Effects of Benzo(a)pyrene and Ethanol on Morphology and Antioxidant Status and Transaminases in Rat Liver

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Abstract

Ethanol and benzo(a)pyrene cause an increase in lipid peroxidation either by producing the reactive oxygen species or decreasing the level of endogenous antioxidant enzymes that leads to cellular damage and cellular dysfunction. The aim of this study was to investigate both physiological and histological changes in liver tissue after administration of benzo(a)pyrene and ethanol. Male Sprague Dawley rats were divided into four groups. First group (control group). Second group treated with benzo(a)pyrene [B(a)P], third group treated with benzo(a)pyrene[B(a)P] plus ethanol (EtOH) and fourth group was given ethanol(EtOH). Superoxide dismutase (SOD), alanin aminotransferase (ALT), aspartat aminotransferase (AST), gamma-glutamyl transferase (GGT), glutathione (GSH), malondialdehyde (MDA) levels as well as histological examination were evaluated to demonstrate the liver response following administration of [B(a)P] and (EtOH) separately and together. SOD activities of the liver tissue in the experimental groups were decreased when compared to the first group. Activities of ALT, AST and GGT of the liver tissue in all experimental groups were found significantly higher than that of the first group. GSH levels of the liver tissue of the experimental groups were lower than the first group especially in fourth group. When we compared MDA levels among study groups, MDA levels of experimental groups were found significantly higher than the first group. Exposure [B(a)P] to resulted in hepatocellular changes in the periportal area and inflammatory cell infiltration . On the other hand, liver tissue in third group and fourth group, which was treated with [B(a)P] plus EtOH and EtOH alone respectively, showed seldom inflammatory cell infiltrations. [B(a)P] and EtOH administration alone or together discretely determined changes in the GSH, MDA levels and SOD ALT, AST and GGT enzyme activities in the liver tissues. Additionally, we noted [B(a)P] induced hepatocellular changes in the periportal area.

Key Words: Liver, antioxidant enzymes, EtOH, [B(a)P], MDA, ALT, AST, GGT

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Introduction

Living organism can be exposed to many various chemical substances that present in the occupational environment or by consuming tinned food and ingestion of alcohol. On the other hand, living organism has a balance between oxidant and antioxidant system but many things can disrupt this balance in favor of oxidant system. Numerous previous studies showed that free radicals and reactive oxygen species could contribute to various diseases including cancer, neurodegenerative disorders, cataract and AIDS, etc [1-3].

Ethanol is a small molecule and react with water and lipid. it passes from the cell membrane of all body tissues and affects most vital functions of virtually all organs including liver, kidney, brain, heart and pancreas. Therefore, ethanol ingestion leads to a wide variety of pathological disturbances affecting the organs [4-7]. Moreover, its effects on the living organism are summarized in a few ways: 1.It disrupts the balance between oxidant and antioxidant enzymes which is present in living organism, 2.it induces free radical generation, 3.It has profound adverse effects on nutritional status, food consuming, digestion and absorption [4, 8]. Effect of EtOH on the tissue and organs is derived directly either from itself and its metabolite or indirectly from metabolic products of EtOH oxidation [9.] the action mechanism of ethanol on oxidant system remains unclear, but there is little knowledge about mechanism of ethanol on DNA repair enzymes. EtOH itself or its metabolite acetaldehyde contribute to suppress this the repair enzyme and the repair or removal of tissue damage DNA repair enzyme is induced by [B(a)P] [10].

[B(a)P] is a polycylic aromatic hydrocarbon and presents in living environment. It is a chemical carcinogen and teratogenic agent, and is produced spontaneously by environmental pollution such as traffic clustering and industrial production [11-14]. The carcinogenesis mechanism of [B(a)P] has been reported to involve DNA adduct formation during initiation stages. Moreover, oxidative metabolites of [B(a)P] can produce many different chemicals including free radicals, peroxides, quinines or other reactive intermediates [15-16].

