsomal CYP2E1 expression from the control and "AA" groups were detected by Western blot analysis and quantitated by densitometry. The value obtained from control animals were taken to be 100%, and the values obtained from the "AA" group were expressed as a percentage of control.

3.3.1.2 Changes in Cytochrome P4502E1 Protein Levels in Resveratrol Treated Rabbit Liver Microsomes

The western blot analysis of liver microsomes of "RESV" group rabbits administered intragastrically with 25 mg/kg b.w. resveratrol at day 1, 5, 8 and controls were represented in Figure 3.15. Intensity of each band was quantified as an arbitrary unit, relative peak area (R.P.A.) per mg microsomal protein by Scion Image software program. Band intensities of each lane and average values (Mean±SEM) are given in Table 3.14. Resveratrol treatment of rabbits led to 1.25-fold increase in CYP2E1 protein level of liver microsomes with respect to controls; however, this fold increase is not a statistically significant value. The effect of resveratrol treatment on rabbit liver microsomal CYP2E1 protein level is given in Figure 3.16.

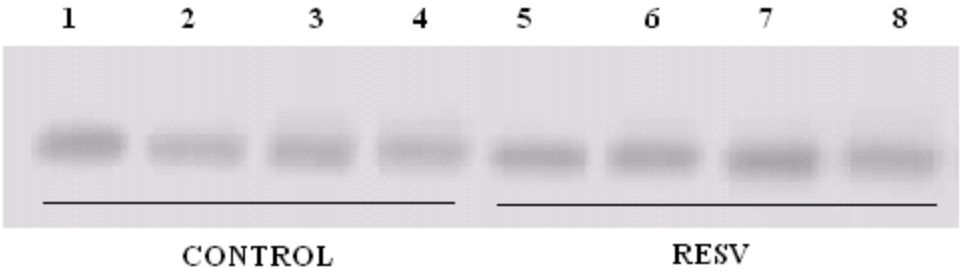

Figure 3.15 Immunochemical detection of liver microsomal cytochrome P4502E1 of control (Lane 1-4) and "RESV" group rabbits (25 mg/kg b.w. intragastrically at day 1, 5, 8; Lane 4-8).

Table 3.14 Band intensities (presented as arbitrary unit of R.P.A/mg microsomal protein) of each sample in the western analysis of rabbit liver microsomal cytochrome P4502E1 (control and "RESV" groups)

Lane No	**1**	**2**	**3**	**4**	**Average (Mean±SEM)**
Control R.P.A/mg protein	1339	1085	1303	1185	1228±58 (N=4)
Lane No	**5**	**6**	**7**	**8**	**Average**
RESV R.P.A/mg protein	1134	1394	1847	1544	1529±115 [b] (N=4)

b: no change with respect to controls

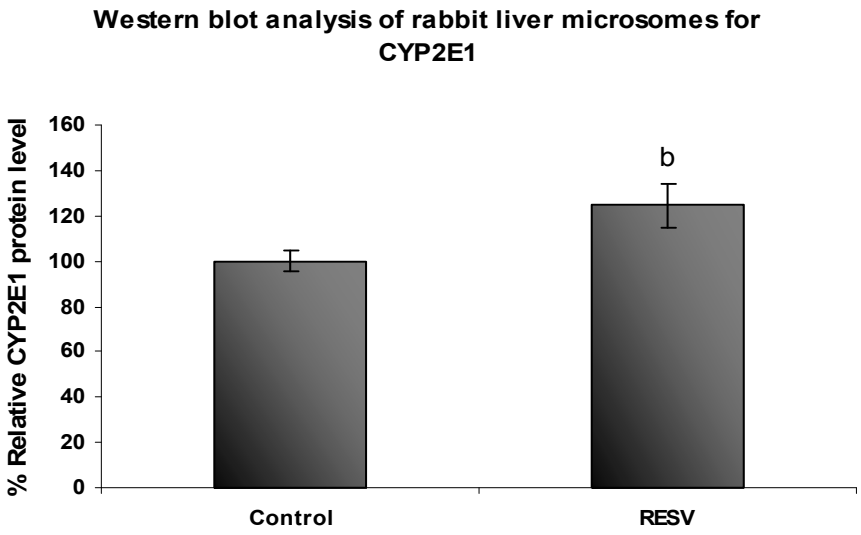

Figure 3.16 Effect of resveratrol treatment on CYP2E1 protein level of rabbit liver microsomes as determined by western blot analysis. Liver microsomal CYP2E1 expression from the control and "RESV" groups were detected by Western blot analysis and quantitated by densitometry. The value obtained from control animals were taken to be 100%, and the values obtained from "RESV" group were expressed as a percentage of control.

3.3.1.3 Changes in Cytochrome P4502E1 Protein Levels in both Acrylamide and Resveratrol Treated Rabbit Liver Microsomes

The western blot analyses of liver microsomes of rabbits are given in Figure 3.17 for "AA", "RESV", "RESV+AA" and "AA+RESV" groups. Intensity of each band was quantified as an arbitrary unit, relative peak area (R.P.A.) per mg microsomal protein by Scion Image software program. Band intensities of each lane and average values (Mean±SEM) are given in Table 3.15. According to these results, in "AA" group the CYP2E1 protein level of liver microsomes resulted in a statistically significant increase (1.50-fold, $p<0.01$) with respect to controls. In addition, statistically significant decrease was observed in CYP2E1 protein levels in "RESV+AA" (1.74-fold ($p<0.05$)) and "AA+RESV" (1.74-fold ($p<0.01$)) groups

compared to “AA” group. The effects of acrylamide, resveratrol and combined treatments on rabbit liver microsomal CYP2E1 protein levels are given in Figure 3.18.

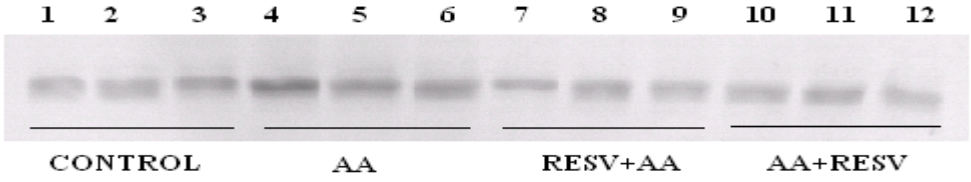

Figure 3.17 Immunochemical detection of liver microsomal cytochrome P4502E1 of control (Lane 1-3), “AA” (Lane 4-6), “RESV+AA” (Lane 7-9), “AA+RESV” (Lane 10-12) groups.

Table 3.15 Band intensities (represented as arbitrary unit of R.P.A/mg microsomal protein) of each sample in the western analysis of rabbit liver microsomal cytochrome P4502E1 (control; “AA”; “RESV+AA”, “AA+RESV” groups)

Lane No	**1**	**2**	**3**	**Average (Mean±SEM)**
Control (R.P.A./mg protein)	611	719	786	705.3±51 (N=3)
Lane No	**4**	**5**	**6**	
AA (R.P.A/mg protein)	1120	1053	1010	$1061\pm32^{a**}$(N=3)
Lane No	**7**	**8**	**9**	
RESV+AA (R.P.A/mg protein)	528	737	561	$609\pm65^{c*}$(N=3)
Lane No	**10**	**11**	**12**	
AA+RESV (R.P.A/mg protein)	600	694	532	$608.6\pm47^{c***}$(N=3)

a**: $p<0.01$, with respect to controls

c^{**}: $p<0.05$ with respect to “”AA” group

c^{***}: $p<0.01$, with respect to “AA” group

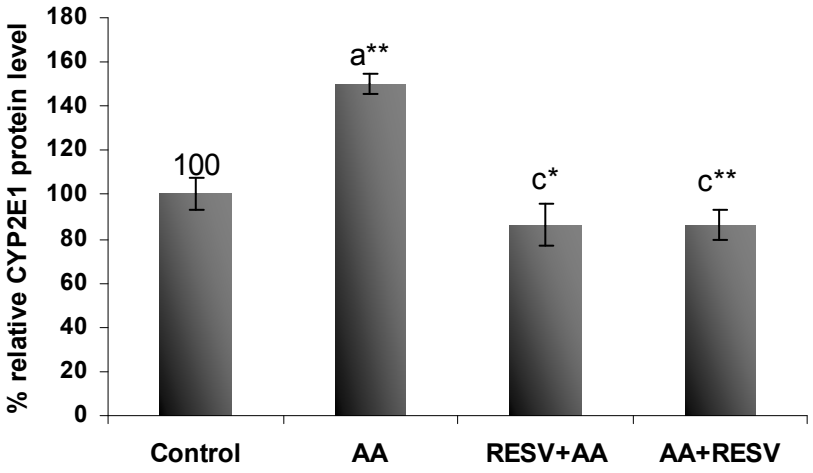

Figure 3.18 Effect of acrylamide, resveratrol and combined treatments on CYP2E1 protein level of rabbit liver microsomes as determined by western blot analysis. Liver microsomal CYP2E1 expression from the control and treated groups ("AA", "RESV", "RESV+AA", "AA+RESV") were detected by Western blot analysis and quantitated by densitometry. The value obtained from control animals were taken to be 100%, and the values obtained from treated groups ("AA", "RESV", "RESV+AA", "AA+RESV") were expressed as a percentage of control.

3.3.2 Changes in Cytochrome P4502E1 Protein Levels in Rabbit Kidney Microsomes

3.3.2.1 Changes in Cytochrome P4502E1 Protein Levels in Acrylamide Treated Rabbit Kidney Microsomes

The western blot analysis of kidney microsomes of "AA" group rabbits injected subcutaneously with 100 mg/kg b.w. acrylamide at day 1, 5, 8 and controls are given in Figure 3.19. Intensity of each band was quantified as an arbitrary unit, relative peak area (R.P.A.) per mg microsomal protein by Scion Image software program. Band intensities of each lane and average values (Mean±SEM) are given in Table 3.16. Thus, CYP2E1 protein level of rabbit kidney microsomes in "AA" group resulted in statistically significant increase, 1.97-fold (p<0.01), compared to controls.

The effect of acrylamide treatment on rabbit liver microsomal CYP2E1 protein level is given in Figure 3.20.

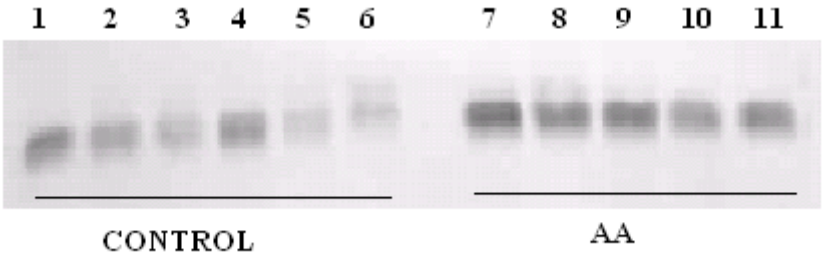

Figure 3.19 Immunochemical detection of kidney microsomal cytochrome P4502E1 of control rabbits (Lane 1-6) and "AA" rabbits (100 mg/kg b.w. at day 1, 5, 8; Lane 7-11).

Table 3.16 Band intensities (presented as arbitrary unit of R.P.A/mg microsomal protein) of each sample in the western analysis of rabbit kidney microsomal cytochrome P4502E1 (controls and "AA")

Lane No	**1**	**2**	**3**	**4**	**5**	**6**	**Average**
Control R.P.A/mg protein	1700	1264	853	1615	618	897	1158±179 (N=6)
Lane No	**7**	**8**	**9**	**10**	**11**		
AA R.P.A/mg protein	2924	2448	2246	1600	2197		2283±214^{a**} (N=5)

a**: $p<0.01$, with respect to controls

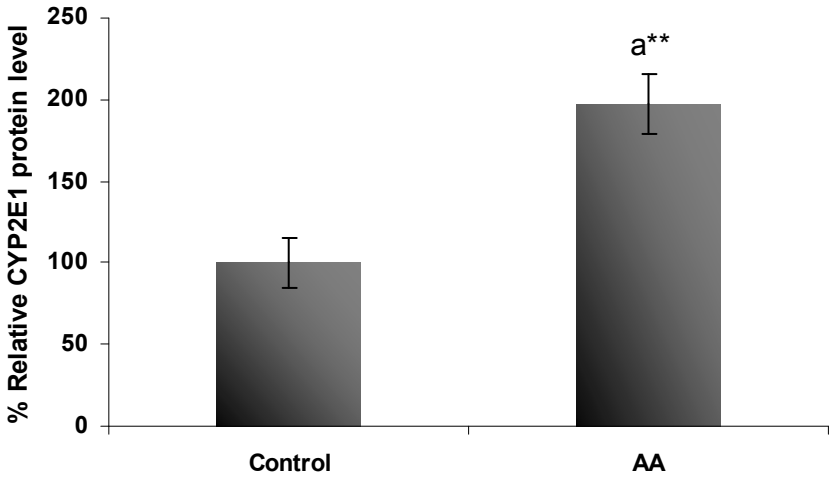

Figure 3.20 Effect of acrylamide treatment on CYP2E1 protein level of rabbit kidney microsomes as determined by western blot analysis. Kidney microsomal CYP2E1 expression from control and "AA" groups were detected by Western blot analysis and quantitated by densitometry. The value obtained from control animals were taken to be 100%, and the values obtained from the "AA" group were expressed as a percentage of control.

3.3.2.2 Changes in Cytochrome P4502E1 Protein Levels in Resveratrol Treated Rabbit Kidney Microsomes

The western blot analysis of kidney microsomes of "RESV" group rabbits administered intragastrically with 25 mg/kg b.w. resveratrol at day 1, 5, 8 and controls are given in Figure 3.21. Intensity of each band was quantified as an arbitrary unit, relative peak area (R.P.A.) per mg microsomal protein by Scion Image software program. Band intensities of each lane and average values (Mean±SEM) are given in Table 3.17. Resveratrol treatment of the rabbits led to 1.35 fold increase in CYP2E1 protein level of kidney microsomes with respect to controls which is not a statistically significant change. The effect of resveratrol treatment on rabbit liver microsomal CYP2E1 protein level is given in Figure 3.22.

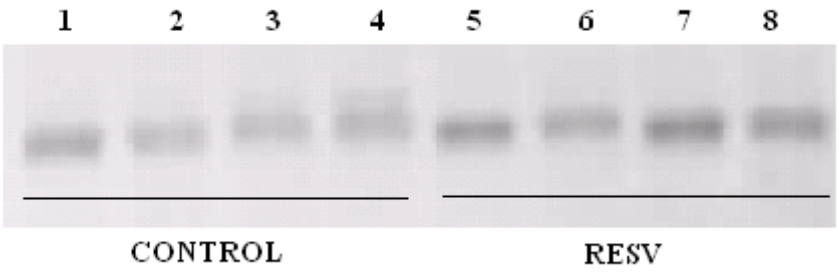

Figure 3.21 Immunochemical detection of kidney microsomal cytochrome P4502E1 of control rabbits (Lane 1-4) and "RESV" group rabbits (25 mg/kg b.w. at day 1, 5, 8; Lane 1-8).

Table 3.17 Band intensities (represented as arbitrary unit of R.P.A/mg microsomal protein) of each sample in the western analysis of rabbit kidney microsomal cytochrome P4502E1 (control and "RESV" groups).

Lane No	**1**	**2**	**3**	**4**	**Average (Mean±SEM)**
Control R.P.A/mg protein	966	678	715	996	839±83 (N=4)
Lane No	**5**	**6**	**7**	**8**	**Average (Mean±SEM)**
RESV R.P.A/mg protein	1108	815	1498	1125	1137±140[b] (N=4)

b: no change with respect to controls

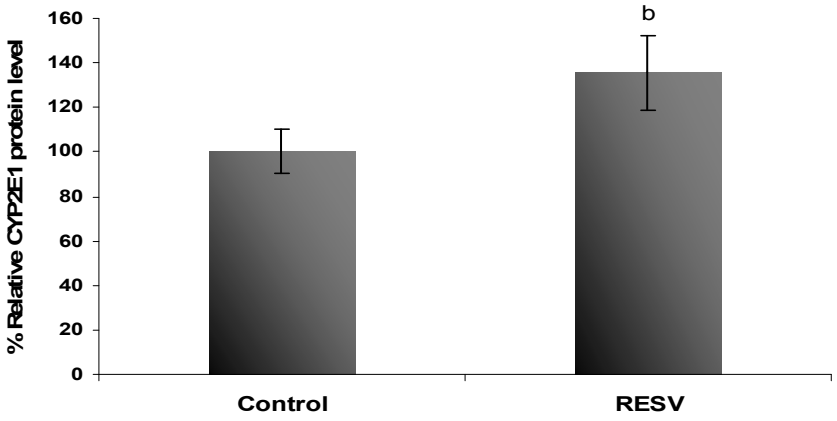

Figure 3.22 Effect of resveratrol treatment on CYP2E1 protein level of rabbit kidney microsomes as determined by western blot analysis. Kidney microsomal CYP2E1 expression from the control and "RESV" groups were detected by Western blot analysis and quantitated by densitometry. The value obtained from control animals were taken to be 100%, and the values obtained from the "RESV" group were expressed as a percentage of control.

3.3.2.3 Changes in Cytochrome P4502E1 Protein Levels in both Acrylamide and Resveratrol Treated Rabbit Kidney Microsomes

The western blot analysis of kidney microsomes of rabbits are given in Figure 3.23 for control, "AA", "RESV", "RESV+AA" and "AA+RESV" groups. Intensity of each band was quantified as an arbitrary unit, relative peak area (R.P.A.) per mg microsomal protein by Scion Image software program. Band intensities of each lane and average values (Mean±SEM) are given in Table 3.18. According to these results, in "AA" group the CYP2E1 protein level of kidney microsomes resulted in a statistically significant increase (2.42-fold, $p<0.001$) with respect to controls. Moreover, in "RESV+AA" group CYP2E1 protein levels of kidney microsomes decreased 2.53-fold ($p<0.001$) and in "AA+RESV" group CYP2E1 protein level of kidney microsomes decreased 2.00 fold ($p<0.01$) with respect to "AA" group. The effects of acrylamide, resveratrol and combined treatments on rabbit kidney microsomal CYP2E1 protein levels are given in Figure 3.24.

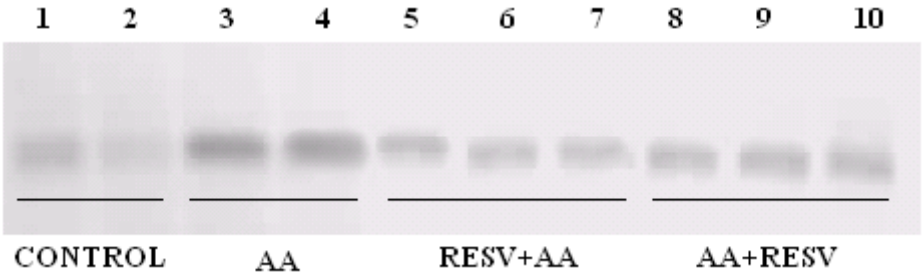

Figure 3.23 Immunochemical detection of kidney microsomal cytochrome P4502E1 of control rabbits, "AA", "RESV", "RESV+AA" and "AA+RESV" groups.

Table 3.18 Band intensities (represented as arbitrary unit of R.P.A/mg microsomal protein) of each sample in the western analysis of rabbit kidney microsomal cytochrome P4502E1 (control; "AA"; "RESV+AA", "AA+RESV" groups)

Lane No	**1**	**2**	**3**	**Average (Mean±SEM)**
Control (R.P.A./mg protein)	886	475		682±207 (N=2)
Lane No	**4**	**5**	**6**	
AA (R.P.A/mg protein)	1631	1672		$1652\pm21^{a***}$ (N=2*
Lane No	**7**	**8**	**9**	
RESV+AA (R.P.A/mg protein)	605	686	666	652 ± 24 (N=3)^{c***}
Lane No	**10**	**11**	**12**	
AA+RESV (R.P.A/mg protein)	779	909	785	$824\pm42^{c**}$ (N=3)

a***: $p<0.001$ with respect to controls

c***: $p<0.001$ with respect to "AA"group

c**: $p< 0.01$ with respect to "AA" group

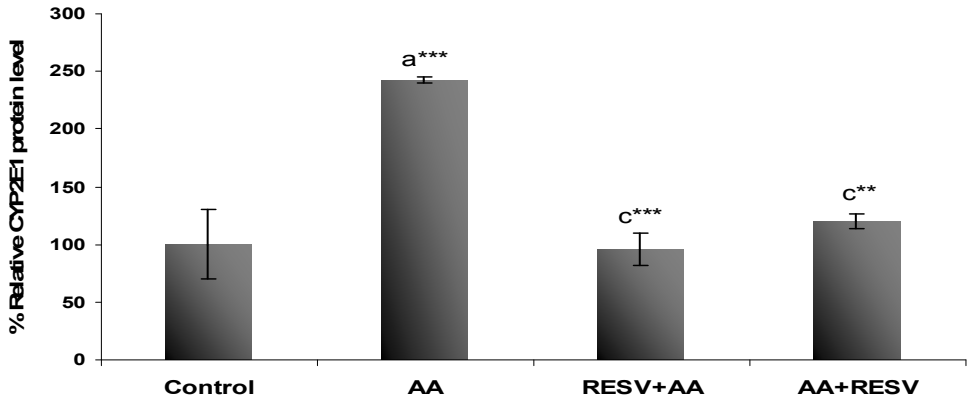

Figure 3.24 Effects of acrylamide, resveratrol and combined treatments on CYP2E1 protein level of rabbit kidney microsomes as determined by western blot analysis. Kidney microsomal CYP2E1 expression from the control and treated groups ("AA", "RESV", "RESV+AA", "AA+RESV") were detected by Western blot analysis and quantitated by densitometry. The value obtained from control animals were taken to be 100%, and the values obtained from treated groups (AA, RESV, RESV+AA, AA+RESV) were expressed as a percentage of control.

3.3.3 Changes in Cytochrome P4503A6 Protein Levels in Rabbit Liver Microsomes

3.3.3.1 Changes in Cytochrome P4503A6 Protein Levels in Acrylamide Treated Rabbit Liver Microsomes

The western blot analysis of liver microsomes of "AA" group rabbits injected subcutaneously with 100 mg/kg b.w. acrylamide at day 1, 5, 8 and controls are given in Figure 3.25. Intensity of each band was quantified as an arbitrary unit, relative peak area (R.P.A.) per mg microsomal protein by Scion Image software program. Band intensities of each lane and average values (Mean±SEM) are given in Table 3.19. According to these results, liver microsomal CYP3A6 protein levels of "AA" group rabbits resulted in statistically significant increase, 1.69-fold (p<0.05),

compared to controls. The effects of acrylamide treatmen on rabbit liver microsomal CYP3A6 protein levels are given in Figure 3.26.

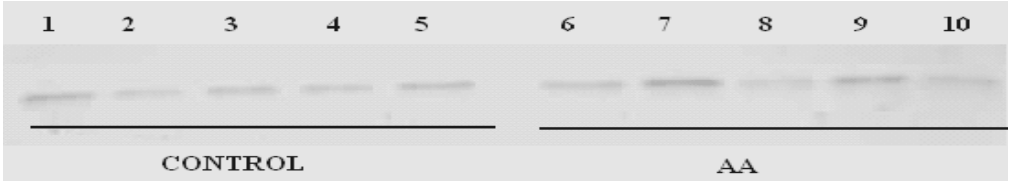

Figure 3.25 Immunochemical detection of liver microsomal cytochrome P4503A6 of control rabbits (Lane 1-5) and "AA" group rabbits (100 mg/kg b.w. s.c at day 1, 5, 8; Lane 6-10)

Table 3.19 Band intensities (represented as arbitrary unit of R.P.A/mg microsomal protein) of each sample in the western analysis of rabbit liver microsomal cytochrome P4503A6 (controls and "AA")

Lane No	**1**	**2**	**3**	**4**	**5**	**Average (Mean±SEM)**
Control$^{\gamma}$ R.P.A/mg protein	497	261	318	304	421	360.2±43 (N=5)
Lane No	**6**	**7**	**8**	**90**	**10**	
AA$^{\gamma}$ R.P.A/mg protein	467	890	436	672	588	611±82^{a*} (N=5)

a*: $p<0.05$, with respect to controls

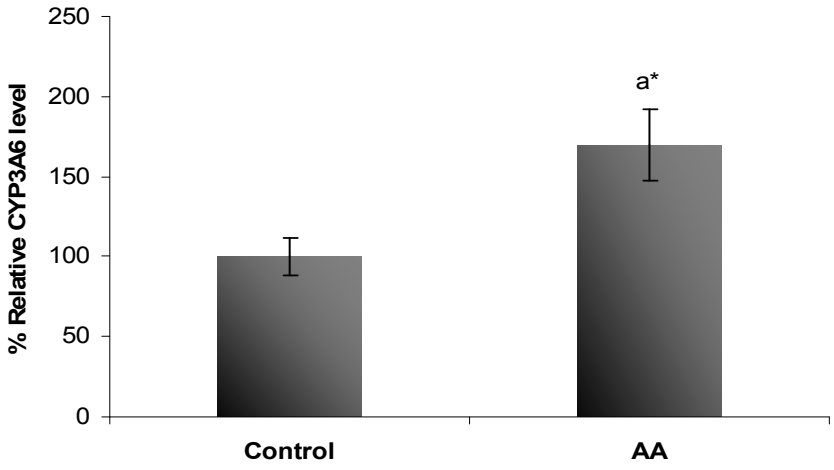

Figure 3.26 Effect of acrylamide treatment on CYP3A6 protein level of rabbit liver microsomes as determined by western blot analysis. Liver microsomal CYP3A6 expression from the control and "AA" groups were detected by Western blot analysis and quantitated by densitometry. The value obtained from control animals were taken to be 100%, and the values obtained from the "AA" group were expressed as a percentage of control.

3.3.3.2 Changes in Cytochrome P4503A6 Protein Levels in Resveratrol Treated Rabbit Liver Microsomes

The western blot analysis of liver microsomes of "RESV" group rabbits injected subcutaneously with 25 mg/kg b.w. acrylamide at day 1, 5, 8 and controls are given in Figure 3.27. Intensity of each band was quantified as an arbitrary unit, relative peak area (R.P.A.) per mg microsomal protein by Scion Image software program. Band intensities of each lane and average values (Mean±SEM) are given in Table 3.20. According to these results, liver microsomal CYP3A6 protein level of "RESV" group resulted in no statistically significant increase compared to controls. The effects of resveratrol treatment on rabbit liver microsomal CYP3A6 protein levels are given in Figure 3.28.

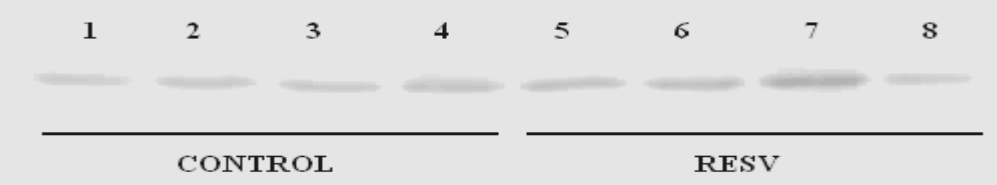

Figure 3.27 Immunochemical detection of liver microsomal cytochrome P4503A6 of control rabbits (Lane 1-4) and "RESV" group rabbits (25 mg/kg b.w. s.c at day 1, 5, 8; Lane 5-8).

Table 3.20 Band intensities (represented as arbitrary unit of R.P.A/mg microsomal protein) of each sample in the western analysis of rabbit liver microsomal cytochrome P4503A6 (controls and "RESV")

Lane No	**1**	**2**	**3**	**4**	**Average (Mean±SEM)**
Control R.P.A/mg protein	1016	1029	1007	1374	1107±89 (N=4)
Lane No	**5**	**6**	**7**	**8**	
AA R.P.A/mg protein	1171	1312	1095	928	1127±80 (N=4)

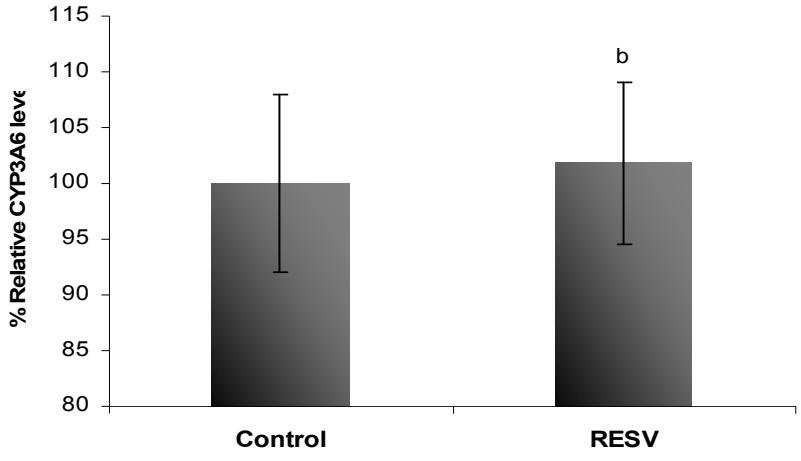

Figure 3.28 Effect of resveratrol treatment on CYP3A6 protein level of rabbit liver microsomes as determined by western blot analysis. Liver microsomal CYP3A6 expression from the control and "RESV" groups were detected by Western blot analysis and quantitated by densitometry. The value obtained from control animals were taken to be 100%, and the values obtained from the "RESV" group were expressed as a percentage of control.

3.4 Effects of Acrylamide and Resveratrol Treatment on NQO1 (DT-Diaphorase) Activity

3.4.1 Effects of Acrylamide and Resveratrol Treatment on NQO1 (DT-Diaphorase) Activity of Rabbit Liver Cytosols

All measured NQO1 (DT-Diaphorase) activity of each rabbit liver values are given in Table 3.22 for both control and treated rabbits. According to these results, rabbit liver cytosolic NQO1 (DT-Diaphorase) activity of "AA" and "RESV" group did not significantly change with respect to controls. In addition, no change was observed in the "RESV+AA" and "AA+RESV" group with respect to "AA" group in liver cytosolic NQO1 (DT-Diaphorase) activity. The effects of *in vivo* acrylamide and resveratrol treatments on rabbit liver cytosolic NQO1 enzyme activity are given in Figure 3.31.

Table 3.21 NQO1 activity of rabbit liver cytosols

	NQO1 activities (µmol/min/mg protein)						
Treatments	**Rabbits**						
	1	**2**	**3**	**4**	**5**	**6**	**Average (Mean±SEM)**
Control	0.12	0.15	0.12	0.13	0.14	0.07	0.12±0.01 (N=6)
AA	0.07	0.24	0.12	0.16	0.11	-	0.14±0.03[a] (N=5)
RESV	0.12	0.11	0.11	0.05	-	-	0.10±0.01[b] (N=4)
RESV+AA	0.05	0.15	0.23	0.19	-	-	0.16±0.04[c] (N=4)
AA+RESV	0.10	0.19	0.07	0.12	-	-	0.12±0.03[c] (N=4)

a: no change with respect to controls

b: no change with respect to controls

c: no change with respect to "AA" group

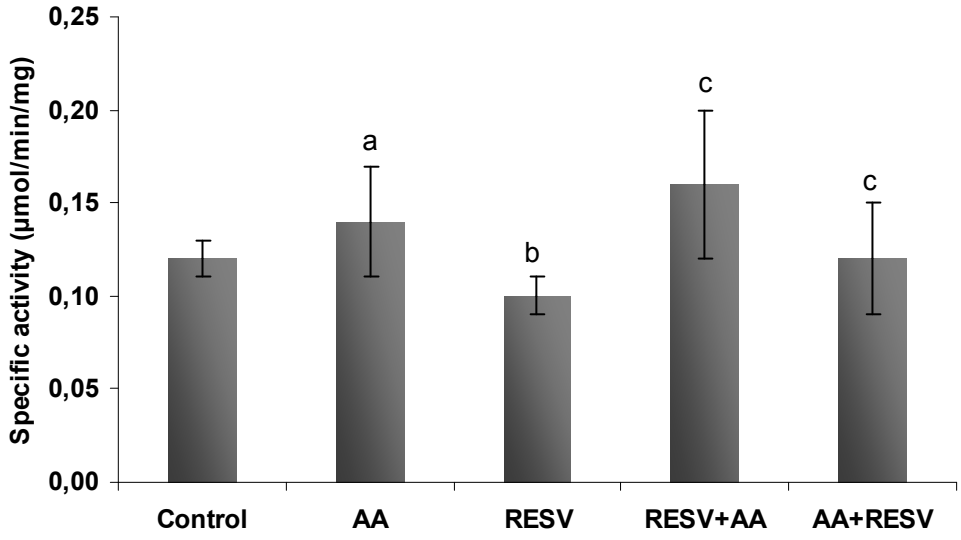

Figure 3.29 NQO1 activities of rabbit liver microsomes

3.4.2 Effect of Acrylamide and Resveratrol Treatment on DT-Diaphorase (NQO1) Activity of Rabbit Kidney Cytosols

All measured NQO1 (DT-Diaphorase) activity of each rabbit kidney values for both control and treated rabbits are given in Table 3.23. According to the results, a statistically significant increase (1.64-fold, $p<0.01$) in "AA" group was observed in NQO1 activity compared the control group. No statistically significant change was observed in the "RESV" group with respect to controls. In addition, there was no statistically significant change in the "RESV+AA" and "AA+RESV" groups with respect to "AA" group in kidney cytosolic NQO1 activity. The effects of *in vivo* acrylamide and resveratrol treatments on rabbit kidney cytosolic NQO1 enzyme activity are given in Figure 3.32.

Table 3.22 NQO1 activities of rabbit kidney cytosols

	NQO1 activity (µmol/min/mg protein)					
Rabbits	**Treatments**					
	1	**2**	**3**	**4**	**5**	**Average(Mean±SEM)**
Control	0.021	0.031	0.034	0.025	-	0.028±0.003 (N=4)
AA	0.056	0.049	0.052	0.040	0.032	0.046±0.004 **a**** (N=5)
RESV	0.032	0.021	0.031	0.019	-	0.026±0.003**b** (N=4)
RESV+AA	0.015	0.038	0.032	0.047	-	0.033±0.007**c** (N=4)
AA+RESV	0.040	0.054	0.040	0.025	-	0.039±0.006**c** (N=4)

a**: $p<0.01$ with respect to controls

b: no change with respect to controls

c: no change with respect to "AA" group

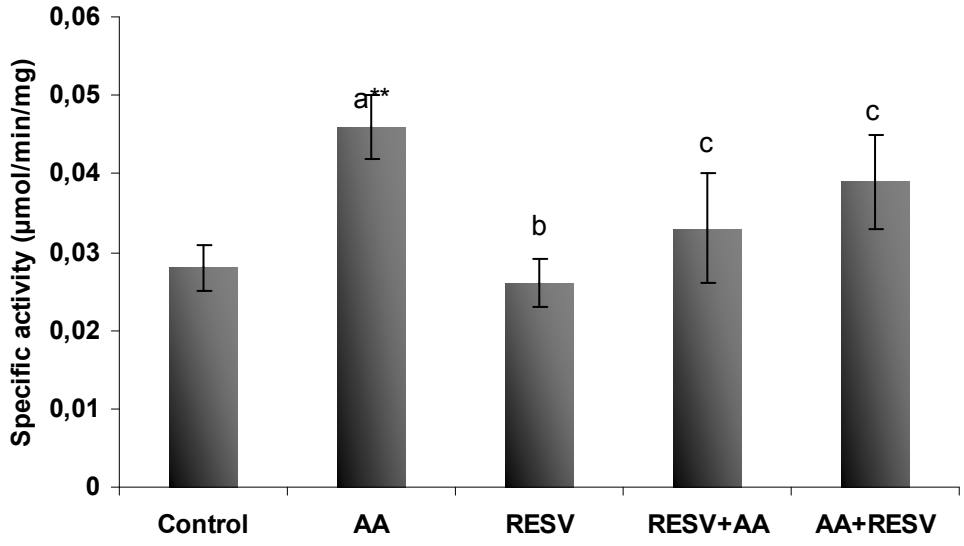

Figure 3.30 NQO1 activities of rabbit kidney cytosols

3.4.3 Effects of Acrylamide and Resveratrol Treatment on DT-Diaphorase (NQO1) Activity of Rabbit Lung Cytosols

All measured NQO1 (DT-Diaphorase) activity of each rabbit lung values are given in Table 3.33 for both control and treated rabbits. According to the results, no statistically significant change was observed in the "AA" and "RESV" group with respect to controls in lung cytosolic NQO1 activity. In addition, there was no statistically significant change in the "RESV+AA" and "AA+RESV" group with respect to "AA" group in lung cytosolic NQO1 activity. The effects of *in vivo* acrylamide and resveratrol treatments on rabbit lung cytosolic NQO1 enzyme activities are given in Figure 3.24.

Table 3.23 NQO1 activities of rabbit lung cytosols

	NQO1 activity (µmol/min/mg protein)					
Rabbits	**Treatments**					
	1	**2**	**3**	**4**	**5**	**Average (Mean±SEM)**
Control	0.09	0.16	0.11	0.07	-	0.11±0.02 (N=4)
AA	0.07	0.11	0.08	0.13	0.14	0.11±0.01[a] (N=5)
RESV	0.09	0.05	0.12	0.05	-	0.08±0.02[b] (N=4)
RESV+AA	0.07	0.19	0.14	0.15	-	0.14±0.03[c] (N=4)
AA+RESV	0.14	0.14	0.06	0.10	-	0.11±0.02[c] (N=4)

a: no change with respect to controls

b: no change with respect to controls

c: no change with respect to "AA" group

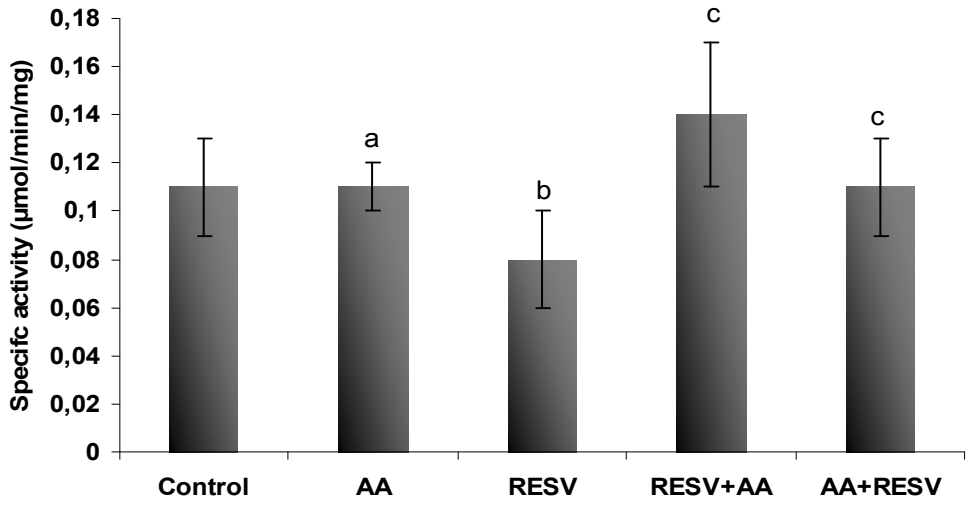

Figure 3.31 NQO1 activities of rabbit lung microsomes

3.5 Effect of Acrylamide and Resveratrol Treatment on Glutathione S-Transferase Activity

3.5.1 Effect of Acrylamide and Resveratrol Treatment on GST Activity of Rabbit Liver Cytosols

3.5.1.1 Total GST Activity

Total GST activity was determined by using CDNB as substrate. All measured total GST activity of each rabbit liver values are given in Table 3.25 for both control and treated rabbits. The results indicated that there was no significant change in the cytosolic total GST activities of rabbit liver microsomes of "AA" and "RESV" groups compared to controls. In addition, no significant change was observed in "RESV+AA" and "AA+RESV" groups with respect to "AA" group. The effects of *in vivo* acrylamide and resveratrol treatments on cytosolic total GST activity are given In Figure 3.34.