It is known that the liver is the main site for EtOH biotransformation and a target organ of xenobiotics [1,17]. Therefore, The aim of the present study was to evaluate the effects of the administration of [B(a)P] and EtOH separately and together on liver tissue enzyme (SOD ,ALT,AST , GGT), liver functions (GSH, TBARS ,) and liver histology in rats. On the other

hand, the goal of our study is directed to the awareness on the effect of wrong habits, diet and environmental pollution on human health.

Material and Methods

Animals

Male Sprague Dawley rats (155-220 g) were used in this study. The rats were bred in animal laboratory at İnönü University. The rats were kept for 2 days before the experiments for acclimatization to the experimental conditions with free access to food (standard diet) and water, but food was withdrawn 24 h before the experiment. The experiment protocol was conducted after obtaining permission from local ethical committee.

The animals were divided into four groups of seven each and maintained as follows:

First group (Control group) : Received i.p. corn oil

Second group [B(a)P] : Received i.p 200mg/kg [B(a)P] with corn oil

Third group [B(a)P] +EtOH: Received EtOH at a dosage of 3 g /kg and 200 mg/kg [B(a)P] under the condition mentioned above

Fourth group: Received EtOH at a dosage of 3 g /kg

EtOH administered 2 h before the injection of [B(a)P] in the [B(a)P] +EtOH group. 24 h after the injection of [B(a)P], blood samples were withdrawn by cardiac puncture and the rats were sacrificed. Blood samples were centrifuged at 3000 x g for 10 min to obtain serum. Serum samples were used to determine the ALT, AST and GGT activities. On the other hand, liver of each rat was promptly removed and part of it was used to determine the tissue levels of SOD, GSH and TBARS.

Determination of malondialdehyde

The MDA content of homogenates was determined spectrophotometrically [18] by measuring the presence of thiobarbituric acid reactive substances. Three ml of 1 % phosphoric acid and 1 ml of 0.6 % thiobarbituric acid solution were added to 0.5 ml of plasma pipetted into a tube. The mixture was heated in boiling water for 45 min. After cooling, the color was extracted into 4 ml of n-butanol. The absorbance was measured in spectrophotometer (shimadzu UV-1601, Japan) at 532 nm. The amounts of lipid peroxides were calculated as thiobarbituric acid

reactive substances of lipid peroxidation. The results were expressed as nanomole per g wet tissue (nmol/g wet tissue) according to a standard graph which was prepared from the measurements done with a standard solution (1, 1, 3, 3-tetramethoxypropane).

Determination of superoxide dismutase (SOD) activity

Total (Cu-Zn and Mn) SOD (EC 1.15.1.1) activity was determined according to the method of Sun et al. [19]. One unit of SOD was defined as the enzyme amount causing 50% inhibition in the NBT reduction rate. SOD activity was expressed as units per milligram protein (U/mg protein).

Determination of glutathione (GSH)

Glutathione was determined by the spectrophotometric method. The principle of the method is based on reaction taking place among DTNB (5,5′□-dithiobis-(2-nitrobenzoic acid) and thiols. Results are expressed as nmol/mg tissue [20].

Determination of serum ALT, AST and GGT activities

Alanine aminotransferase (ALT), aspartate aminotransferase (AST) and gamma-glutamyl transferase (GGT) levels were determined in serum by Olympus AU 2700 autoanalyzer.

Determination of protein content

Protein measurements were made at all stages according to the Lowry's method [21].

Histologic Examination

Liver tissue samples were fixed in phosphate buffered solution 10% formalin and processed for light microscopic evaluation. Slices of the tissue were cut from paraffin embedded samples and stained with hematoxylin-eosin (H-E). The liver tissues were examined for inflammatory cell infiltration and hepatocellular changes by a blinded observer to the animal treatment groups.

Statistical analysis

The data obtained were analyzed by One-way analysis of variance (ANOVA) and Tukey-Kramer Posthoc tests for the significant interrelation between the various groups using Instat computer software. $P < 0.05$ was considered to be significant from the control.