Table 3.24 Total GST activities of rabbit liver cytosols

	Total GST activity (µmol/min/mg protein)						
Treatments	**Rabbits**						
	1	**2**	**3**	**4**	**5**	**6**	**Average (Mean±SEM)**
Control	5.18	8.04	8.25	5.14	5.12	6.32	6.34±0.60 (N=6)
AA	5.71	8.32	5.80	8.18	4.62		6.53±0.73[a] (N=5)
RESV	5.85	5.10	5.58	6.89			5.86±0.38[b] (N=4)
RESV+AA	5.84	5.71	5.57	6.17			5.82±0.13[c] (N=4)
AA+RESV	6.74	5.76	8.47	7.36			7.08±0.57[c] (N=64)

a: no change with respect to controls

b: no change with respect to controls

c: no change with respect to "AA"group

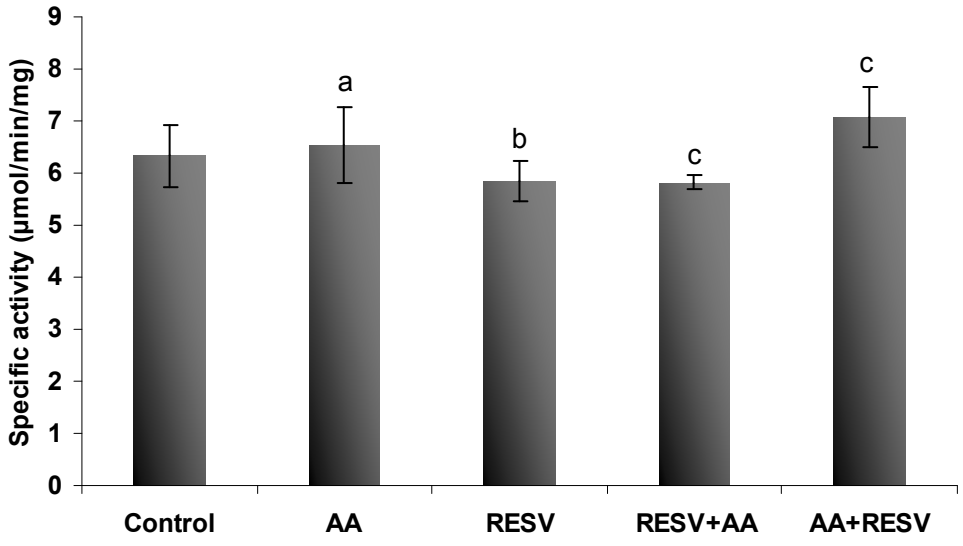

Figure 3.32 Total GST activities of rabbit liver cytosols

3.5.1.2 GST-Mu Activity

GST-Mu activity was determined by using DCNB as substrate. All measured GST-Mu activity of each rabbit liver values are given in Table 3.26 for both control and treated rabbits. According to these results, there was no statistically significant change in the activity of liver cytosolic GST-Mu in "AA" or "RESV" groups compared to control group. Additionally, no significant change in the liver cytosolic GST-Mu activity in groups "RESV+AA" and "AA+RESV" was observed with respect to "AA" group. The effects of *in vivo* acrylamide and resveratrol treatments on rabbit liver cytosolic GST-Mu activity are given in Figure 3.35.

Table 3.25 GST-Mu activity of rabbit liver cytosols

	GST-Mu activity (nmol/min/mg protein)						
Treatments	**Rabbits**						
	1	**2**	**3**	**4**	**5**	**6**	**Average (Mean±SEM)**
Control	4.42	4.44	4.97	3.31	4.37	3.39	4.15±0.27 (N=6)
AA	3.05	4.80	2.65	4.20	5.39		4.24±0.52[a] (N=5)
RESV	4.73	3.05	3.77	5.39			4.39±0.64[b] (N=4)
RESV+AA	3.79	6.29	3.92	3.56			4.41±0.29[c] (N=4)
AA+RESV	4.08	3.77	5.02	4.79			4.15±0.27[c] (N=4)

a: no change with respect to controls

b: no change with respect to controls

c: no change with respect to "AA" group

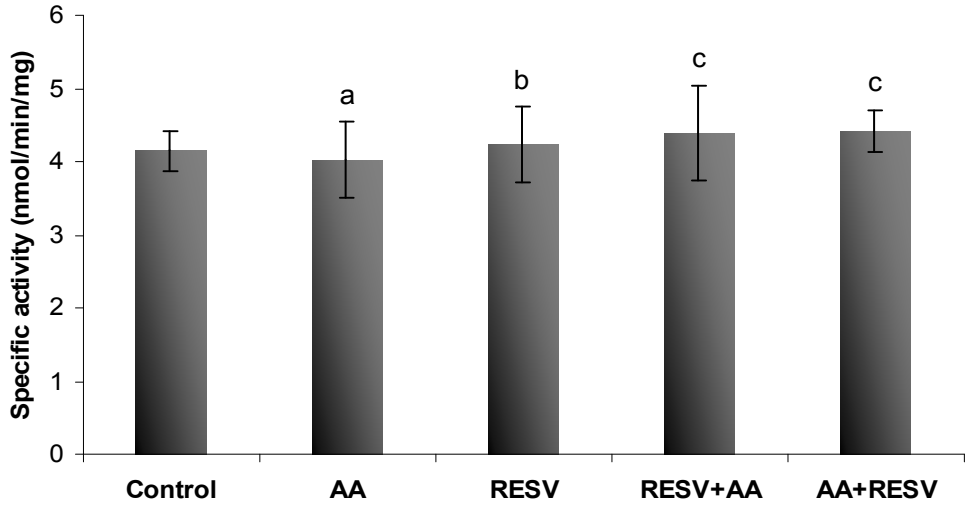

Figure 3.33 GST-Mu activity of rabbit liver cytosols

3.5.2 Effect of Acrylamide and Resveratrol Treatment on GST Activity of Rabbit Kidney Cytosols

3.5.2.1 Total GST Activity

Total GST activity was determined by using CDNB as substrate. All measured Total GST activity of each rabbit kidney values are given in Table 3.27 for both control and treated rabbits. Thus, rabbit kidney cytosolic total GST activity of "AA" group did not significantly change with respect to controls. A statistically significant increase in the enzyme activity (1.50-fold, $p<0.05$) was observed in "RESV" group. On the other hand, no change was observed in the "RESV+AA" and "AA+RESV" group with respect to "AA" group in kidney cytosolic total GST activity. The effects of *in vivo* acrylamide and resveratrol treatments on rabbit kidney total GST activity are given in Figure 3.36.

Table 3.26 Total GST activity of rabbit kidney cytosols

	Total GST activities (µmol/min/mg protein)					
Treatments	**Rabbits**					
	1	**2**	**3**	**4**	**5**	**Average (Mean±SEM)**
Control	1.30	0.98	1.48	0.78	-	1.14±0.16 (N=4)
AA	1.50	1.76	1.49	1.18	1.11	1.41±0.12 (N=5)
RESV	1.69	2.02	1.68	1.56	-	1.74±0.09^{b*} (N=4)
RESV+AA	1.48	1.24	1.30	1.55	-	1.39±0.07 (N=4)
AA+RESV	1.65	1.69	1.84	0.96	-	1.54±0.20 (N=4)

a: no change with respect to controls

b*: $p<0.05$, with respect to controls

c: no change with respect to "AA" group

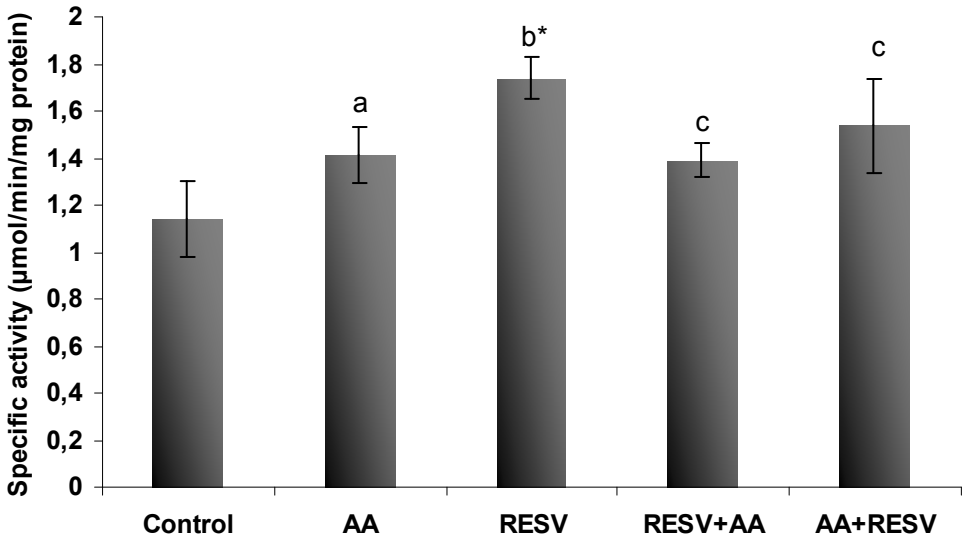

Figure 3.34 Total GST activity of rabbit kidney cytosols

3.5.2.2 GST-Mu Activity

GST-Mu activity was determined by using DCNB as substrate. All measured GST-Mu activity of each rabbit kidney values are given in Table 3.28 for both control and treated rabbits. According to these results, rabbit kidney cytosolic GST-Mu activity of "AA" group did not significantly change with respect to controls. A statistically significant increase in the enzyme activity (1.50-fold, $p<0.05$) was observed in "RESV" group. On the other hand, no change was observed in the "RESV+AA" and "AA+RESV" group with respect to "AA" group in kidney GST-Mu activity. The *in vivo* effects of acrylamide and resveratrol treatments on rabbit kidney cytosolic GST-Mu activity are given in Figure 3.37.

Table 3.27 GST-Mu activity of rabbit kidney cytosols

	GST-Mu activities (nmol/min/mg protein)					
Treatments	**Rabbits**					
	1	**2**	**3**	**4**	**5**	**Average (Mean±SEM)**
Control	1.36	1.33	1.65	1.11		1.36±0.11 (N=4)
AA	1.92	2.40	1.74	1.46	1.28	1.76±0.19 (N=5)
RESV	1.74	2.61	2.05	2.07		2.12±0.18[b*] (N=4)
RESV+AA	2.11	1.37	1.83	1.96		1.82±0.16 (N=4)
AA+RESV	1.19	2.16	1.87	1.21		1.61±0.24 (N=4)

a: no change with respect to controls

b*: $p<0.05$, with respect to controls

c: no change with respect to "AA" group

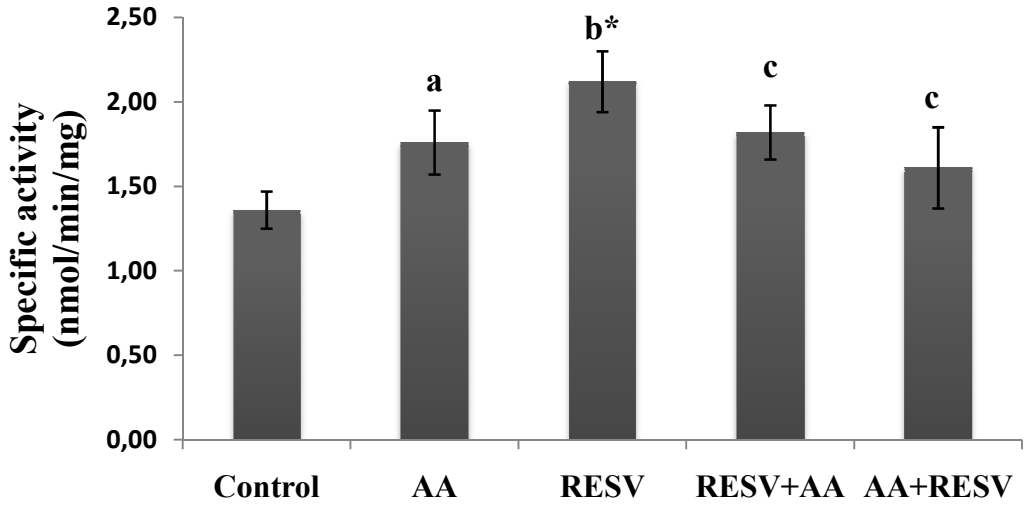

Figure 3.35 GST-Mu activities of rabbit kidney cytosols

3.5.3 Effect of Acrylamide and Resveratrol Treatment on GST Activity of Rabbit Lung Cytosols

3.5.3.1 Total GST Activity

Total GST activity was determined by using CDNB as substrate. All measured total GST activity of each rabbit lung values are given in Table 3.29 for both control and treated rabbits. According to these results, rabbit lung cytosolic total GST activity of "AA" group did not significantly change with respect to controls. A 1.60-fold ($p<0.05$) significant increase was observed in "RESV" group with respect to controls. In addition, no statistically significant change was observed in "RESV+AA" and "AA+RESV" groups compared to "AA" group in lung cytosolic total GST activity. The effects of *in vivo* acrylamide and resveratrol treatments on rabbit lung cytosolic total GST activity are given in Figure 3.38.

Table 3.28 Total GST activity of rabbit lung cytosols

	Total GST activity (µmol/min/mg protein)					
Treatments	**Rabbits**					
	1	**2**	**3**	**4**	**5**	**Average (Mean±SEM)**
Control	0.56	0.75	0.58	0.54		0.61±0.05 (N=4)
AA	0.57	0.44	0.36	0.66	0.57	0.52±0.03[a] (N=5)
RESV	0.79	0.89	1.02	1.20		0.98±0.09[b*] (N=4)
RESV+AA	0.56	0.69	0.66	0.63		0.64±0.03[c] (N=4)
AA+RESV	0.68	0.65	0.57	0.60		0.63±0.03[c] (N=4)

a: no change with respect to controls

b*: $p<0.05$, with respect to controls

c: no change with respect to "AA" group

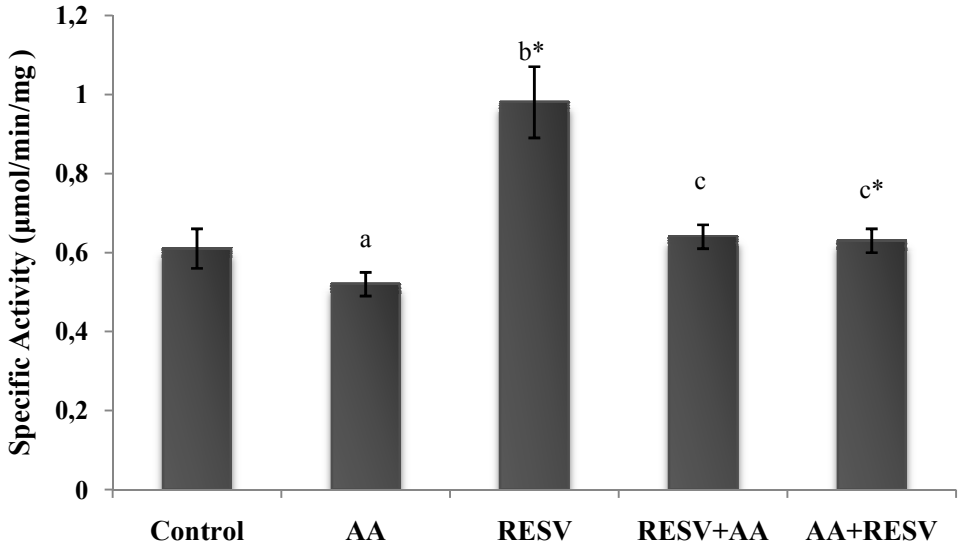

Figure 3.36 Total GST activity of rabbit lung cytosols

3.5.3.2 GST-Mu Activity

GST-Mu activity was determined by using DCNB as substrate. All measured GST-Mu activity of each rabbit lung values are given in Table 3.30 for both control and treated rabbits. According to these results, rabbit kidney cytosolic GST-Mu activity of "AA" and "RESV" group did not significantly change with respect to controls. In addition, no change was observed in the "RESV+AA" and "AA+RESV" group with respect to "AA" group in lung cytosolic GST-Mu activity. The effects of *in vivo* acrylamide and resveratrol treatments on rabbit lung cytosolic GST-Mu activity are given in Figure 3.39.

Table 3.29 GST-Mu activity of rabbit lung cytosols

	GST-Mu activity (nmol/min/mg protein)					
Treatments	**Rabbits**					
	1	**2**	**3**	**4**	**5**	**Average (Mean±SEM)**
Control	1.26	1.32	1.00	1.00		1.14±0.09 (N=4)
AA	1.01	0.49	0.58	0.66	1.2	0.79±0.14[a] (N=5)
RESV	0.49	1.05	0.56	1.29		0.85±0.19[b] (N=4)
RESV+AA	1.15	1.28	1.06	0.83		1.08±0.09[c] (N=4)
AA+RESV	0.68	0.65	0.57	0.60		0.63±0.03[c] (N=4)

a: no change with respect to controls

b*: no change with respect to controls

c: no change with respect to "AA" group

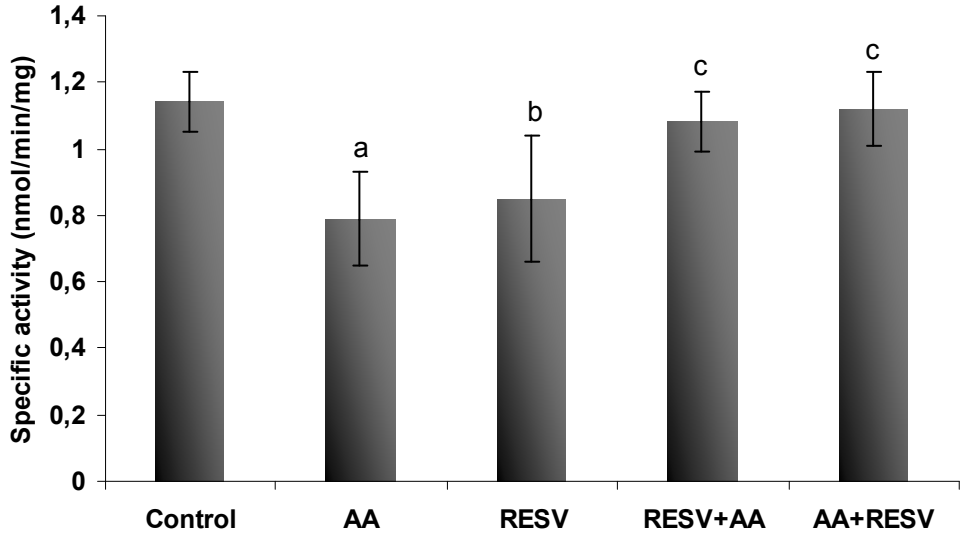

Figure 3.37 GST-Mu activities of rabbit lung cytosols

CHAPTER 4

DISCUSSION

Acrylamide, an industrially produced compound, is known as an animal neurotoxin, a reproductive toxin and a carcinogen. The metabolism of acrylamide is complex and not fully understood yet (Sczerbina *et al.,* 2008). However, several studies have demonstrated that acrylamide is metabolized to its epoxide glycidamide by CYP2E1. In addition, it was also recently suggested that mutagenicity and carcinogenicity of acrylamide is due to the conversion of acrylamide to epoxide glycidamide, by liver CYP2E1 ((Besaratinia and Pfeifer, 2004; Adler *et al.*, 2000; Manjanatha *et al.*, 2006; Ghanayem *et al.,* 2005b; Rice, 2005). Thus, the aim of the present study was to investigate *in vivo* interaction of carcinogenic acrylamide with Phase I (CYP2E1, CYP3A6, CYP2B4 and NQO1) and Phase II enzymes (GSTs) in rabbit liver, kidney and lung for the first time. In addition to acrylamide, resveratrol which is a phenolic compound found in grapes, peanuts and red wine was also selected for the present study. Phenolic compounds have known to affect carcinogenesis with their anti-oxidant properties. One important route of phenolic compounds to implement their chemoprevention effect is the modulation of enzymes (such as CYP2E1, CYP3A6, CYP2B4) which metabolizes the procarcinogens or carcinogens to more carcinogenic compounds or induction of detoxification enzymes such as GSTs. Thus, *in vivo* protective effects of resveratrol on acrylamide induced toxicity was also investigated on acrylamide toxicity on rabbit liver, kidney and lung Phase I and Phase II enzymes, in the present study.

A proposed mechanism for the acrylamide metabolism in mice has been demonstrated in the study conducted by Sumner *et al.* (1999) in which epoxidation of acrylamide to glycidamide has been demonstrated to be catalyzed by CYP2E1. This metabolic pathway has also been demonstrated in rats (Calleman *et al.*, 1990; Sumner *et al.,* 1997), mice (Sumner *et al.,* 1999; Ghanayem *et al.,* 2000) and humans (Bergmark *et al.,* 1993) both *in vivo* and *in vitro*. In addition, several studies carried out in recent years demonstrated that mutagenicity and carcinogenicity associated

with acrylamide is mostly attributed to the conversion of acrylamide to its epoxide glycidamide, by liver CYP2E1. (Besaratinia and Pfeifer, 2004; Adler *et al.,* 2000; Manjanatha *et al.*, 2006; Ghanayem *et al.,* 2005b; Rice, 2005). To best of our knowledge, although acrylamide carcinogenicity is mostly attributed to the conversion of acrylamide to its epoxide glycidamide, by liver CYP2E1; there is no study investigating the effect of *in vivo* acrylamide treatment on CYP2E1 enzyme activity and its protein levels.

In the present study, rabbits were administered with acrylamide at a dose of 100 mg/kg b.w. subcutaneously on days 1, 5 and 8 (presented as "AA" group). The CYP2E1 enzyme activities measured by aniline 4-hydroxylase, *p*-nitrophenol hydroxylase and NDMA N-demethylase reactions were resulted in 1.80 fold ($p<0.05$), 3.16 fold ($p<0.001$) and 2.6 fold ($p<0.001$) statistically significant increase, respectively in "AA" group rabbit liver microsomes with respect to controls. This induction effect of *in* vivo acrylamide treatment not only observed in liver microsomal CYP2E1 but also observed in kidney microsomal CYP2E1. Rabbit kidney CYP2E1 enzyme activity measured by aniline 4-hydroxylase and *p*-nitrophenol hydroxylase reactions resulted in 1.60 fold ($p<0.001$) and 1.56 fold ($p<0.05$) significant increase in "AA" group with respect to controls. In the present study, the induction of rabbit liver and kidney CYP2E1 protein level by *in vivo* acrylamide treatment was also confirmed by western blot analysis. According to the results, there were statistically significant 2.06 fold ($p<0.01$) and 1.97 fold, $p<0.01$) increases in "AA" group liver and kidney microsomal CYP2E1 protein level with respect to controls. In the present study, the CYP2E1 enzyme activity and its associated *p*-nitrophenol hydroxylase and NDMA N-demethylase activities were also determined in rabbit lung microsomes. In contrast to rabbit liver and kidney microsomal CYP2E1 enzyme activities, no statistically significant change was observed in lung microsomal CYP2E1 enzyme activity of "AA" group with respect to controls. The effects of acrylamide, resveratrol and their combined treatments on liver, kidney and lung CYP2E1 associated enzyme activities and CYP2E1 protein levels of rabbit liver and kidney microsomes were presented in Table 4.1 and Table 4.2, respectively.

Table 4.1 Effects of acrylamide, resveratrol and their combined treatments on liver, kidney and lung CYP2E1 associated *p*-nitrophenol hydroxylase, NDMA N-demethylase and aniline 4-hydroxylase enzyme activities

	Activity µmol/min/mg pro.	**Control**	**AA**	**RESV**	**AA+RESV**	**RESV+AA**
LIVER	Aniline 4-hydroxylase	0.67±0.04	1.20±0.11	0.75±0.05	0.79±0.07	0.82±0.08
	Change (Fold)		**1.80 X ↑** [a*]	-	**1.51 X ↓** [c*]	**1.48 X ↓** [c*]
	p-nitrophenol hydroxylase	0.19±0.01	0.60±0.01[a***]	0.30±0.05	0.42±0.01	0.36±0.06
	Change (Fold)		**3.16 X ↑** [a***]	-	**1.43 X ↓** [c**]	1.66 **X ↓** [c*]
	NDMA N-demethylase	0.22±0.04	0.57±0.04	0.30±0.01	0.39±0.03	0.37±0.04
	Change (Fold)		**2.6 X ↑** [a***]	**-**	1.80 **X ↓** [c*]	1.60 **X ↓** [c*]
KIDNEY	Aniline 4-hydroxylase	0.025±0.001	0.040±0.002	0.026±0.004	0.039±0.005	0.038±0.004
	Change (Fold)		**1.60 X ↑** [a***]	-	-	-
	p-nitrophenol hydroxylase	0.016±0.001	0.025±0.003	0.018±0.001	0.025±0.002	0.021±0.002
	Change (Fold)		**1.56 X ↑** [a***]	-	-	-
LUNG	*p*-nitrophenol hydroxylase	0.18±0.01	0.20±0.02	0.16±0.02[b]	0.19±0.01	0.18±0.01
	Change (Fold)		-	-	-	-
	NDMA N-demethylase	0.07±0.02	0.11±0.01	0.10±0.01	0.09±0.01	0.10±0.04
	Change (Fold)		-	-	-	-

a*: significant change with respect to controls, $p<0.05$
a**: significant change with respect to controls, $p<0.01$
a***: significant change with respect to control, $p<0.001$
c*: significant change with respect to "AA" group, $p<0.05$
c**: significant change with respect to "AA" group, $p<0.01$
c***: significant change with respect to "AA" group, $p<0.001$

Table 4.2 The effects of acrylamide and resveratrol treatments on rabbit liver and kidney CYP2E1 protein levels

Tissue		CYP2E1 protein level (RPA)	Change (Fold)		CYP2E1 protein level (RPA)	Change (Fold)
LIVER	Control	1594±113		AA	1061±32	
	AA	3286±268	**2.06 X** $\uparrow^{a**}$	RESV+AA	609±65	**1.74 X** $\downarrow^{c*}$
	Control	1228±58		AA+RESV	608.6±47	**1.74** $\downarrow^{c***}$
	RESV	1529±115	-			
KIDNEY	Control	1158±179		AA	1652±21	
	AA	2283±214	1.97 **X** $\uparrow^{a**}$	RESV+AA	652±24	2.53 **X** $\downarrow^{c***}$
	Control	839±83		AA+RESV	824±42	2.00 **X** $\downarrow^{c**}$
	RESV	1137±140				

a**: significant change with respect to controls, $p<0.01$
c*: significant change with respect to "AA" group, $p<0.05$
c**: significant change with respect to "AA" group, $p<0.01$
c***: significant change with respect to "AA" group, $p<0.001$

Previous studies have demonstrated that the only CYP enzyme involved in the acrylamide metabolism is CYP2E1 (Sumner *et al.,* 1999; Ghanayem *et al.,* 2000; Calleman *et al.,* 1990; Sumner *et al.,* 1997; Bergmark *et al.,* 1993). Thus, acrylamide is a substrate of CYP2E1. However, it is well-known phenomenon that some substrates of CYP2E1; such as acetone, pyridine and benzene are also its inducers (Yang and Hong, 1993; Lieber, 1997; Arınç *et al.,* 2000a, b). Thus, in the present study, it was also demonstrated that acrylamide is an inducer of rabbit liver and kidney microsomal CYP2E1 enzyme both in activity and protein level.

As previously mentioned, several studies suggested that mutagenicity and carcinogenicity associated with acrylamide is due to the conversion of acrylamide to its epoxide glycidamide, by liver CYP2E1. (Besaratinia and Pfeifer, 2004; Adler *et al.*, 2000; Manjanatha *et al.*, 2006; Ghanayem *et al.,* 2005b; Rice, 2005). It is known

that, CYP2E1 is one of the few forms of cytochrome P450s which are mainly metabolizes toxic and carcinogenic compounds to their toxic derivatives. Besides its endogenous substrates, CYP2E1 predominantly metabolizes exogenous substrates such as industrial solvents, protoxins and procarcinogens (Table 1.3). In addition, CYP2E1 is of critical importance in the bioactivation of low molecular weight compounds (Yang and Hong, 1993; Arınç *et al.,* 1991; Yamazaki *et al.,* 1992; Arınç *et al.,* 2000a, b). Thus, CYP2E1 has received great attention due to this vital role in the activation of many toxic chemicals participated in cancer. For instance, among this toxic chemicals, benzene is an important environmental carcinogen preferentially metabolized by CYP2E1 (Post and Synder, 1983). In addition, CYP2E1 metabolic activation of benzene has known to cause cancer such as leukemia, lymphosarcoma, lung, liver, stomach, esophagus, nasopharynx and intestine cancers (Mehlman, 2002). Similar to the present study which was observed by acrylamide treatment, previous studies have demonstrated the induction of rabbit liver CYP2E1 by benzene treatment (Arınç *et al.,* 1991). Another example for the induction of liver and kidney CYP2E1 enzyme and its associated NDMA N-demethylase, *p*-nitrophenol hydroxylase and aniline 4-hydroxylase activities was the administration of rabbits with pyridine which is one of the carcinogenic substrates of CYP2E1. The study conducted by Arınç *et al.* (2000a) have demonstrated that *in vivo* administration of pyridine resulted in increase in the activity of CYP2E1 enzyme and its associated NDMA N-demethylase, *p*-Nitrophenol hydroxylase and aniline 4-hydroxylase enzyme activities. Not only benzene and pyridine but also CYP2E1 metabolism of *p*-Nitrophenol, NDMA and aniline has been also associated with cancer. The formation of catechols from *p*-Nitrophenol has been implicated in the carcinogenic activity (Billing, 1985). *p*-Aminophenol and a product of 4-hydroxylation of aniline have been shown to be nephrotoxic (Gartland *et al.,* 1989, 1990), and NDMA, a procarcinogen, after bioactivation by NDMA N-demethylase has been shown to be carcinogenic in various tissues including kidney. Thus, the bioactivation of procarcinogens, carcinogens and toxic chemicals to its more toxic metabolites via CYP2E1 metabolism demonstrates the involvement of CYP2E1 enzyme in cancer. Thus, the present study results also suggested that acrylamide carcinogenicity may be due to the conversion to its epoxide glycidamide by CYP2E1 metabolism.

The induction of CYP2E1 due to acrylamide exposure leads to undesirable imbalance between rates of "toxification" versus "detoxification". Firstly, the induction of CYP2E1 enzyme due to acrylamide exposure can result in further formation of epoxide glycidamide. Glycidamide is a mutagenic agent which can easily bind to DNA and hemoglobin, which in turn forms DNA/hemoglobin adducts and leads to organ toxicity, hepatotoxicity and cancer. (Adler *et al.,* 2000; Yousef and El-Demerdash, 2006). Furthermore, exposure to chemicals that stimulates the activity of CYP2E1 could increase the rate of acrylamide metabolism and formation of epoxide glycidamide. Besides the chemicals stimulating the CYP2E1 enzyme activity, some pathophysiological conditions such as diabetes, and obesity, also increase the level of CYP2E1 (Raucy *et al.,* 1990; Shimojo *et al.,* 1993; Yang and Hong, 1995; Arınç *et al.*, 2005, 2007) which leads to further metabolism of acrylamide by liver or kidney CYP2E1. Moreover, induction of CYP2E1 by acrylamide can further potentiate the toxic and/or carcinogenic effects of the xenobiotics metabolized by CYP2E1 (such as benzene, pyridine, *p*-nitrophenol, NDMA and aniline), through their induction metabolism by CYP2E1.

During the course of CYP catalytic cycle, O_2 is used leading to the production of H_2O_2 and superoxide anion radical. Another reason for the acrylamide carcinogenicity due to CYP2E1 metabolism can be the formation of reactive oxygen species; since, CYP2E1 is the most active cytochrome P450 in this process. In addition, CYP2E1 metabolism of a number of substrate is known to lead to increased reactive oxygen species thereby oxidative stress (Gonzalez, 2005). Even in the absence of a substrate CYP2E1 expression can generate ROS. CYP2E1 also involved in the kidney toxicity due to oxidative stress. In this regards, the induction of CYP2E1 by acrylamide exposure can lead to further formation of reactive oxygen species which leads to damaged mitochondria, DNA modification, lipid peroxidation and cell death and cancer.

Since CYP2E1 gene shows several genetic polymorphisms, CYP2E1 enzyme is also associated with cancer (Lieber 1997; Bolt *et al.*, 2003, Ulusoy *et al.,* 2007a, b). Thus, these genetic polymorphisms in CYP2E1 gene may alter the activities or expression levels of this enzyme. Several case-control studies have described the influence of these polymorphisms with increased risk for various cancer types in different populations (El Zein *et al.*, 1997; Wu *et al.,* 1998; Farker *et al.*, 1998; Liu *et*

al., 2001). As previously mentioned acrylamide carcinogenicity is associated with its metabolism by CYP2E1. In addition, in the present study, it was demonstrated that acrylamide is an inducer of CYP2E1 enzyme in both activity and protein level in rabbit liver and kidney. Thus, the polymorphisms in CYP2E1 gene may alter the expression of CYP2E1, and further metabolism of acrylamide with induced CYP2E1 leads to formation of epoxide glycidamide which in turn resulted in acrylamide induced toxicity and cancer.

In the present study, although *in vivo* acrylamide administration induced the CYP2E1 enzyme in rabbit liver and kidney for both activity and enzyme level; no change was observed in lung CYP2E1 enzyme activity. Thus, the present study result demonstrated the existence of tissue specifity. As in the present study, previous studies of *in vivo* benzene treatment of rabbits demonstrated significant increase in liver microsomes whereas no induction was observed in lung microsomes (Arınç, 1991). In addition, in the study conducted by Arınç *et al.* (2000), although a significant increase in rabbit lung microsomal NDMA N-demethylase activity by *in vivo* pyridine treatment was observed (5.2 fold); no significant change was observed in lung microsomal CYP2E1 associated *p*-nitrophenol and aniline 4-hydroxylase activities.

A recently important flavonoid, found mainly grapes, peanuts, mulberries and redwine, with its well-known anti-oxidant and cancer chemoprevention effects is resveratrol (Soleas *et al.*, 1997; Sanders *et al.*, 2000; Fauconneau *et al.,* 1997, Sengottuvelan *et al.*, 2006; Schneider *et al.*, 2000). Thus, in the present study the protective effects of resveratrol on acrylamide-induced toxicity were also investigated. Rabbits were administered with resveratrol intragastrically at a dose of 25 mg/kg b.w on days 1, 5 and 8 (presented as "RESV" group). The combined effects of acrylamide and resveratrol were also investigated in two groups. In "RESV+AA" group, rabbits were administered with resveratrol intragastrically at a dose of 25 mg/kg b.w at days 1, 5 and 8; and then 6 hours following administration of resveratrol, rabbits were injected with acrylamide subcutaneously (s.c.) at a dose of 100 mg/kg b.w on days 1, 5 and 8. In "AA+RESV" group, rabbits were injected with acrylamide at a dose of 100 mg/kg b.w on days 1, 5 and 8; and then 6 hours following last injection of acrylamide, rabbits were administered with a single dose of 100 mg/kg b.w. resveratrol intragastrically on day 8.

According to the results, CYP2E1 enzyme activities measured by aniline 4-hydroxylase, *p*-nitrophenol hydroxylase and NDMA N-demethylase reactions were resulted in no statistically significant change in "RESV" group rabbit liver microsomes with respect to controls. In "RESV+AA" group rabbit liver microsomes CYP2E1 enzyme activities measured by aniline 4-hydroxylase activity, *p*-nitrophenol hydroxylase activity and NDMA N-demethylase activity were decreased significantly 1.51 fold ($p<0.05$), 1.43 fold ($p<0.05$) and 1.80 fold ($p<0.05$), repectively compared to "AA" group. In addition, the CYP2E1 associated enzyme activities measured by aniline 4-hydroxylase, *p*-nitrophenol hydroxylase and NDMA N-demethylase reactions were significantly decreased (1.48 fold ($p<0.05$), 1.66 fold ($p<0.05$) and 1.6 fold ($p<0.05$), respectively) in "AA+RESV" group rabbit liver microsomes compared to "AA" group. The western blot analysis of liver microsomes also confirmed these results that no significant change was observed in CYP2E1 protein level of "RESV" group compared to controls. According to western blot analysis including all "AA", "RESV+AA" and "AA+RESV" groups, results demonstrated that the liver CYP2E1 protein level of "AA" group increased 1.50 fold ($p<0.01$) significantly with respect to controls. In addition, the liver CYP2E1 protein level of "RESV+AA" (1.74 fold, $p<0.05$) and "AA+RESV" groups (1.74 fold, $p<0.05$) were decreased significantly with respect to "AA" group.