Results

The results of the statistical evaluation of hepatic antioxidant enzyme and liver function test are expressed in Table 1.

The activity of SOD of liver tissues were significantly lower in groups second, third and fourth which were treated with[B(a)P], [B(a)P] +EtOH and EtOH respectively than that of the first group ($p<0.001$).

Although the GSH levels in liver tissue of all the experimental groups were lower than the first group, only fourth group showed statistically significant decrease when compared to the first group($p<0.05$).

MDA levels of the experimental groups were statistically higher than the first group. MDA levels of the third and fourth groups were significantly higher than the second group as well ($p <0.001$).

Table 1. Effect of ethanol on ALT, AST, GGT, lipid peroxidation, enzymic and non-enzymic antioxidants induced by[B(a)P] in livers of mice.

Parameters	First group (Control)	Second group [B(a)P]	Third group [B(a)P] +(EtOH)	Fourth group (EtOH)
ALT	72.8 ± 5.6	92.8 ± 1.7aNS	143.6 ± 9.4b#	164.2 ± 22.2a*, b@
AST	36.5 ± 3.8	138.8 ± 6.9a@	238.2 ± 28.5b@	184.4 ± 15.2a*,NS
GGT	8.5 ± 1.5	14.0 ± 2.1aNS	40.8 ± 8.6b@	8.8 ± 1.2aNS, bNS
MDA	117.1 ± 5.9	149.9 ± 6.2a#	225.5 ± 10.5b*	246.9 ± 9.2a*, b*
SOD	4.26 ± 0.3	0.98 ± 0.3a*	1.6 ± 0.3bNS	1.3 ± 0.1a*, bNS
GSH	1.12 ± 0.1	1.04 ± 0.2aNS	0.72 ± 0.1bNS	0.54 ± 0.1a#, bNS

a: compared with control, b; compared with[B(a)P], NS: not significant
#: p<0.05; @: p<0,01; *:p<0,001

The activity of serum ALT increased in the second group when compared to the first but it was not significant. However, ALT activities of third group ($p < 0.05$) and fourth IV ($p < 0.001$) were significantly higher than the first.

AST activity of the liver tissue in the second group and forth group were significantly higher than the first ($p < 0.01$). The AST activity of third group was statistically higher than both the first group ($p < 0.05$) and the second group ($p < 0.01$).

We did not determine any significant differences between the first and the second group for GGT activities. However the GGT activity of the third group was significantly higher than the first and the second group ($p < 0.01$) although GGT activity of fourth group was close to the first group and lower than both the second and third groups.

The first group showed normal liver histology (Figure 1). Whereas, Second group which treated with [B(a)P] showed inflammatory cell infiltration and hepatocellular changes. Hepatocellular changes were observed in the periportal area and included hydropic hepatocytes and necrotic hepatocytes (Figure 2, 3). In the livers of the third and fourth groups, there were not any evidence of hepatocellular changes but seldom inflammatory cell infiltration were detected (Figure 4,5).