The protective effect of resveratrol on the acrylamide induced toxicity was also determined in rabbit kidney. No statistically significant change was observed in rabbit kidney microsomal CYP2E1 enzyme activity in "RESV" group compared to controls for both aniline 4-hydroxylase and *p*-nitrophenol hydroxylase reactions. However; in contrast to liver microsomal CYP2E1 enzyme activity and protein level; no change was observed in "RESV+AA" and "AA+RESV" groups in rabbit kidney microsomal CYP2E1 enzyme activity with respect to "AA" group for both aniline 4-hydroxylase and *p*-nitrophenol hydroxylase reactions. Furthermore, no significant change was observed in kidney CYP2E1 protein levels determined by western blot analysis of "RESV" group. According to western blot analysis investigating controls, "AA", "RESV+AA" and "AA+RESV" groups together demonstrated that the kidney microsomal CYP2E1 protein levels of "AA" group increased 2.42 fold ($p<0.001$) significantly with respect to controls. In addition, there was statistically significant decrease in kidney microsomal CYP2E1 protein levels of "RESV+AA" (2.53 fold,

$p<0.001$) and "AA+RESV" groups (2.00 fold, $p<0.01$) with respect to "AA" group; although no significant decrease was observed in CYP2E1 activity in rabbit kidney by resveratrol treatment.

According to the present study results, it can be suggested that resveratrol may have inhibitory effects on acrylamide induced toxicity in liver microsomal CYP2E1 enzyme activity and protein levels. However, the same result cannot be concluded for rabbit kidney microsomal CYP2E1 enzyme activity and protein level. Phenolic compounds, like resveratrol have known to be important in cancer chemoprevention in several ways. One way to introduce their chemoprevention effects is their modulatory effects on CYPs. Thus, one target of chemoprevention effect of resveratrol could be the inhibition of cytochrome P450s (CYPs) that leads to toxication processes. Several *in vitro* studies have demonstrated that resveratrol have inhibitory effect on CYP2E1 enzyme activity in mouse liver (Mikstacka *et al.,* 2002), in rat and human liver microsomes (Piver *et al.*, 2001). These studies suggested that, CYP2E1 modulation by resveratrol can dramatically affect (decrease) the toxicity and carcinogenesis of the toxic and carcinogenic compounds metabolized by CYP2E1. According to the results of the present study, the inhibition of CYP2E1 enzyme by resveratrol may cease the conversion of acrylamide to its more toxic metabolite epoxide glycidamide which is a mutagenic compound, and probably responsible for the mutagenicity and carcinogenicity of acrylamide (Adler *et al.,* 2000; Yousef and El-Demerdash, 2006) in rabbit liver. Thus, inhibition of CYP2E1 by resveratrol may lead to less formation of glycidamide and protects acrylamide induced carcinogenesis.

In contrast to *in vitro* studies, an *in vivo* study conducted by Hebbar *et al.* (2005) suggested that rat hepatic microsomal CYP2E1 activity was unchanged by *in vivo* resveratrol treatment. According to present study results, although prevention effect of resveratrol may be suggested on acrylamide induced CYP2E1 enzyme activity and protein level for rabbit liver; it did not change the acrylamide-induced CYP2E1 enzyme activity of rabbit kidney. Thus, this result also demonstrates the existence of tissue specifity. In addition, although *in vitro* studies have demonstrated the inhibition of CYP2E1 by resveratrol (Mikstacka *et al.,* 2002; Piver *et al.*, 2001), there is inconsistency with the *in vitro* and *in vivo* studies (Hebbar *et al.,* 2005). To understand the effects of resveratrol on acrylamide induced toxicity and CYP2E1

enzyme, *in vivo* studies are crucial. Thus, further *in vivo* studies are required to examine the inhibitory effects of resveratrol on CYP2E1 enzyme activity and also its protective effect on acrylamide induced toxicity.

As previously mentioned, among the P450s, the most active enzyme in the process of generating reactive oxygen species is CYP2E1. Thus, it is logical in this study to investigate the AST and ALT activities in rabbit serum in order to examine whether there is tissue damage due to chemical induced toxicity and metabolism of acrylamide by CYP2E1. According to the present study results, no statistically significant change was observed in the measured ALT and AST activities of "AA" and "RESV" groups with respect to controls. In addition, no statistically significant change was observed in the "AA+RESV" and "RESV+AA" groups with respect to "AA" group. However, the results of the present study demonstrated that no activity change in ALT and AST by acrylamide administration in these dose regimens. This can be interpreted as anti-oxidant defense system of rabbits may overcome this oxidative damage. In addition, it is also known that, besides the CYP2E1 metabolism of acrylamide, another pathway in the metabolism of acrylamide is the acrylamide conjugation with GSH. Thus, no change in the ALT and AST activities may be due to this second metabolism.

In the present study, we have also investigated whether acrylamide, resveratrol and their combined treatments stimulate microsomal CYP3A6 dependent drug metabolizing enzyme activity, erythromycin N-demethylase and its protein level in rabbit liver for the first time. According to the results, rabbit liver CYP3A6 enzyme activity was resulted in 1.85 fold ($p<0.01$) significant increase in "AA" group with respect to controls. There was no statistically significant change in rabbit liver CYP3A6 activity of "RESV" group with respect to controls. In addition, the rabbit liver CYP3A6 enzyme activities of "RESV+AA" and "AA+RESV" groups were resulted in 2.17 fold ($p<0.01$) and 1.85 fold ($p<0.01$) significant decrease, respectively compared to "AA" group. Furthermore, western blot analysis of liver microsomes were demonstrated that the liver CYP3A6 protein level of "AA" group was found to be significantly increased (1.69 fold, $p<0.05$) compared to controls. Secondly, no statistically significant change was observed in CYP3A6 protein levels of "RESV" group with respect to controls. The effects of acrylamide, resveratrol and

their combined treatments on CYP3A6 associated erythromycin N-demethylase activities and CYP3A6 protein levels were summarized in Table 4.3.

Table 4.3 The effects of acrylamide and resveratrol treatment on CYP3A6 associated enzyme activities of rabbit liver microsomes

	Erythromycin N-demethylase (nmol HCHO/min/mg protein)	Change (Fold)		CYP3A6 protein levels (RPA)	Change (Fold)
Control	0.20±0.03	-	Control	360.2±43	
AA	0.37±0.04	**2.17 X** $\uparrow^{a**}$	AA	611±82	**1.69 X** $\uparrow^{a*}$
RESV	0.22±0.03	-	Control	1107±89	
RESV+AA	0.17±0.02	**2.17 X** $\downarrow^{c**}$	RESV	1127±80	-
AA+RESV	0.20±0.02	**1.85 X** $\downarrow^{c**}$			

a*: significant with respect to controls, $p<0.05$

a**: significant with respect to controls, $p<0.01$

c**: significant with respect to "AA" group, $p<0.01$

To our best knowledge, the present study was the first study demonstrating the induction of CYP3A6 enzyme by administration of acrylamide in rabbit liver. The CYP3A6 isoform in rabbits has similar P450 predominance and substrate specifity as human isoform of CYP3A4 (Guengerich, 1997; Chirulli *et al.,* 2005; Weber *et al.,* 2001). It is well-known that CYP3A4 mainly participates in drug metabolism and approximately 50% of the marketed drugs are metabolized by CYP3A4 in humans. In addition, it is the most abundantly expressed CYP isozymes in liver and gastrointestinal tract (40% and 80% of total P450 content, respectively) (Cupp and Tracy, 1998; Nebert and Russel, 2002; Lamba *et al.,* 2002; Xie *et al.,* 2004). Thus, suppression or induction of this enzyme leads to clinical drug toxicity or alteration of drug clearance. According to the present study results, it was demonstrated that acrylamide induces CYP3A6 (CYP3A4 in humans) enzyme activity and protein level in rabbit liver; thus, the ingestion of acrylamide containing foods with drugs metabolized by CYP3A4 may alter drug clearance.

CYP3A4 is the first defense of the body to limit drug entry into the general circulation. However, inactivators of CYP3A4 may potentially modulate the intestinal absorption of CYP3A4 substrates by inhibiting their intestinal CYP3A4-dependent metabolism. Resveratrol has been studied on CYP3A4 in several *in vitro* studies to investigate whether it is such an inactivator of CYP3A4. These studies, performed on human microsomes (Chang *et al.,* 2000; Chan and Delucchi, 2000) and both in human and rat microsomes (Piver *et al.,* 2001), have demonstrated that resveratrol has inhibitory effects on CYP3A4 enzyme activity. According to the present study results, it was demonstrated that resveratrol may have protective effect on acrylamide-induced CYP3A6 (CYP3A4 in humans) enzyme in activity level in rabbit liver. Regarding this data, it can be suggested that ingestion of acrylamide with dietary flavonoid resveratrol may return the inducer effect of acrylamide on CYP3A4 activity. However, further *in vivo* studies should be performed to demonstrate the inhibitory effects of resveratrol on CYP3A4 enzyme activity.

In the present study, among CYPs, *in vivo* effects of acrylamide and resveratrol were also investigated on CYP2B4 associated benzphetamine N-demethylase anzyme activity. No significant change was observed in "RESV" with respect to controls and in "RESV+AA" and "AA+RESV" groups with respect to "AA" in liver. In addition, there was no statistically significant change in rabbit kidney microsomal CYP2B4 enzyme activity in "AA" group with respect to controls and in "RESV+AA" and "AA+RESV" with respect to "AA" group. For lung microsomal CYP2B4 enzyme activities of rabbit lung microsomes; there was no statistically significant change in "AA" or "RESV" groups with respect to controls and "RESV+AA" and "AA+RESV" groups with respect to "AA" group. The CYP2B4 enzyme activities of "AA" group of liver and "RESV" group of kidney were increased significantly compared to controls (1.19 fold, $p<0.05$; 1.52, $p<0.05$; respectively). However, this fold increases were not considered as important elevations. It can be concluded that *in vivo* acrylamide administration has no effect on CYP2B4 enzyme activity in rabbit liver, lung and kidney in this dose regimens. In addition, *in vivo* resveratrol administration has also no effect on CYP2B4 enzyme activity in rabbit liver, lung and kidney in these dose regimens.

In addition to cytochrome P450s, another important cytochrome P450 independent Phase I enzyme is NQO1. In the present study the *in vivo* effects of

acrylamide and resveratrol were also determined on cytosolic NQO1 activities of rabbit liver, kidney and lung. According to the results, no statistically significant change was observed in rabbit liver cytosolic NQO1 activities of "AA" and "RESV" groups with respect to controls and rabbit liver cytosolic NQO1 activities of "RESV+AA" and "AA+RESV" groups with respect to "AA" group. Kidney cytosolic NQO1 enzyme activity increased 1.64 fold ($p<0.01$) significantly in "AA" group with respect to controls. No statistically significant change was observed in kidney cytosolic NQO1 activity of "RESV" group with respect to controls and kidney cytosolic NQO1 activities of "RESV" and "AA+RESV" groups compared to "AA" group. NQO1 enzyme activity was also determined in rabbit lung cytosols. According to results, no statistically significant change was observed in rabbit lung cytosolic NQO1 enzyme activities of "AA" or "RESV" groups with respect to controls and in lung cytosolic NQO1 activities of "RESV+AA" and "AA+RESV" groups compared to "AA" group.

Although NQO1 generally categorized as a detoxification enzyme and an anti-oxidant enzyme, it also participates in the bioactivation of chemically reactive metabolites. They reduce quinones to hydroquinones bypassing the potentially toxic semiquinone radical intermediates (Lind *et al.,* 1982) and some redox-labile hydroquinones generated by NQO1 can react with molecular oxygen to form semiquinones and generate reactive oxygen species (Cadenas, 1995). According to the present study results, it can be concluded that *in vivo* administration of acrylamide has no effect in rabbit liver and lung cytosolic NQO1 activities. The only effect of acrylamide was observed in rabbit kidney cytosolic NQO1 activity. Furthermore, resveratrol, as an anti-oxidant phenolic compound, did not change the cytosolic NQO1 enzyme activity and demonstrate no protective effect in rabbit liver, kidney and lung. Another important feature of this enzyme is that high amounts of this enzyme found in lung, liver, breast and colon tumors (Schlager and Powis, 1990; Malkinson, 1992; Cresteil and Jaiswal, 1991, Jarrett *et al.,* 1998). Its ability to reduce quinone-containing alkylating agents, which are used in cancer chemoprevention, and its high amounts in tumor tissues increase the attention towards this enzyme in cancer treatment. Thus, induction of this enzyme in tumor tissues is important in the cancer treatment, for instance induction of this enzyme by anti-oxidant compounds

such as resveratrol. However, in the present study, no *in vivo* effect of resveratrol was observed on this enzyme in rabbit liver, lung and kidney in these dose regimens.

It is well-known that Phase II enzymes participate in the detoxification process of most carcinogens and procarcinogens. In the present study, *in vivo* effects of acrylamide and resveratrol were investigated on rabbit liver, kidney and lung cytosolic total GST and GST-Mu enzyme activities which are Phase II enzymes.

The results demonstrated that no significant change was observed in liver cytosolic total GST activities of "AA" or "RESV" groups with respect to controls and rabbit liver cytosolic total GST activities of "RESV+AA" and "AA+RESV" groups compared to "AA" group. In addition, the GST-Mu activities of rabbit liver cytosols were resulted in no significant change in "AA" or "RESV" groups compared to controls and in "RESV+AA" and "AA+RESV" groups with respect to "AA" group.

According to the results, there was no change in kidney cytosolic total GST activities of "AA" group with respect to controls and in "RESV+AA" and "AA+RESV" groups with respect to "AA" group. However, kidney cytosolic total GST activities of "RESV" group was increased 1.50 fold ($p<0.05$) significantly with respect to controls. The GST-Mu activities of kidney cytosols were also determined. No significant change was observed in "AA" group in kidney cytosolic GST-Mu activity with respect to controls and in RESV+AA" and "AA+RESV" groups with respect to "AA" group. Kidney cytosolic GST-Mu activity of "RESV" group increased 1.50 fold ($p<0.05$) significantly with respect to controls.

Finally, in the present study, the total GST and GST-Mu activities were determined on rabbit lung cytosols. According to the results, there was no significant change in rabbit cytosolic total GST and GST-Mu activities of "AA" or "RESV" groups with respect to controls. In addition, there was also no statistically significant change in rabbit lung cytosolic total GST and GST-Mu activities of "RESV+AA" and "AA+RESV" groups with respect to "AA" group. Only significant change was observed in total GST activity of "RESV" group with respect to controls (1.6 fold, $p< 0.05$).

Although major route for acrylamide metabolism is the conversion of acrylamide to its epoxide glycidamide by CYP2E1, another route for acrylamide metabolism is the conjugation of acrylamide with glutathione (Sumner *et al.,* 1997,

1999; Ghanayem *et al.,* 2005a, Kruyebashi and Ohno, 2006). In addition, the glutathione has been suggested as the major scavenger of acrylamide *in vivo* (Tong *et al.,* 2004; Kurebayashi and Ohno, 2006). Furthermore, some environmental and dietary carcinogens are genotoxic only after activation *in vivo*. Since; cytochrome P450s are the most efficient enzymes of carcinogen activation, cytochrome P450 catalyzed oxidations are also frequently followed by conjugation with GSH via GST, and other detoxifying enzymes. Thus, among the Phase II enzymes, known as detoxification enzymes, the activation of GSTs expected to be decreased by acrylamide exposure *in vivo*. In the present study, it was found that *in vivo* acrylamide administration did not altered neither total GST or GST-Mu activity in rabbit liver, kidney and lung cytosols in this dose regimens.

As previously mentioned, one important route of phenolic compounds to implement their chemopreventive effect is the modulation of enzymes. Thus, they can increase the activities of detoxification enzymes and lead to detoxification of compounds which are toxic to humans. Resveratrol is an anti-oxidant agent and protective agent against cancer. Studies have been demonstrated that resveratrol has inducer effect on Phase II metabolizing enzymes (Hebbar *et al.,* 2005). In an *in vivo* study performed on rats, it was demonstrated that high doses of resveratrol is required to induce total GST activity in liver (Hebbar *et al.,* 2005). In the present study, both total GST and GST-Mu activities were increased by resveratrol administration in rabbit kidney, and total GST activity in lung whereas no significant difference was observed in liver total GST and GST-Mu activities and lung cytosolic GST-Mu activity in these dose regimens. In addition, no protective effect of resveratrol was observed on total GST and GST-Mu activities in rabbit liver, kidney and lung. It should be noted that studies have been suggested that resveratrol has low bioavailability *in vivo* and whether dietary resveratrol will reach the desirable sites of action is still a matter of debate (Mikstacka *et al.* 2007; Walle *et al.,* 2004). Thus, the present study results also suggest that further *in vivo* studies are required to demonstrate anti-oxidant and anti-cancer activity of resveratrol *in vivo*.

In conclusion, in the present study *in vivo* effects of carcinogenic acrylamide was investigated on rabbit liver, lung and kidney microsomal CYP2E1 enzyme activities and protein levels for the first time and it was demonstrated that acrylamide increases the CYP2E1 enzyme activity and protein levels in rabbit liver and kidney

microsomes. Besides, *in vivo* interaction of carcinogenic acrylamide was investigated on rabbit liver CYP3A6 for the first time and it was also demonstrated that acrylamide increases the CYP3A6 enzyme activity and protein level in rabbit liver. The *in vivo* interaction of resveratrol and its protective effects on acrylamide induced toxicity were investigated and it can be suggested that resveratrol may have a protective effect on acrylamide induced CYP2E1 enzyme activity and protein level in rabbit liver. In addition, resveratrol may have a protective effect on acrylamide induced CYP2E1 protein level whereas it did not demonstrated in rabbit kidney CYP2E1 protein level. The protective effect of resveratrol may be suggested in liver CYP3A6 enzyme activity. In the present study the effects of acrylamide and resveratrol also investigated on NQO1, total GST and GST-Mu enzymes in liver, kidney and lung cytosols. In these dose regimens, no *in vivo* effects of acrylamide and resveratrol were observed on cytosolic rabbit liver, kidney and lung NQO1, total GST and GST-Mu enzymes in the present study, so further studies are needed to clarify this issue.

CHAPTER 5

CONCLUSION

To sum up, in the present study, *in vivo* effects of acrylamide and resveratrol and their combined effects were explored on rabbit liver, kidney and lung microsomal cytochrome P450 dependent drug metabolizing enzyme activities (CYP2E1, CYP3A6 in liver and CYP2B4), and protein levels (CYP2E1 in liver and kidney and CYP3A4 in liver) for the first time. In addition, *in vivo* effects of acrylamide and resveratrol and their combined effects were investigated on NQO1 enzyme activites, on biomarkers used to measure chemical toxicity including aspartate aminotransferase (AST) and alanine Aminotransferase (ALT) and on some oxidative stress enzymes including Glutathione-S-Transferase enzymes (GSTs).

In the present study, rabbits were treated with acrylamide and resveratrol experimentally with different regimens. Rabbits were injected with 100 mg/kg b.w. acrylamide subcutaneously ("AA" group) at day 1, 5 and 8. Resveratrol was administered to rabbits as 25 mg/kg b.w. ("RESV" group) intragastrically at day 1, 5 and 8. The combined effects of acrylamide and resveratrol also investigated in the present study. In one group ("RESV+AA" group), 25 mg/kg b.w. resveratrol was administered to rabbits i.g. at day 1,5 and 8; at the same days 6 hours following resveratrol administration 100 mg/kg b.w. acrylamide was injected s.c to rabbits. In the last group ("AA+RESV" group), 100 mg/kg b.w. acrylamide was injected s.c. at day 1,5 and 8; and at day 8, 6 hours following last injection of acrylamide single dose (100 mg/kg b.w) resveratrol was administered to rabbits. The fifth group constituted control group of rabbits.