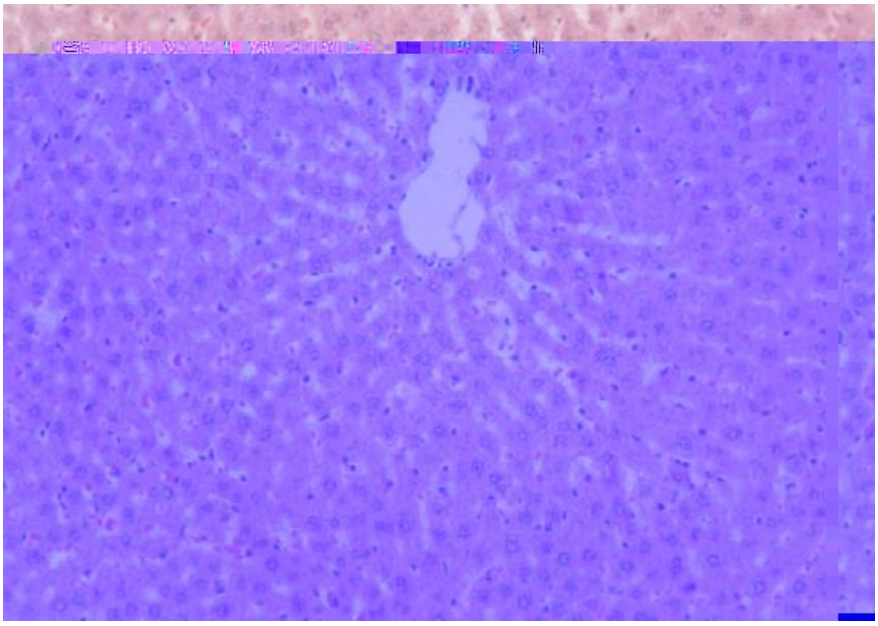

Figure 1. Light micrograph of liver in control group. H-E, X66.

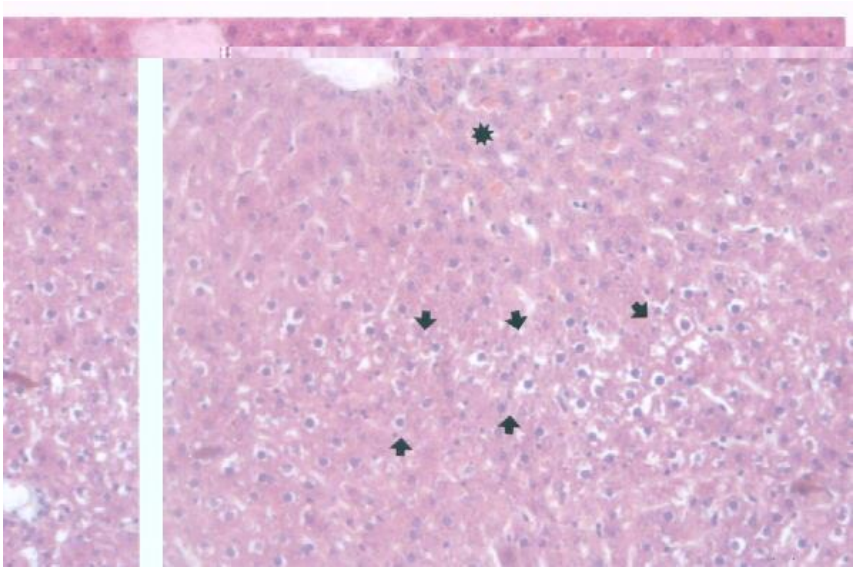

Figure 2: Light micrograph of liver in [B(a)P] group. The hepatocytes (asterisk) in the centrilobular area are seen normal, while hepatocellular changes are visible in the periportal area (between arrows). H-E, X66.