CYP2E1 dependent aniline 4-hydroxylase, *p*-nitrophenol hydroxylase and NDMA N-demethylase were increased 1.79, 3.16 and 2.59 fold, respectively in "AA" group in rabbit liver microsomes with respect to controls. These were parralled with a smilar increase (2.06-fold) in liver CYP2E1 protein level as determined by immunoblot analysis. Similarly, CYP2E1 dependent aniline 4-hydroxylase and *p*-

nitrophenol hydroxylase activities of rabbit kidney microsomes were increased 1.6-fold and 1.56-fold in “AA”which is confirmed by immunublot analysis resulted in 1.94 fold increase in CYP2E1 protein level of rabbit kidney microsomes compared to controls. In contrast to liver and kidney microsomal CYP2E1 activities, no significant change was observed in lung microsomal CYP2E1 activities determined by *p*-nitrophenol hydroxylase and NDMA N-demethylase by *in vivo* acrylamide treatment.

The combined effects of acrylamide and resveratrol were also investigated in the present study. In “RESV+AA” group the CYP2E1 dependent aniline 4-hydroxylase, *p*-nitrophenol hydroxylase and NDMA N-demethylase were decreased 1.51, 1.42 and 1.46-fold significantly in rabbit liver with respect to “AA” group. In “AA+RESV” group 0.82, 1.67, and 1.54-fold significant decrease were observed in CYP2E1 dependent aniline 4-hydroxylase, *p*-nitrophenol hydroxylase and NDMA N-demethylase in rabbit liver with respect to “AA” group. These results also confirmed by immunoblot analysis that 1.74-fold decrease was observed in both “RESV+AA” and “AA+RESV” groups with respect to “AA” group in rabbit liver. In kidney microsomes, no statistically significant decrease was observed in CYP2E1 dependent aniline 4-hydroxylase and *p*-nitrophenol hydroxylase activites in “RESV+AA” and “AA+RESV” groups with respect to “AA” group; however, 2.5-fold and 2.0-fold significant decreases were observed in immunoblot analysis for “RESV+AA” and “AA+RESV” groups, respectively.

For lung microsomal CYP2E1 activities determined by *p*-nitrophenol hydroxylase and NDMA N-demethylase reactions, no significant change was observed in “RESV+AA” and “AA+RESV” groups compared to “AA” group.

In the present study, another cytochrome P450 dependent drug metabolizing enzyme investigated was CYP2B4 isozyme. The CYP2B4 associated benzphetamine N-demethyase activity of rabbit liver, kidney and lung micromsomes were investigated in all groups. Liver microsomal CYP2B4 of “AA” group was found to be significantly increased compared to control groups; however, the same result was not obtained for kidney and lung CYP2B4 enzyme actvity. In addition, no significant changes was observed in “RESV” groups compared to controls and “RESV+AA”/“AA+RESV” groups compared to “AA” group in rabbit liver, kidney and lung microsomal benzphetamine N-demethylase enzyme activities.

In addition to CYP2E1 and CYP2B4 isozymes, the effects of acrylamide and resveratrol treatments was also established on CYP3A6 isozyme of CYP in liver. CYP3A6 associated erythromycin N-demethylase enzyme activity of rabbit liver microsomes were increased significantly (1.85-fold) in “AA” group with respect to controls. The CYP3A6 protein level of rabbit liver microsomes were demonstrated to be increased (1.69-fold) by immunoblot analysis. The resveratrol treatment (“RESV” group), resulted in no statistically significant change in CYP3A6 associated erythromycin N-demethylase enzyme activity as confirmed by immunoblot analysis. Moreover, In “AA+RESV” group 2.18-fold significant decrease and) in “AA+RESV” group 1.85-fold decrease were observed in CYP3A6 dependent erythromycin N-demethylase activity in rabbit liver compared to “AA”.

In the present study, Aspartate aminotransferase (AST) and Alanine Aminotransferase (ALT) activites wich are biomarkers used to measure chemical toxicity including). In these dose regimens were also determined in all groups; however, no statistically significant difference was observed in the activities of these biomarkers in rabbit serum.

We also examined the effects of acrylamide and resveratrol and also their combined effects on some oxidative enzymes including Total GST, Mu-GST and NQO1 activities in rabbit liver, kidney and lung cytosols. Accept kidney cytosolic Total GST and GST-Mu activities, no significant change was observed in “AA” and “RESV” group with respect to controls and “RESV+AA” and “AA+RESV” group with respect to “AA” group in Total GST and GST-Mu activities of rabbit liver, lung and kidney cytosols. The Total GST activity and GST-Mu activities of “RESV” group were increased significantly (1.53 and 1.56-fold, respectively) with respect to controls in kidney cytosols. Moreover, the NQO1 activities of rabbit liver, kidney and lung cytosols were not significantly change in “AA” and “RESV” group with respect to controls and “RESV+AA” and “AA+RESV” group with respect to “AA” group in these dose regimens, except NQO1 enzyme activity of “AA” group of kidney were increased significantly (1.64-fold) with respect to controls.

In conclusion, the most prominent feature of the present study is the demonstration of induction of liver CYP2E1 both in activity and protein level enzyme by the *in vivo* administration of acrylamide in rabbit liver and kidney microsomes, for the first time. Studies have demonstrated that acrylamide is

metabolized to its epoxide glycidamide by liver CYP2E1 and carcinogenicity associated with acrylamide is mostly attributed to this metabolism. In addition, it should be noted that humans can easily exposed to acrylamide in their diet; since, acrylamide has found to be formed in heat-processed foods, specifically carbohydrate rich food cooked at higher temperatures. Thus, induction of CYP2E1 by acrylamide may lead to formation of epoxide glycidamide which is mutagenic, can bind to DNA easily and cause genetic damage and cancer. Furthermore, among the cytochrome P450s, it is well-known that CYP2E1 mainly participate in the bioactivation of several procarcinogen and carcinogens. Therefore, the induction of CYP2E1 by acrylamide can further stimulate the metabolic activation of other toxic compounds and procarcinogens metabolized by CYP2E1 enzyme which leads to the formation of reactive metabolites, reactive oxygen species. The formation of epoxide glycidamide and bioactivation of other reactive metabolites and reactive oxygen species by CYP2E1 may in turn further potentiate the risk of hepatotoxicity, organ toxicity and mutagenicity and carcinogenicity. Thus, all of these support the association of acrylamide toxicity with metabolism by CYP2E1. In addition, the present study results also demonstrated for the first time that *in vivo* acrylamide treatment induces rabbit liver microsomal CYP3A6 enzyme in both activity and protein level which may in turn lead to undesired drug-drug interactions, primarily drug clearence and clinical drug toxicity. Studies have been demonstrated that other route for acrylamide metabolism is its conjugation through GSH. According to the present study results, further *in vivo* studies required to demonstrate the effects of *in vivo* acrylamide on GSt enzyme activities to show whether they are major scavanger of acrylamide *in vivo*. Finally, according to the present study results, resveratrol may be suggested as a protective phenolic compound in acrylamide induced CYP2E1 toxicity in rabbit liver and kidney. However, further *in vivo* studies required to clearly define the effect of resveratrol on acrylamide induced toxicity and on anti-oxidant enzyme systems.

REFERENCES

Adalı, O, Arınç, E. 1990. Electrophoretic, spectral, catalytic and immunochemical properties of highly purified cytochrome P450 from sheep lung. *Int J Biochem* 22:1433-1444.

Adalı, O, Abu-Baker, T, Arınç, E. 1996. Immunochemical and sub-structural characterization of sheep lung cytochrome P450LgM2. *International Journal of Biochemistry and Cell Biology* 28:363-372.

Adler, ID, Baumgartner, A, Gonda, H, Friedman, MA, Skerhut, M. 2000. *Mutagenesis* 15: 133-136.

Anzenbacher, P, Anzenbacherová, E. 2001. Cytochromes P450 and metabolism of Xenobiotics. *Cell Mol Life Sci* 58:737-747.

Arınç, E, Adalı, O, Özkan-Gençler, AM. 2000a. Induction of N-Nitrosodimethylamine metabolism in liver and lung by in vivo pyridine treatment of rabbits. *Arch Toxicol* 74: 329-334.

Arınç, E., Adalı, O., Özkan-Gençler, AM. 2000b. Stimulation of aniline, p-nitrophenol and N-Nitrosodimethylamine metabolism in kidney by pyridine pretreatments of rabbits. *Arch Toxicol* 74: 527-532.

Arınç, E., Arslan, S., Adali, O. 2007. Differential effects of diabetes on CYP2E1 and CYP2B4 proteins and associated drug metabolizing enzyme activities in rabbit liver. *Arch Toxicol* 79: 427-433.

Arınç, E., Arslan, S., Bozcaarmutlu, A., Adali, O. 2007. Effects of diabetes on rabbit kidney and lung CYP2E1 and CYP2B4 expression and drug metabolism and potentiation of carcinogenic activity of N-nitrosodimethylamine in kidney and lung. *Food and Chemical Toxicology* 45: 107-118.

Arınç, E., Aydoğmus, A. 1990. Lung microsomal p-nitrophenol hydroxylase-Characterization and reconstitution of its activity. *Comp Biochem Physiol* 97: 455-460.

Arınç, E., Çelik, H. 2002. Biochemical Characteristics of Purified Beef Liver NADPH-Cytochrome P450 Reductase. *Journal of Biochemical and Molecular Toxicology* 16:286-297.

Arınç, E., İşcan, MV. 1983. Comperative studies of sheep liver and lung microsomal aniline 4-hydroxylase. *Comp Biochem Physiol* 74: 151-158.

Arinç, E. 1993. Extrahepatic microsomal forms: Lung microsomal cytochrome P450 isozymes. In *Handbook of experimental pharmacology, cytochrome P450* ed. Schenkman J.B. and Greim H., 373–386. New York: Springer, Berlin Heidelberg.

Arinç, E., Adalı, O., İşcan, M., Güray, T. 1991. Stimulatory effects of benzene on rabbit liver and kidney microsomal cytochrome P-450 drug metabolizing enzymes. *Archives of Toxicology* 65:186-190.

Arinç, E., Philpot, RM. 1976. Preparation and properties of partially purified pulmonary cytochrome P-450 from rabbits. *J Biol Chem* 25: 3213–3220

Arinç, E., Adalı, O. 1983. Solubilization and partial purification of two forms of cytochrome P-450 from trout liver microsomes. *Comparative Biochemistry and Physiology* 76:653-662.

Arınç, E., Bozcaarmutlu, A. 2003. Catalyzation of cocaine N-demethylation by cytochromes P4502B, P4503A, and P4502D in fish liver. *J Biochem Mol Toxicol* 17(3): 169-176.

Armstrong, RN. 1997. Structure, catalytic mechanism, and evolution of the Glutathione transferases. *Chem Res Toxicol* 10: 2-18.

Bergmark, E., Calleman, C. J., Costa L.G. 1991. Formation of hemoglobin adducts of acrylamide and its epoxide metabolite glycidamide in the rat. *Toxicol Appl Pharmacol* 111: 352-363.

Bergmark, E., Calleman, C.J., He, F., Costa, L.G. 199. Determination of hemoglobin adducts in humans occupationally exposed to acrylamide. *Toxicol Appl Pharmacol* 120: 45-54.

Bergmark, E. 1997. Hemoglobin adducts of acrylamide and acrylonitrile in laboratory workers, smokers and nonsmokers. *Chemical Reseach in Toxicology* 10:78-84.

Bertelli, A.A., Giovannini, L., Stradi, R., Bertelli, A., Tillement, J.P. 1996. Plasma, urine and tissue levels of trans- and cis-resveratrol (3,4',5-trihydroxystilbene) after short-term or prolonged administration of red wine to rats. *Int J Tissue React* 18: 67-71.

Besaratinia, A., Pfeifer, G.P. 2004. Genotoxicity of acrylamide and glycidamide. Journal of the National Cancer Institute 96: 1023-1029.

Billing, R.E. 1985. Mechanism of catechol formation from aromatic compounds in isolated rat hepatocytes. *Metab Disp* 13: 287-290.

Black, S.D., Coon, M.J. 1986. Comparative structures of p-450 cytochromes. In *Cytochrome P-450 structure, mechanism and biochemistry,* ed. Ortiz de Mantellano PR, 161-216. New York: plenum Press.

Black, S.D., Coon, M.J. 1982. Structural features of liver microsomal NADPH-cytochrome P450 reductase. *J Biol Chem* 257:5729-5738.

Boland, M.P., Knox, R.J., Roberts, J.J. 1991. The differences in kinetics of rat and human DT diaphorase result in a differential sensitivity of derived cell lines to CB 1954 (5-(aziridin-1-yl)-2,4-dinitrobenzamide). *Biochem Pharmacol* 41: 867-75.

Bolt, H.M. 2003. Genotoxicity—Threshold or not? Introduction of cases of industrial chemicals. *Toxicol Lett* 140–141:43–51.

Bolt, H.M., Hengstler, JG. 2008. A new series of review articles on drug metabolizing enzymes: nomenclature, of isozyme families, genetic organizations, ğpolymorphisms, substrate specifities, clinical relevance and role in carcinogenesis. *Arch Toxicol* 82: 413-414.

Bonfils, C., Combalbert, J., Pineau, T., Angevin, J., Larroque, C., Derancourt, J., Capony, J.P., Maurel, P. 1990. Ontogenesis of rabbit liver cytochrome P450. Evidence for a cytochrome P450-IIE (3a)-related form prevailing during the post-natal period. *Eur J Biochem* 188:187–194

Bozcaarmutlu A and Arinç E. Purification of CYP2B-like protein from feral leaping mullet (Liza saliens) liver microsomes and its biocatalytic, molecular, and

immunological characterization. Journal of Biochemical Molecular Toxicology. 2008; 22(4):284-98.

Bull, R.J., Robinson, M., Laurie, R.D., Stoner, G.D., Greisiger, E., Meier, J.R., Stober, J. 1984a. Carcinogenic effects of acrylamide in Sencar and A/J mice. *Cancer Res*; 44:107–111.

Bull, R.J., Robinson, M., Stober, J.A. 1984b. Carcinogenic activity of acrylamide in the skin and lung of Swiss-ICR mice. *Cancer Lett* 24: 209–212.

Cadenas, E. 1995. Antioxidant and prooxidant functions of DT-diaphorase in quinone metabolism. *Biochem Pharmacol* 49:127-140.

Calleman, C.J., Bergmark, E., Costa, L.G. 1990. Acrylamide is metabolized to glycidamide in the rat: evidence from hemoglobin adduct formation. *Chemical Research in Toxicology* 3: 406-12.

Calleman, C.J. 1996. The metabolism and pharmacokinetics of acrylamide: implications for mechanism of toxicity and human risk estimation. *Drug Metabolism Reviews* 28:527–590.

Chan, W.K., Delucchi, A.B. 2000. Resveratrol, a red wine constituent, is a mechanism based inactivator of cytochrome P450 3A4. *Life Sciences* 67: 3103-3112.

Chapin, R.E., Fail, P.A., George, J.D., Grizzle, T.B., Heindel, J.J., Harry, G.J., Collins, B.J., Teague, J. 1995. The reproductive and neural toxicities of acrylamide and three analogues in Swiss mice, evaluated using the continuous breeding protocol. *Fundam Appl Toxicol* 27: 9-24.

Cheng, D., Harris, D., Reed, J.R., Backes, W.L. 2007. Inhibition of CYP2B4 by 2-ethynylnaphthalene: evidence for the co-binding of substrate and inhibitor within the active site. *Arch Biochem Biophys* 468: 174-82.

Chirulli, V., Longo, V., Marini, S., Mazaccarro, A., Fiorio, R., Gervasi, P.G. 2005. CAR and PXR expression and inducibility of CYP2B and CYP3A activities in rat and rabbit lungs. *Life Sci* 76: 2535-2546.

Chun, Y.J., Kim, M.Y., Guengerich, F.P. 1999. Resveratrol is a selective human cytochrome P450 1A1 inhibitor. *Biochem Biophys Res Commun* 262: 20-4.

Ciolino, H.P., Yeh, G.C. 1999. Inhibition of aryl hydrocarbon-ınduced cytochrome p-450 1a1 enzyme activity and cyp1a1 expression by resveratrol. *Molecular Pharmacology* 56: 760–767.

Cochin, J., Axelrod, J. 1959. Biochemical and pharmacological changes in the rat following chronic administration of morphine, nalorphine, and normorphine. *Pharmacol Exp Ther* 125: 105-110.

Coles, B.F., Kadlubar, F.F. 2003. Detoxification of electrophillic compounds by glutathione S-transferase catalysis: Determinants of individual response to chemical carcinogens and chemotherapeutic drugs. *BioFactors* 17: 115-130.

Constant, J. 1997. Alcohol, ıschemic heart disease and French paradox. *Coronary Artery Dis* 8: 645-649.

Costa, L.G. 1996. Biomarker research in neurotoxicology: the role of mechanistic studies to bridge the gap between the laboratory and epidemiological investigations. *Environ. Health Perspect* 104: 55-67.

Cresteil, T., Jaiswal, A.K. 1991. High levels of expression of the NAD(P)H:quinone oxidoreductase (NQO1) gene in tumor cells compared to normal cells of the same origin. *Biochem Pharmacol* 42:1021-1027.

Cupp, M.J., Tracy, T. 1998. Cytochrome P450: New nomenclature and clinical applications. *Clin Pharmacol* 57: 107-116.

Danielson P.B. 2002. The cytochrome P450 superfamily: biochemistry, evolution and drug metabolism in humans. *Curr Drug Metab* 3: 561-597.

Ding, X., Koop, D.R., Crumb, B.L., Coon, M.J. 1986. Immunochemical identification of cytochrome P450 isozyme 3a (p450ALC) in rabbit nasal and kidney microsomes and evidence for differential induction by alcohol. *Mol pharmacol* 30: 370-378.

Ding, X., Pernecky, J.S., Coon, M.J. 1991. Purification and characterization of cytochrome P4502E2 from hepatic microsomes of neonatal rabbits. *Arch Biochem Biophys* 291: 270-276.

Doerge, D.R., Da Costa, G., McDaniel, L.P., Churchwell, M.I., Twaddle, N.C., Beland, F.A. 2005. DNA adducts derived from administration of acrylamide and glycidamide to mice and rats. *Mutation Research* 580: 131-141.

Dong, Z. 2003. Molecular mechanism of the chemopreventive effect of resveratrol. *Mutation Research* 523-524: 145-150.

Edwards, P.M., Parker, V.H. 1977. A simple, sensitive, and objective method for early assessment of acrylamide neuropathy in rats. *Toxicol Appl Pharmacol* 40: 589-591.

Edwards, R., Dixon, D.P. 2004. Metabolism of natural and xenobiotic substrates substrates by the plant glutathione S-transferase superfamily. In *Molecular Ecotoxicology of Plants*, ed. H Sandermann, 17-50. Heidelberg: Springer Verlag

Ekstrom, G., Ingelman-Sundberg, M. 1989. Rat liver microsomal NADPH-supported oxidase activity and lipid peroxidation dependent on ethanol-inducible cytochrome P-450 (P-450IIE1). *Biochem Pharmacol* 38: 1313–1319.

El-Zein, R., Zwischenbergher, J.B., Wood, T.G., Abdel Rahman, S.Z., Brekelbaum, C., Au, W.W. 1997. Combined genetic polymorphism and risk for development of lung cancer. *Mutat Res* 381: 189–200.

Ernster, L., Navazio, F. 1958. *Acta Chem Scand* 12: 595.

Ernster, L. 1967. DT-diaphorase. *Methods in Enzymology* 10: 309-317.

European Commission. Opinion of the Scientific Commitee on Food on New findings regarding the presence of acrylamide in food. Expressed on 3 July 2002. (http://ec.europa.eu/food/fs/sc/scf/out131_en.pdf) Last accessed date: 28. 12.2008

Exon, J.H. 2006. A review of the toxicology of acrylamide. *Journal of Toxicology and Enviromental Health* 9: 397-412.

Ey, P.L., Ashman, L.K. 1986. The use of alkaline phosphatase-conjugated anti-immunoglobulin with immunoblots for determining the specificity of monoclonal antibodies to protein mixtures. *Methods Enzymol* 121: 497-509.

Farker, K., Lehmann, M.H., Oelschlagel, B., Haerting, J., HoVmann, A., Janitzky, V., Schubert, J. 1998. Impact of CYP2E1 genotype in renal cell and urothelial cancer patients. *Exp Toxicol Pathol* 50:425–431.

Fauconneau, B., Waffo-Teguo, P., Huguet, F., Barrier, L., Decendit, A., Merillon, J.M. 1997. Comparative study of radical scavanger and anti-oxidant properties of phenolic compounds from visits finera cell cultures using in vitro tests. *Life Sci* 61: 2103-2110.

Field, E.A., Price, C.J., Sleet, R.B., Marr, M. C., Schwetz, B.A., Morissey, R.E. 1990. Developmental toxicity evaluation of acrylamide in rats and mice. *Fundam Appl Toxicol* 14: 502-512.

Frankel, E.N., Waterhouse, A.L., Kinsella, J.E. 1993. Inhibition of human LDL oxidation by resveratrol. *Lancet* 341: 1103-1104.

Friedman, M. 2003. Chemistry, biochemistry and safety of acrylamide. A review. *Journal of Agricultural and Food Chemistry* 51: 4504-4526.

Friedman, M.A., Duak, L.H., Stedha, M.A.A. 1995. Lifetime oncogeniciy sudy in rats with acrylamide. *Fundam Appl Toxicol* 27: 95-105.

Gamboa da Costa, G., Churchwell, M.I., Hamilton, L.P., Von Tungeln, L.S., Beland, F.A., Marques, M.M., Doerge, D.R. 2003. DNA adduct formation from acrylamide via conversion to glycidamide in adult and neonatal mice. *Chem Res Toxicol* 16: 1328-37.

Gartland, K.P.R., Bonner, F.W., Timbrell, J.A., Nicholson, J.K. 1989. The biochemical characterization of p-aminophenol-induced nephrotoxic lesions in the F344 rat. *Arch Toxicol* 63: 97–106.

Gartland, K.P.R., Eason, C.T., Bonner, F.W., Nicholson, J.K. 1990. Effects of biliary cannulation and buthionine sulphoximine pretreatment on the nephrotoxicity of para-aminophenol in the F344 rat. *Arch Toxicol* 64: 14–25.

Ghanayem, B.I., McDaniel, L.P., Churchwell, M.I., Twaddle, C.N., Snyder, R., Fennel, T.R., Doerge, D.R. 2005a. Role of CYP2E1 in the epoxidation of acrylamide to glycidamide and formation of DNA and hemoglobin adducts. *Toxicological Sciences* 88 (2): 311-318.

Ghanayem, B.I., Wang, H., Sumner, S.C.J. 2000. Using cytochrome P450 gene knock-out mice to study chemical metabolism, toxicity, and carcinogenicity. *Toxicol Pathol* 28: 839–850.

Ghanayem, B.I., Witt, K.L., El-Hadri, L., Hoffler, U., Kissling, G.E., Shelby, M.D., Bishop, J.B. 2005b. Comparison of germ cell mutagenecity in male CYP-2E1-null and wild-type mice treated with acrylamide: Evidence supporting a glycidamide–mediated effect. *Biology of Reproduction* 72: 157-163.

Goldberg, D.M. 1996 More on antioxidant activity of resveratrol in red wine. *Clinical Chemistry* 1:113-114.

Gonzalez, F.J., Kimura, S. 2003. Study of P450 function using gene knockout and transgenic mice. *Archives of Biochemistry and Biophysics* 409: 153-158.

Gonzalez FJ. Role of cytochromes P450 in chemical toxicity and oxidative stress: studies with CYP2E1. Mutation Research 2005; 569: 101-110.

Gorsky, L.D., Hollenberg, P.F. 1989. Metabolism of N-nitrosodimethyl- and N-nitrosodiethylamine by rat hepatocytes: effects of pre-treatment with ethanol. *Chemical Researsch in Toxicology* 2: 436-441.

Gorsky, L.D., Hollenberg, P.F. 1989. Metabolism of N-nitrosodimethyl- and N-nitrosodimethylamine by rat hepatocytes: Effects of pretreatment with ethanol. *Chem Res Toxicol* 2: 436-441.

Guengerich, F.P., Kim, D.H., Iwasaki, M. 1991. Role of human Cytochrome P450 IIE1 in the oxidation of many low molecular weight cancer suspects. *Chem Res Toxicol* 4: 168-179.

Guengerich, F.P., Shimada, T. 1998. Activation of procarcinogens by human CYP450 enzymes. *Mutat Res* 400:201-213.

Guengerich, F.P. 1997. Comparisons of catalytic selectivity of cytochrome P450 subfamily enzymes from different species. *Chem Biol Interact* 106: 161-182.

Gum, J.R., Strobel, H.W. 1981. Isolation of the membrane binding peptide of NADPH-cytochrome P450 reductase. *J Biol Chem* 256: 7478-7486.

Habig, W.H., Pabst, M.J., Jakoby, W.B. 1974. Glutathione S-transferases. The first enzymatic step in mercapturic acid formation. *J Biol Chem* 249: 7130-9.

Hagmar, L., Törnquvist, M., Nordander, C., Rosèn, I., Bruze, M., Kautiainen, A. 2001. Health effects of occupational exposure to acrylamide using hemoglobin adducts as biomarkers of internal dose. *Scand J Work Environ Health* 27: 219-226.

Hajos, A.K., Winston, G.W. 1991. Dinitropyrene nitroreductase activity of purified NAD(P)H-quinone oxidoreductase: role in rat liver cytosol and induction by Aroclor-1254 pretreatment. *Carcinogenesis* 12: 697-702.

Hanasono, G.K., Witschi, H., Plaa, G.L. 1975. Potentiation of the hepatotoxic responses to chemicals in alloxan diabetic rats. *Proc Soc Exp Biol Med* 149: 903–907.

Hayes, J.D., Flanagan, J.U., Jowsey, I.R. 2005. Glutathione Transferases. *Annu Rev Pharmacol Toxicol* 45: 51-88.

He, F.S., Zhang, S.L., Wang, H.L., Li, G., Zhang, Z.M., Li, F.L., Dong, X.M., Hu, F. 1989. Neurological and electroneuromyographic assessment of the adverse effects of acrylamide on occupationally exposed workers. *Scand J Work Environ Health* 15:125-129.

Hebbar, V., Shen, G., Hu, R., Kim, B., Chen, C., Korytko, P.J., Crowell, J.A., Levine, B.S., Kong, A.T. 2005. Toxicogenomics of resveratrol in rat liver. *Life Sciences* 76: 299-234.

Hodek, P., Trefil, P., Stiborova, M. 2002. Flavanoids-potent and versatile biologically active compounds interacting with Cytochromes P450. *Chem Biol Interact* 139: 1-21.

Hollmann, P.C., Katan, M.B. 1997. Absorption, metabolism and health effects of dieatry flavonoids in man. *Biomedical Pharmacotherapy* 51: 305-310.

Huang, M.T., Miwa, G.T., Cronheim, N., Lu, A.Y. 1979. Rat liver cytosolic azoreductase. Electron transport properties and the mechanism of dicumarol inhibition of the purified enzyme. *J Biol Chem* 254: 11223-7.

Hursting, S.D., Slaga, T.J., Fischer DiGiovanni, J., Phang, J.M. 1999. Mechanism-based cancer prevention approaches: Targets, examples, and the use of transgenic mice. *J Natl Cancer Inst* 91: 215–225.

Imai, Y., Ito, A., Sato, R. 1966. Evidence for biochemically different types of vesiclesin the hepatic microsomal fraction. *J Biochem* 60: 417-428.

Ingelman-Sundberg, M., Johansson, I., Yin, H., Terelius, Y., Eliasson, E., Clot, P., Albano, E. 1993. Ethanol-inducible cytochrome P4502E1: genetic polymorphism, regulation, and possible role in the etiology of alcohol-induced liver disease. *Alcohol* 10: 447-52.

International Agency for Research on cancer (IARC). 1994. Acrylamide. *IARC Monogr Eval Carcinog Risks Hum* 60: 389-433.

İşcan, M.Y., Arınç, E. 1988. Comparison of highly purified sheep liver and lung NADPH-cytochrome P450 reductases by the analysis of kinetic and catalytic properties. *Int J Biochem* 20: 1189-1196.

İşcan, M.Y., Arınç, E. 1986. Kinetic and structural properties of biocatalytically active sheep lung microsomal NADPH-cytochrome c reductase. *Int J Biochem* 18: 731-741.

Jaiswal, A.K. 2000. Regulation of genes encoding NAD(P)H:quinone oxidoreductases. *Free Radic Biol Med* 29: 254-62.

Jakobsson, P-J., Morgenstern, R., Mancini, J., Ford-Hutchinson, A., Persson, B. 1999. Common structural features of MAPEG—a widespread superfamily of membrane associated proteins with highly divergent functions in eicosanoid and glutathione metabolism. *Protein Sci* 8: 689–92.

Jang, M., Cai, L., Udeani, G.O., Slowing, K.W., Thomas, C.F., Beecher, C.W.W., Fong, H.H.S., Farnsworth, A.D., Kinghorn, A.D., Mehta, R.G., Moon, R.C., Pezzuto, J.M. 1997. *Science* 275: 218-220.

Jarrett, C.M., Bibby, M.C., Phillips, R.M. 1998. Bioreductive enzymology of malignant and normal human tissues. *Proc Am Assoc Cancer Res* 39: 429.

Johansson, I., Ingelman-Sundberg, M. 1998. Benzene metabolism by ethanol-inducible, acetone-inducible, and benzene-inducible cytochrome P-450 (IIE1) in rat and rabbit liver microsomes. *Cancer Res* 48: 5387–5390.

Johnson, K.A., Gorzinski, S.J., Bodner, K.M., Campbell, R.A., Wolf, C.H., Friedman, M.A., Mast, R.W. 1986. Chronic toxicity and oncogenicity study on acrylamide incorporated in the drinking water of Fischer 344 rats. *Toxicol Appl Pharmacol* 85: 154–168.

Keen J.H., Jakoby, W.B. 1978. Glutathione transferases. Catalysis of nucleophilic reactions of glutathione. *J Biol Chem* 253: 5654–57.

Kharasch, E.D., Thummel, K.E. 1993. Identification of cytochrome P450 2E1 as the predominant enzyme catalyzing human liver microsomal defluorination of sevoflurane, isoflurane, and methoxyflurane. *Anesthesiology* 79: 795–807.

Koop D.R., Casazza, J.P. 1985. Identification of ethanol-inducible P-450 isozyme 3a as the acetone and acetol monooxygenase of rabbit microsomes. *J Biol Chem* 260: 13607-13612.

Koop, D.R., Morgan, E.T., Tarr, G.E., Coon, M.J. 1982. Purificationa and characterization of a unique isozyme of cytochrome P-450from liver microsomes of ethanol treated rabbits. *J Biol Chem* 257: 8472-8480.

Kurebayashi, H. Ohno, Y. 2006. Metabolism of acrylamide to glycidamide and their cytotoxicity in isolated rat hepatocytes: protective effects of GSH precursors. *Arch Toxicol* 80: 820-828.

Ladner, J.E., Parsons, J.F., Rife, C.L., Gilliland, G.L., Armstrong, R.N. 2004. Parallel evolutionary pathways for glutathione transferases: structure and mechanism of the mitochondrial class Kappa enzyme rGSTK1-1. *Biochemistry* 43:352–61.

Laemmli, U.K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 227: 0-685.

Lamba, J.K., Lin, Y.S., Schuetz, E.G., Thummel, K.E. 2002. Genetic contribution to variable human CYP3A-mediated metabolism. *Adv Drug Deliv Rev* 54: 1271–1294.

Lehninger, A.L. 1975. *Biochemistry. The molecular basis of cell structure and function.* Second eds. New York: Worth Publisher.

Lewis, D.F., Lake, B.G. 1997. Molecular modelling of mammalian CYP2B isoforms and their interaction with substrates, inhibitors and redox partners. *Xenobiotica* 27: 443-78.

Li,Y., Jaiswal, A.K. 1992. Regulation of human NAD(P)H:quinone oxidoreductase gene. Role of AP1 binding site contained within human antioxidant response element. *J Biol Chem* 267:15097-15104

Lieber, C.S. 1997. Cytochrome P-4502E1: Its physiological and Pathological Role. *Biological Reviews* 77 (2): 517-544.

Lind, C., Cadenas, E., Hochstein, P., Ernster, L. 1990. DT-diaphorase: purification, properties, and function. *Methods in Enzymology* 186:287-301.

Lind, C., Hochstein, P., Ernster, L. 1982. DT-diaphorase as a quinone reductase: a cellular control device against semiquinone and superoxide radical formation. *Arch Biochem Biophys* 216: 178-185.

Liu, S., Park, J.Y., Schantz, S.P., Stern, J.C., Lazarus, P. 2001. Elucidation of CYP2E1 5_ regulatory RsaI/PstI allelic variants and their role in risk for oral cancer. *Oral Oncol* 37: 437–445.

Liu, Y., Hao, H., Liu, C., Wang, G., Xie, H. 2007. Drugs as Cyp3A probes, inducers and inhibitors. Drug Metabolism Reviews 39: 699-721.

Lopachin, R.M. 2004. The changing view of acrylamide neurotoxicity. *NeuroToxicology* 25: 617-630.

LoPachin, R.M. 2002. The role of fast axonal transport in acrylamide pathophysiology: Mechanism or epiphenomenon? *Neurotoxicology* 23: 253-257.

Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randal, R.J. 1951. Protein measurement with the Folin Phenol reagent. *J Biol Chem* 243: 1331-1332.

Lu, A.Y., Coon, M.J. 1968. Role of hemoprotein P-450 in fatty acid omega-hydroxylation in a soluble enzyme system from liver microsomes. *J Biol Chem* 243: 1331-1332.

Lu, A.Y.H., Junk, K.W., Coon, J.M. 1969. Resolution of the Cytochrome P-450-containing w-Hydroxylation System of Liver Microsomes into Three Components. *The Journal of Biological Chemistry* 244:3714-3721.

Lu, A.Y.H., Levin, W. 1974. The resolution and reconstitution of the liver microsomal hydroxylation system. *Biochem Biophys Acta* 344: 205-240.

Malkinson, A.M.D., Siegel, G.L., Forrest, A.F., Gazdar, H.K., Oie, D.C., Chan, P.A., Bunn, M., Mabry, D.J., Dykes, S.D. 1992. Elevated DT-diaphorase activity and messenger RNA content in human non-small cell lung carcinoma: relationship to the response of lung tumor xenografts to mithomycin C. *Cancer Res* 52: 4752-4757.

Manjanatha, M.G., Aidoo, A., Shelton, S.D., Bishop, M.E., McDaniel, L.P., Lyn-Cook, L.E., Doerge, D.R. 2006. Genotoxicity of acrylamide and its metabolite glycidamide administered in drinking water to male and female big blue mice. *Environmental and Molecular Mutagenesis* 47: 6-17.

Marsh, G.M., Lucas, L.J., Youk, A.O., Schall, L.C. 1999. Mortality patterns among workers exposed to acrylamide. *Occup Environ Med* 56: 181-190.

Martins, S.I.F.S., Jongen, W.M.F., van Boekel M.A.J.S. 2001. A review of Maillard reaction in food and implications to kinetic modelling. *Trends in Food Science and Technology* 11: 364–373.

McCollister, D., Oyen, F., Rowe, V. 1964. Toxicology of acrylamide. *Toxicology and Applied Pharmacology* 6:172-181.

Mehlman, M.A. 2002. Carcinogenic effects of benzene. *CesaN Y Acad Sci* 982: 137-144.

Melnick, R.L. 2002. Carcinogenicity and mechanistic insights on the behaviour of epoxides and epoxide-forming chemicals. *Annals of the New York Academy of Sciences* 982: 177-189.

Miatello, R., Vázquez, M., Renna, N,, Cruzado, M., Zumino, A.P., Risler, N. 2005. Chronic administration of resveratrol prevents biochemical cardiovascular changes in fructose-fed rats. American Journal of Hypertension 18: 864-870.

Mikstacka, R., Gnojkowski, J., Baer-Dubowska, W. 2002. Effect of natural phenols on the catalytic activity cytochrome P4502E1. *Acta Biochimica Polonica* 49: 917-925.

Mikstacka, R., Przybylska, D., Rimando, A.M., Baer-Dubowska, W. 2007. Inhibition of human recombinant cytochromes P450 CYP1A1 and CYP1B1 by trans-resveratrol methyl esters. *Mol Nutr Food Res* 51. 517-24.

Moon, Y.J., Wang, X., Morris, M.E. 2006. Dieatry flavonoids. Effects on Xenobiotic and carcinogen metabolism. *Toxicology in Vitro* 20: 187-210.

Mottram, D.S., Wedzicha, B.L., Dodson, A.T. 2002. Acrylamide is formed in the Maillard reaction. *Nature* 419: 448-449.

Mucci, L.A., Dickman, P.W., Steinek, G., Adami, H.O., Augustsson, K. 2003. Dietary acrylamide and cancer of the large bowel, kidney, and bladder. Absence of an association in a population based study in Sweden. *Br J Cancer* 88: 84-89.

Mucci, L.A., Lindblad, P., Steineck, G., Adami, H.O. 2004. Dietary acrylamide and risk of renal cell cancer. *Int J Cancer* 109:774-776.

Nash, T. 1953. The colorimmetric estimation of formaldehyde by means of the Hantzsch reaction. *Biochem J* 55: 416-421.

Nebert, D.W., Adesnik, M., Coon, M.J., Estabrook, R.W., Gonzales, F.J., Guengerich, F.P., Gunsalus, I.C., Johnson, E.F., Kemper, B., Levin, W., Phillips, I.R., Sato, R., Waterman, M.R. 1987. The P450 gene superfamily. Recomended nomenclature. *DNA* 6: 1-13.

Nebert, D.W., Nelson, D.R., Coon, M.J., Estabrook, R.W., Feyereisen, R., Fujiikuriyama, Y., Gonzalez, F.J., Guengerich, F.P., Gunsalus, I.C., Johnson, E.F., Loper, J.C., Sato, R., Waterman, M.R., Waxman, D.J. 1991. 'The P450 superfamily: Update on new sequences, gene mapping, and recommended nomenclature. *DNA Cell Biol* 10: 1-14.

Nebert, D.W., Russel, D.W. 2002. Clinical imporatance of cytochromes P450. *Lancet* 360: 1155-1162.

Nelson, D.R., Kamataki, T., Waxman, D.J., Guengerich, F.P., Estabrook, R.W., Feyereisen, R., Gonzalez, F.J., Coon, M.J., Gunsalus, I.C., Gotoh, O., Okuda, K., Nebert, D.W. 1993. The P450 superfamily: update on new sequences, gene mapping, accesion numbers, early trivial names of enzymes, and nomenclature. *DNA Cell Biol* 12: 1-51.