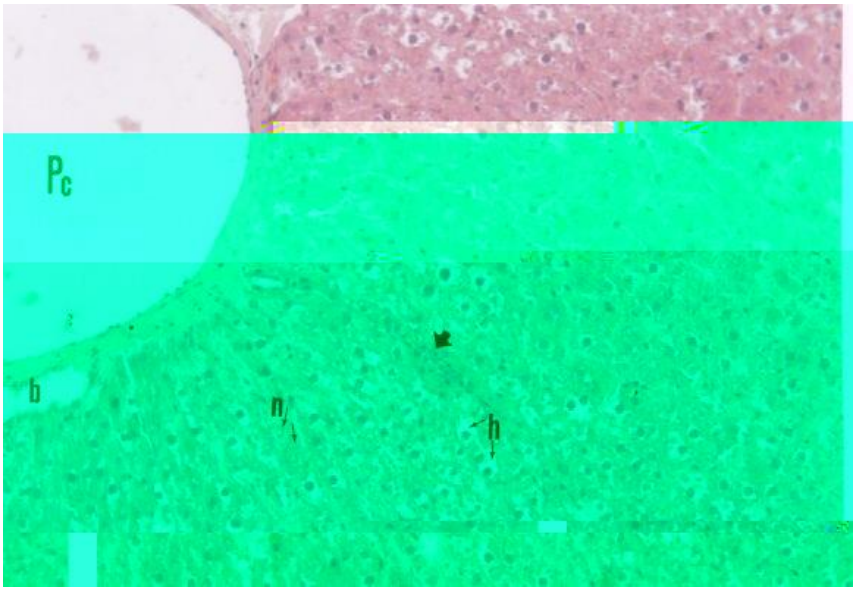

Figure 3: Light micrograph of liver in [B(a)P] group. Inflammatory cell infiltration (arrow) and periportal hepatocellular changes are seen. Hydropic (h) and necrotic (n) hepatocytes are visible around portal canal (Pc) and bile duct (b). H-E, X66.

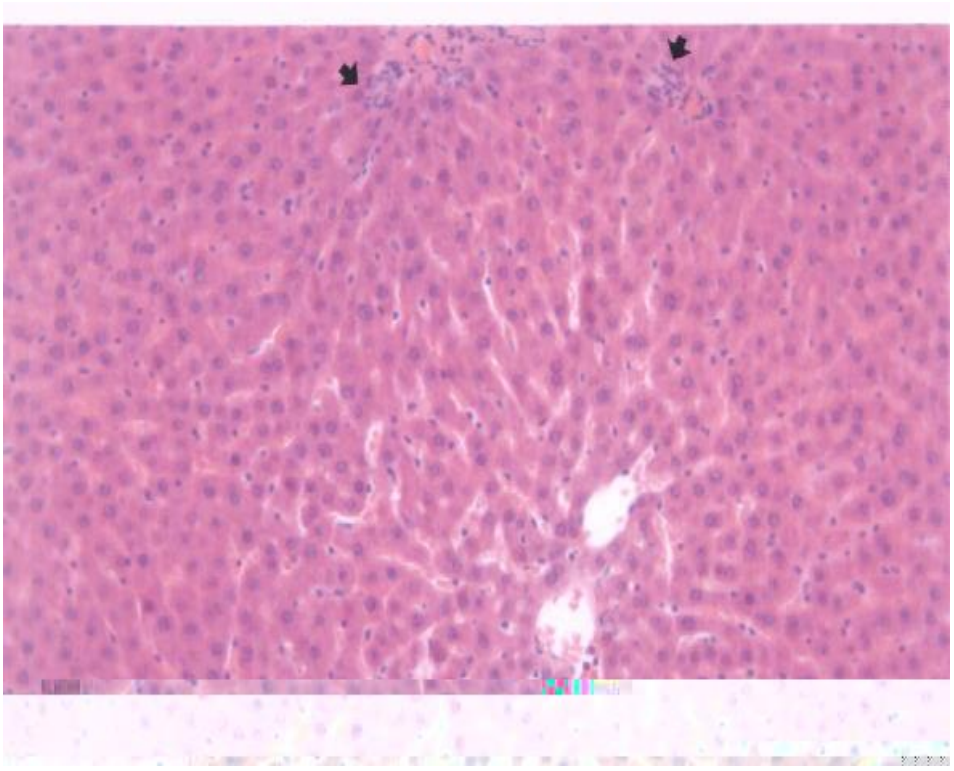

Figure 4: Light micrograph of liver in ethanol group. The histology of the liver is normal. Seldom inflammatory cell infiltrations are detected (not visible in this photo). H-E, X66.

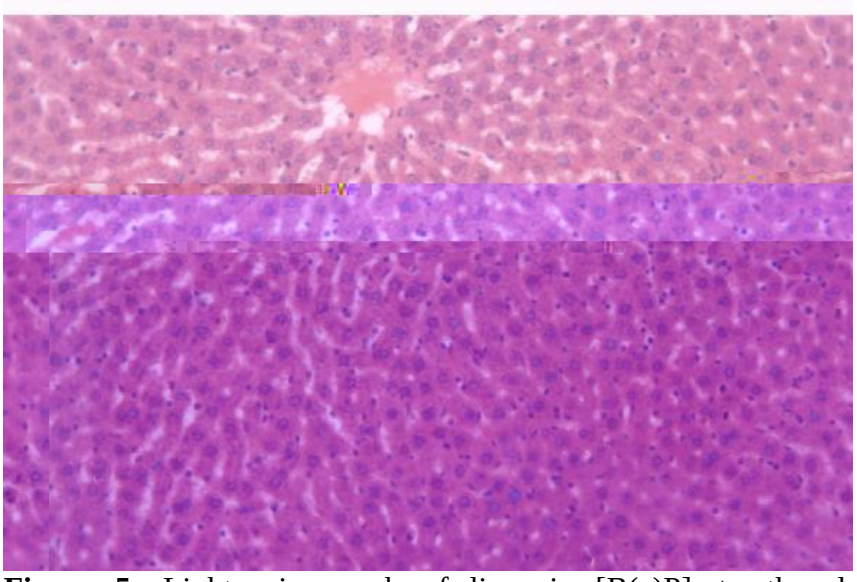

Figure 5: Light micrograph of liver in [B(a)P] + ethanol group. Inflammatory cell infiltrations are seen in the portal area (arrows). Hepatocellular changes are not evident in this group. H-E, X66.

Discussion

The aim of this study was to evaluate histological structure and the oxidative status of liver tissue during exposure to[B(a)P] and EtOH separately or together in all groups. For this purpose, the activity of SOD, ALT, AST, GGT, the concentration of GSH and MDA were determined in Table 1.

[B(a)P] and EtOH administration alone or together causes changes in the antioxidant enzyme status and transaminases enzyme activities in the liver tissues. At the same time, we determined [B(a)P] induced hepatocellular changes in the periportal area.

SOD activity of the second study group decreased when compared with the first group. This result is concordant with the result of Kim and Devi [15,22]. We also determined some hepatocellular changes in the second experimental group. However, our results were different from that of Kim and Devi because a single dose of [B(a)P] was given in our study. SOD activity in the third group, which was treated with [B(a)P] plus EtOH together, was also lower than the first group while it was higher than that of the second group. When we discussed about the results of the third experimental group, we must consider the interaction between [B(a)P] and EtOH. Numerous studies indicate that [B(a)P] is a carcinogen for human and animals [3,12, 23]. However, [B(a)P] itself does not cause deleterious damage but its metabolites produce enzyme like prostaglandin H synthase and etc. and induces many biochemical changes [12, 23]. Mechanism of [B(a)P] in carcinogenesis has been reported to be related to DNA damage. On the other hand, it has been shown that EtOH alters the activity of several signal transduction system. Therefore, it is probable that a reduce an ionic transfer through alterations in the monovalent cations pump and the antiport system. At the same time, EtOH induces generation of free radical species by either increase cytochrome P450's, NADPH reductase-dependent microsomal oxidizing system or xanthine oxidase [7,9,24]. Normally, EtOH and acetaldehyde, which is metabolite of EtOH, suppress DNA repair enzymes. But in our study the third group SOD activity was high and histological changes were slight when compared to the second study group. This finding is possibly relate to the blocking of [B(a)P] - effect on the DNA repair enzymes because EtOH alters several signal transduction system in the cells. In the fourth study group, SOD activity was significantly lower than the first group but it was higher than the second group. However, this difference was not significant. This finding can be related to either ratio of cytoplasmic NAD/NADH

and xanthine or EtOH induced-depleteion of glutathione reductase which is important for glutathione and glucose -6-phospate dehydrogenase, the enzyme responsible from NAD [9]. At least, it appears that three metabolic pathways produce oxygen reactive species depending on EtOH [25]. The results of the histological alterations are presented in Figs 1-5. Histopathological changes determined in the fourth group are harmonious with literature and supported by the decrease in antioxidant enzymes which is caused by ethanol [26].