Omura, T., Sato, R. 1964. The carbon monoxide of liver binding pigment of liver microsomes. II. Solubilization, purification, and properties. *Journal of Biological Chemistry* 419: 2379-2385.

Ötles, S., Ötles, S. 2004. Acrylamide in food (Chemical Structure of Acrylamide). *EJEAFChe* 3: 723-730.

Patten, C.J., Ning, S.M., Lu, A.Y.H., Yang, C.S. 1986. Acetone inducible cytochrome P-450: purification, catalytic activity and interaction with cytochrome b5. *Arch Biochem Biophys* 251: 629-638.

Paulsson, B., Kotova, N., Grawe, J., Henderson, A., Granath, F., Golding, B., Tornqvist, M. 2003. Induction of micronuclei in mouse and rat by glycidamide, genotoxic metabolite of acrylamide. *Mutat Res* 535: 15–24.

Pelucchi, C., Franceschi, S., Levi,. F., Trichopoulos, D., Bosetti, C., Negri, E., La Vecchia. C. 2003. Fried potatoes and human cancer. *Int J Cancer* 105.

Penumathsa, S.V., Thirunavukkarasu, M., Koneru, S., Juhasz, B., Zhan, L., Pant, R., Menon, V.P., Otani, H., Maulik, N. 2006. Statin and resveratrol in combination induces cardioprotection against myocardial infarction in hypercholesterolemic rat. *Journal of Molecular and Cellular Cardiology*.

Persson, J.O., Terelius, Y., Ingelman-Sundberg, M. 1990. Cytochrome P-450-dependent formation of reactive oxygen radicals: isozyme- specific inhibition of P-450-mediated reduction of oxygen and carbon tetrachloride. *Xenobiotica* 20: 887–900

Peter, R., Bocker, R., Beaune, P.H., Iwasaki, M., Guengerich, F.P., Yang, C.S. 1990. Hydroxylation of chlorzoxazone as a specific probe for human liver cytochrome P-450IIE1. *Chem Res Toxicol* 3: 566–573.

Philpot, R.M., Arinç, E. 1976. Separation and purification of two forms of hepatic cytochrome P-450 from untreated rabbits. *Molecular Pharmacology* 12: 483-493.

Piver, B., Berthou, F., Dreano, Y., Lucas Danièle, L. 2003. Differential inhibition of human cytochrome P450 enzymes by epsilon-viniferin, the dimer of resveratro: comparizon with resveratrol and polyphenols from alcholized beverages. *Life Sci* 73(9): 1199-1213.

Piver, B., Berthou, F., Dreano, Y., Lucas Danièle, L. 2001. Inhibition of CYP3A, CYP1A and CYP2E1 activities by resveratrol and other non volatile red wine components. *Toxicology Letters* 125: 83-91.

Pustylnyak, V.O., Gulyaeva, L.F., Lyakhovich, V.V. 2007. Induction of CYP2B: Role of regulatory elements and nuclear receptors. *Biochemistry* 72(6): 608-617.

Raucy J.L., Lasker, J.M., Kraner, J.C., Salazar, D.E., Lieber, C.S., Corcoran, G.B. 1990, Induction of cytochrome P450IIE1 in the obese over-fed rat. *Mol Pharmacol* 39: 275–280.

Ray, P.S., Maulik, G., Cordis, G.A., Bertelli, A.A.E., Bertelli, A., Das, D.K. 1999. The red wine anti-oxidant resveratrol protects rat heart from ischemia reperfusion injury. *Free Radic Biol Med* 27: 160-169.

Reinke, L.A., Moyer, M.J. 1985. P-nitrophenol hydroxylation. A micrsomal oxidation which is highly inducible by ethanol. *Drug Metab Dispos* 13: 548-552.

Reitman, S., Frankel, S. 1957. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. *Am J Clin Pathol* 28(1): 56-63.

Rice, J.M. 2005. The carcinogenicity of acrylamide. *Mutation Research* 580: 3-20.

Rich, J.R., Boobis, A.R. 1997. Expression and inducibility of P450 enzymes during liver ontogeny. *Microsc Res Tech* 39: 424-435.

Robinson, A., Huttley, G.A., Booth, H.S., Board, P.G. 2004. Modelling and bioinformatics studies of the human Kappa class glutathione transferase predict a novel third transferase family with homology to prokaryotic 2-hydroxychromene-2-carboxylate isomerases. *Biochem J* 379: 541–52.

Rosén, J., Hellenäs, K. 2002. Analysis of acrylamide in cooked foods by liquid chromatography tandem mass spectrometry. *Analyst* 127: 880–882.

Ross, D. 1997. Quinone reductases. Vol 3 of *Comprehensive Toxicology*, ed. Guengerich F. P., 179. New York: Pergamon.

Ryan, D.E., Iida, S., Wood, A.W., Thomas, P.E., Lieber, C.S., Lewin, W. 1984 Characterization of highly purified cytochrome P-450 from hepatic microsomes of adult male rats. *J Biol Chem* 259: 1239-1250.

Sanders, T.H., McMichael, R.W., Hendrix, K.W. Occurence of resveratrol in edible peanuts. *J Agric Food Chem* 48: 1243-1246.

Schenkman, JB. 1999. Molecular and Applied Aspects of Oxidative Drug Metabolizing Enzymes, In *NATO ASI Series A, Life Sciences,* ed. Arinç E, Hodgson E, 1-20. New York: Plenum Press.

Schettgen, T., Kutting, B., Hornig, M., Beckmann, M.W., Weiss, T., Drexler, H., Angerer, J. 2002. Trans-placental exposure of neonates to acrylamide – a pilot study. *Int Arch Occupat Environ Health* 77: 213–216.

Schlager, J.J., Powis. G. 1990. Cytosolic NAD(P)H:quinone acceptor oxidoreductase in human normal and tumor tissue: effects of cigarette smoking and alcohol. *Int J Cancer* 45: 403-409.

Schneider, Y., Vincent, F., Duranton, B., Badolo, L., GosseÂ, F., Bergmann, C., Seiler, A., Raul, F. 2000. Anti-proliferative effect of resveratrol, a natural component of grapes and wine, on human colonic cancer cells. *Cancer Letters*; 158: 85-91.

Segerbäck, D., Calleman, C.J., Schroeder, J.L., Costa, L.G., Faustman, E.M. 1995. Formation of N-7-(2-carbamoyl-2-hydroxyethyl)guanine in DNA of the mouse and the ratfollowing intraperitoneal administration of [14C] acrylamide.*Carcinogenesis* 16: 1161–1165.

Segura-Aguilar, J., Lind, C. 1989. On the mechanism of the Mn3(+)-induced neurotoxicity of dopamine:prevention of quinone-derived oxygen toxicity by DT diaphorase and superoxide dismutase. *Chem Biol Interact* 72(3): 309-24.

Sengattuvelan, M., Shenthilkumar, R., Nalini, N. 2006. Modulatary influence of dietary resveratrol during different phases of 1,2-dimethylhydrazine induced mucosal lipid-peroxidation, anti-oxidant status and abberant crypt foci development in rat colon carcinogenesis. *Biochimica et Biophysica Acta* 1760: 1175-1183.

Serabjit-Singh, C.J., Philip, A.W., Robertson, I.G.C., Philpot, R.M. 1983. Interactions between xenobiotics that increase or decrease the levels of cytochrome P-450 isozymes in rabbit lung and liver. *J Biol Chem* 258: 12827-12834.

Sheehan, D., Meade, G., Foley, V.M., Dowd, C.A. 2001. Structure, function and evolution of glutathione transferases: implications for classification of nonmammalian members of an ancient enzyme superfamily. *Biochem J* 360: 1–16.

Shelby, M.D., Cain, K.T., Cornett, C.V., Generoso, W.M. 1987. Acrylamide: induction of heritable translocations in male mice. *Environ Mutagen* 9: 363-368.

Shimojo, N., Ishizaki, T., Imaoka, S., Funae ,Y., Fuji, S., Okuda, K. 1993. Changes in amount of cytochrome P450 isozymes and levels of catalytic activities in hepatic and renal microsomes of rats with streptozotocin-induced diabetes. *Biochem Pharmacol* 46: 621–627.

Smith, C.J., Perfetti, T.A., Rumple, M.A., Rodgman, A., Doolittle, D.J. 2000. IARC Group 2A Carcinogens reported in cigarette mainstream smoke. *Food and Chemical Toxicology* 38: 371-383.

Sobel, W., Bond, G.G., Parsons, T.W., Brenner, F.E. 1986. Acrylamide cohort mortality study. *Br J Ind Med* 43: 785–788.

Soleas, G.J., Diamandis, E.P., Goldberg, D.M. 1997. Resveratrol: A molecule whose time has come? And gone. *Clinical Biochemistry* 30(2): 91-113.

Solomon, J.J., Fedyk, J., Mukai, F., Segal, A. 1985. Direct alkylation of 2-deoxynucleosides and DNA following in vitro reaction with acrylamide. *Cancer Res* 45: 3465-3470.

Solomon, J.J. 1999. Cyclic adducts and intermediates induced by simple epoxides. *IARC Sci Publ* 150: 123-135.

Song, B.J., Gelboin, H.V., Park, S.S., Yang, C.S., Gonzales, F.J. 1986. Complemantary Dna and protein sequences of ethanol-inducible rat and human P450s: transcriptional and post-transcriptional regulationthe rat enzyme. *J Biol Chem* 261:16689-16697.

Sörgel, F., Weissenbacher, R., Kinzig-Schippers, M., Hofmann, A., Illauer, M., Skott, A., Landersdorfer, C. 2002. Acrylamide: increased concentrations in homemade food and first evidence of its variable absorption from food, variable metabolism and placental and breast milk transfer in humans. *Chemotherapy* 48: 267–274.

Spencer, P.S., Schaumburg, H.H. 1974. A review of acrylamide neurotoxicity. Part II. Experimental animal neurotoxicity and pathologic mechanisms. *Can J Neurol Science* 1: 152–169.

Sporn, M.B., Suh, N. 2000. Chemoprevention of cancer. *Carcinogenesis* 1: 525-30.

Stadler, R.H., Blank, I., Varga, N., Robert, F., Hau, J., Guy, P.A., Robert, M., Riediker, S. 2002. Acrylamide from Maillard reaction products. *Nature* 419-449.

Stewart, J.R., Artime, M.C., O'Brians, A.C. 2003. Resveratrol: a candidate nutritional substance for prostate cancer prevention. *J Nutr* 133(7 Suppl): 2440-2443.

Strange, R.C., Jones, P.W., Fryer, A.A. 2000. Glutathione-S-transferase: genetics and role in toxicology. *Toxicology Letters* 112: 357-363.

Sumner, S.C.J., Fennel, T.R., Moore, T.A., Chanas, B., Gonzales, F., Ghanayem, B.I. 1999. Role of cytochrome P4502E1 in the metabolism of acrylamide and acrylonitrile in mice. *Chem Res Toxicol* 12: 1116-1120.

Sumner, S.C.J., Selvaraj, L., Nauhaus, S.K., Fennell, T.R. 1997. Urinary metabolites from F344 rats and B6C3F1 mice coadministered acrylamide and acrylonitrile for 1 or 5 days. *Chem Res Toxicol* 10: 1152-1160.

Svensson, K., Abramsson, L., Becker, W., Glynn, A., Hellenäs, K.E., Lind, Y., Rosén, J. 2003. Dietary intakke of acrylamide in Sweden. Food and Chemical Toxicology 41: 1581-1586.

Szczerbina, T., Banach, Z., Tylko, G., Pyza, E. 2008. Toxic effects of acrylamide on survival, development and haemocytes of Musca domestica. *Food and Chemical Toxicology* 46: 2316-2319.

Szmitko, P.E., Verma, S. 2005. Red wine and your heart. *Circulation* 111: 10-11.

Şen, A., Arinç, E. 2000. Further immunochemical and biocatalytic characterization of CYP1A1 from feral leaping mullet liver (Liza saliens) microsomes. Comparative Biochemistry and Physiology C. *Pharmacology, Toxicology and Endocrinology* 126C(3): 235-244.

Şen, A., Arinç, E. 1998. Preparation of highly purified cytochrome P4501A1 from leaping mullet (Liza saliens) liver microsomes and its biocatalytic, molecular and immunological properties. *Comparative Biochemistry and Physiology* 121: 249-265.

Taeymans, D., Wood, J., Ashby, P., Blank, I., Studer, A., Stadler, R.H., Gondé, P., van Eijck, P., Lalljie, S., Lingnert, H., Lindblom, M., Matissek, R., Müller, D., Tallmadge, D., O'Brien, J., Thompson, S., Silvani, D., Whitemore, T. A. 2004. Review of acrylamide: an industry perspective on research, analysis, formation, and control. *Critical Reviews in Food Science and Nutrition* 44: 323–347.

Tang, H.Y., Shih, A., Cao, H.J., Davis, F.B., Davis, P.J., Lin, H.Y. 2006. Resveratrol-induced cyclooxygenase-2 facilitates p53-dependent apoptosis in human breast cancer cells. *Mol Cancer The* 5(8): 2034-2042.

Tareke, E., Rydberg, P., Karlsson, P., Eriksson, S., Tornqvist, M. 2002. Analysis of acrylamide, a carcinogen formed in heated foodstuffs. *J Agric Food Chem* 50: 4998-5006.

Tareke, E., Rydberg, P., Karlsson, P., Eriksson, S., Törnquvist, M. 2000. Acrylamide: A cooking carcinogen? *Chem Res Toxicol* 13: 517-522.

Tong, C.G., Cornwell, W.K., Means, G.E. 2004. Reactions of acrylamide with glutathione and serum albumin. *Toxicology Letters* 147: 127-131.

Towbin, H., Staehelin, T., Gordon, J. 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc Natl Acad Sci* 76(9): 4350-4.

Tyl, R.W., Friedman, M.A. 2003. Effects of acrylamide on rodent reproductive performance. *Reproductive Toxicology* 17: 1–13.

Tyl, R.W., Marr, M.C., Myers, C.B., Ross, W.P., Friedman, M.A. 2000. Realtionship between acrylamide reproductive and neurotoxicity in male rats. *Reprod Toxicol* 14: 147-157.

Ulusoy, G., Arinç, E.,·Adali, O. 2007a;Genotype and allele frequencies of polymorphic CYP2E1 in the Turkish population. *Arch Toxicol* 81: 711–718.

Ulusoy, G., Adali, O., Boyunegmez, Tumer T., Sahin, G., Gozdasoglu, S., Arınç, E. 2007b. Significance of Genetic Polymorphisms at Multiple Loci of CYP2E1 in the Risk of Development of Childhood Acute Lymphoblastic Leukemia. *Oncology* 72: 125–131.

Umeno, M., McBridge, O.W., Yang, C.S., Gelboin, H.V., Gonzales, F.J. 1988. Human ethanol-inducible P450IIEI: complete gene sequence, promoter characterization, chromosome mapping and cDNAdirected expression. *Biochemistry* 27: 9006–9013

Wallace, A., Wallace, G.A., Abouzam, A.M. 1986. Effect of excess level of a polymer as a soil conditioner on yield and mineral nutrition of plants. *Soil Science* 141: 377-379.

Wang, H., Napoli, K.L., Strobel, H.W. 2000. Cytochrome P450 3A9 catalyzes the metabolism of progesterone and other steroid hormones. Mol Cell and Biochem 213: 127-135.

Wang, R.W., Newton, D.J., Scheri, T.D., Lu, A.Y. 1997. Human cytochrome P450 3A4-catalyzed testesterone 6 beta-hydroxylation and erythromycin N-demethylation. Competition during catatlysis. *Drug Metab Dispos* 25: 502-507.

Waxman, D.J., Azaroff, L. 1992 *Biochem J* 281: 577-592.

Weber, A., Kaplan, M., Chugtai, S.A., Cohn, L.A., Smith, A.L., Unadkat, J.D. 2001. CYP3A inductive potential of the rifamycins, rifabutin and rifampin, in the rabbit. *Biopharm Drug Dispos* 22: 157-168.

Wise, L.D., Gordon, L.R., Soper, K.A., Duchai, D.M., Morissey, R.E. 1995. Developmenal neurotoxicity evaluation of acrylamide in Sprague-Dawley rats. *Neurotoxicol Teratol* 17: 189-198.

Wood, A.W., Smith, D.S., Chang, R.L., Huang, M.T., Conney, A.H. 1986. Effects of flavonoids on the metabolism of xenobiotics. *Prog Clin Biol Res* 213: 195-210.

Wrighton, S.A., Thomas, P.E., Ryan, D.E., Levin, W. 1987. Purification and characterization of ethanol-inducible hepatic cytochrome P450HLj. *Arch Biochem Biophys* 258: 292-297.

Wu, X., Amos, C.I., Kemp, B.L., Shi, H., Jiang, H., Wan, Y., Spitz, M.R. 1998. Cytochrome P450 2E1 DraI polymorphism in lung cancer in minority populations. *Cancer Epidemiol Biomark Prev* 7: 13–18.

Xie, H.G., Wood, A.J., Kim, R.B., Stein, C.M., Wilkinson, G.R. 2004. Genetic variability in CYP3A5 and its possible consequences. *Pharmacogenomics* 5: 243–272.

Yamazaki, H., Shimada T. 1997. Progesterone and testesterone hydroxylation by cytochromes P450 2C19, 1C9, and 3A4 in human liver microsomes. Arch Biochem Biphys 346: 161-169.

Yang, C.S., Hong, J.Y. 1993. Molecular aspects of cytochrome P4502E1 and its roles in chemical toxicology. In *Molecular Aspects of Oxidative drug Metabolizing Enzymes*, ed. Arınç, E., Schenkman, J. B., Hodson, E., 181-192. NATO ASI Series

Yang CS, Hong JY. 1995. Molecular aspects of P4502E1 and its roles in chemical toxicology, In *Molecular Aspects of Oxidative Drug Metablixing Enzyme,*ed. Arinç E, Schenkman JB, Hodsgon E, 181-192. Berlin: Springer-Verlag.

Yang, C.S., Yoo, J-S.H., Ishizaki, H., Hong, J.Y. 1990. Cytochrome P450 IIEI: Roles in nirosamine metabolism and mechanisms of regulation. *Drug Metab Rev* 22: 147-160.

Yousef, M.I., El-Demerdash, F.M. 2006. Acrylamide-induced oxidative stress and biochemical perturbations in rats. *Toxicology* 219: 13-141

Zeldin, D.C., Plitman, J.D., Kobayashi, J., Miller, R.F., Snapper, J.R., Falck, J.R., Szarek, J.L., Philpot, R.M., Capdevila, J.H. 1995. The Rabbit Pulmonary Cytochrome P450 Arachidonic Acid Metabolic Pathway: Characterization and Significance. *The journal of Clinical Investigation* 95: 2150-2160.

Zhang, Y., Zhang, Y. 2007. Formation and reduction of acrylamid in Maillard reaction: a review based on the current state of knowledge. *Critical Reviews in Food Science and Nutrition* 47: 521–542.

Zyak, D.V., Sanders, R.A., Stojanovic, M., Tallmadge, D.H., Eberhart, B.L., Ewald, D.K., Gruber, D.C., Morsch, T.R., Strothers, M.A., Rizzi, G.P., Villagran, M.D. 2003. Acrylamide formation mechanism in heated foots. Journal of Agricaltural and Food Chemistry 51: 4782–4787.