GSH is a non-enzymatic antioxidant which involves in the protection of normal cell structure and functions by maintenance of the redox homeostasis and participating into many detoxification processes which are catalyzed by GST and the catalytic conversion of organohydroperoxides to alcohols by GPx [5,27]. Therefore, in living organism GHS level has a critical role against the effects of oxidant substances. It is well described that during metabolism of [B(a)P] and its quinines, highly reactive oxygen species such as superoxide anion and consequences their reactive could be generated. Free radicals attack macromolecules such as DNA, protein oxidation of the cell and the living organism resists by antioxidant enzymes and non-enzymatic antioxidants against to free radical damage and it causes decreased liver GSH level. GSH concentration of the second group which was treated by [B(a)P] was not significantly different from the first group. On the other hand, in the present study GSH level of the third group is decreased and it is concordant with the literature [5-6,28]. This result might be related to either the increase in GSH oxidation, which is a direct result of increase in free radical generation or acetaldehyde promotes peroxidation reaction binding to cysteine and/or glutathione, which causes depletion of GSH [5,28]. Moreover, this result might be affected by interaction between EtOH and [B(a)P]. The fourth group's liver GSH concentration was significantly lower when compared to the second group. This result could be related to the effect of EtOH as mentioned above.

Table 1 also shows the levels of lipid production in liver tissue of experimental animals. [B(a)P] presents in urban air particulates, cigarette smoke and cooking oil fumes. Tung et al [29] have reported that cooking oil fumes induces lipid peroxidation. MDA, which is a marker for lipid peroxidation, was higher in the livers of the second group than that of the first group. This finding is parallel to that of Tung and Bhagavathy [29-30]. This result could be related to generation of free radical species by [B(a)P] or its metabolites. MDA level in the third group, which was treated with a combination of [B(a)P] and EtOH, was also significantly higher than the second group. This result is supported by several results from the

literature [1,5]. this result could be related to synergistic effect of combination [B(a)P] and EtOH. The fourth group MDA level was higher when compared to the first and the second group. There are not harmonious results with literature in viewpoint of effect of EtOH on LPO. This difference may be related to the experimental design and the investigated tissue [1,31].

On the other hand, a number of experimental studies have demonstrated that chronic or acute alcohol administration to experimental animals increases lipid peroxidation product and decreases tissue level of antioxidants [5-6,32]. On the other hand, we did not determine any information about the direct effect of [B(a)P] on the liver in the literature. However it is known that liver is the main site of EtOH biotransformation and main target for xenobiotics and a numerous previous experimental studies have shown that [B(a)P] induces many alterations in cell such as DNA damage, adhesion and cytoskeleton etc.[11,13,33]. Therefore, [B(a)P], which is carcinogenic and teratogenic for human and animals, have evident to induce some histopathological changes in the liver.

Activities of AST, ALT and GGT in the first and the experimental animals are presented in table 1. ALT and AST activity values were significantly increased in all experimental groups when compared to the control. These results are concordant with that of Thirunavukkarasu and Lakshmi .([6,34]. On the other hand, GGT values of the third group were significantly higher than the second, fourth and the first group. However, GGT activity in the fourth group did not show any significant difference from the first and second groups. Elevated transaminase activities in the second group can possibly be due to the carcinogenic and mutagenic effect of [B(a)P] . Findings in the third group could be related to either synergistic effects of [B(a)P] and ethanol or depend on the inhibition capacity of DNA repair enzyme by EtOH. GGT activity in the fourth group is not consistent with the literature. This result could be related to either the dosage of ethanol, species of animals or experimental procedures [4].

In conclusion: When liver is exposed to EtOH prior to dosing with [B(a)P] MDA level and transaminase activites increase. However, SOD activity and GSH concentration decrease. To explain these findings further investigations are needed on the interaction between EtOH and [B(a)P] and both [B(a)P] and EtOH's and their metabolites' reaction process in the living organisms.

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