Türk Biyokimya Dergisi [Turkish Journal of Biochemistry–Turk J Biochem] 2015; 40(1):66–73
doi: 10.5505/tjb.2015.22448

Research Article [Araştırma Makalesi]

Yayın tarihi 24 Ocak 2015 © TurkJBiochem.com
[Published online January 24, 2015]

The effects of grape seed extract against toxicity of benzene on liver and kidney tissues of albino mice: biochemical evaluation

[Albino farelerde benzenin böbrek ve karaciğer dokularında toksisitesine karşı üzüm çekirdeği ekstraktının etkisi: biyokimyasal değerlendirme]

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Registered: 28 June 2013; Accepted: 25 September 2014
[Kayıt Tarihi: 28 Haziran 2013; Kabul Tarihi: 25 Eylül 2014]

ABSTRACT

Objective: The objective of the present study was to investigate the possible effects of grape seed extract (GSE) against benzene-induced toxicity in Swiss albino mice.

Methods: The animals were randomly divided into six groups each containing six mice. Group I, treated with distilled water; Group II and III orally treated with 50 mg/kg and 150 mg/kg body weight GSE, respectively. Group IV, orally treated with 250 mg/kg body weight benzene by using feeding cannula; Group V, orally treated with 50 mg/kg body weight GSE + 250 mg/kg body weight benzene; Group VI, orally treated with 150 mg/kg body weight GSE + 250 mg/kg of body weight benzene for 50 consecutive days. At the end of experimental period all mice were sacrificed; blood, liver and kidney tissues were removed after post-mortem examination. Aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), blood urea nitrogen (BUN), creatinine levels were analyzed from serum. Malondialdehyde (MDA) and reduced glutathione (GSH) levels were analyzed from isolated tissues. And also histopathological examinations of liver and kidney tissues were investigated.

Results: Serum AST, ALT, ALP, BUN and creatinine levels were slightly increased in Group IV compared with the other tested groups ($p<.05$). Benzene-induced toxicity caused a significant decrease in GSH levels and a significant rise in MDA levels of liver and kidney tissues. Oral treatment with GSE significantly ameliorated the indices of hepatotoxicity, nephrotoxicity and lipid peroxidation induced by benzene. Both doses of GSE provided significant protection and the strongest effects were observed at the dose level of 150 mg/kg.

Conclusion: Consequently, it was found that GSE has a significant positive effect in benzene-induced toxicity, and its GSE effect is dose dependent.

Key Words: Benzene, GSH, MDA grape seed

Conflict of Interest: The authors have no conflict of interest.

ÖZET

Amaç: Bu çalışmanın amacı albino farelerde benzen tarafından oluşturulan toksisitesiye karşı üzüm çekirdeği ekstraktının muhtemel etkisini araştırmaktır.

Metod: Her bir grupta 6 fare olmak üzere uygulama grupları oluşturulmuştur. Grup I, oral yolla distile su ile; Grup II, oral yolla 50 mg/kg GSE; Grup III, 150 mg/kg GSE; Grup IV, oral yolla kanula kullanılarak 250 mg/kg benzen; Grup V, 50 mg/kg GSE + 250 mg/kg benzen; Grup VI, 150 mg/kg GSE + 250 mg/kg benzen ile 50 gün süre ile beslenmişlerdir. Deney sürelerinin sonunda otopsi yoluyla farelerin kan, karaciğer ve böbrek dokuları alınmıştır. Aspartat aminotransferaz (AST), Alanin aminotransferaz (ALT), Alkalen fosfataz (ALP), kan üre azotu (BUN), kreatinin düzeyleri kan dokudan, malondialdehit (MDA), redükte glutatyon (GSH) ise karaciğer ve böbrek dokularında incelenmiştir. Ayrıca karaciğer ve böbrek dokularında histopatolojik değişimler de araştırılmıştır.

Bulgular: Benzen uygulanan farelerde serum AST, ALT, ALP BUN ve kreatinin seviyelerinde kontrol grubuna kıyasla düşük düzeyde artış belirlenmiştir. Benzen tarafından oluşturulan oksidatif toksisitenin karaciğer ve böbrek dokularında GSH seviyesinde önemli bir azalmaya ve MDA seviyesinde önemli bir artışa neden olduğu belirlenmiştir. Üzüm çekirdeği ekstraktı uygulamasının benzen tarafından oluşturulan hepatotoksisite, nefrotoksisite ve lipid peroksidasyonunda önemli gerilemeye neden olduğu belirlenmiştir. Üzüm çekirdeği ekstraktının test edilen iki dozunun benzen tarafından oluşturulan toksisite üzerine pozitif etkisi olduğu belirlenmiş ve en güçlü etkiyi 150 mg/kg dozunda gösterdiği belirlenmiştir.

Sonuç: Sonuç olarak GSE'nin benzen toksisitesine karşı pozitif etkisi olduğu ve bu etkinin doza bağımlı olduğu belirlenmiştir.

Anahtar Kelimeler: Benzen, GSH, MDA, üzüm çekirdeği ekstraktı

Çıkar Çatışması: Yazarların çıkar çatışması yoktur.

Introduction

Benzene is an important pollutant compound, present in both occupational and general environment. It is a highly flammable liquid with clastogenic and carcinogenic activity [1-3]. It is classified as a "Known" carcinogen "Category A" under the risk assessment Guidelines of 1986 [4]. Many industrial applications have been found for benzene, and since its discovery it has been widely used as an industrial solvent [1]. Potential exposure to benzene can be higher in certain industries, such as shoe factories, petrol stations and the petrochemical industry [2]. Furthermore, lifestyle factors, such as cigarette smoking is the main source of benzene exposure for many people [1]. Humans with acute inhalation exposure to benzene may experience drowsiness, dizziness, headaches and a host of eye, skin, and respiratory tract irritations [3-5]. Many experimental animal studies, support the evidence that benzene exposure increases the risk of cancer in multiple organ systems including the hematopoietic system, liver and stomach [4].

Acute occupational exposure to benzene may cause narcosis: headache, dizziness, drowsiness, confusion, tremors and loss of consciousness. Use of alcohol enhances the toxic effect. Chronic exposure to benzene can reduce the production of both red and white blood cells from bone marrow in humans, resulting in aplastic anaemia [5,6]. Benzene is metabolized, primarily in the liver, to a variety of hydroxylated and ring-opened products that are transported to the periferal tissues where subsequent secondary metabolism occurs. Benzene and its metabolites may damage cellular macromolecules to induce toxicity include the covalent binding of reactive metabolites of benzene [7]. Decreasing the hepatic metabolism of benzene by partial hepatectomy also reduced benzene toxicity, suggesting that hepatic metabolism plays an important role in toxicity. In addition to hepatic metabolism, it appears that filtration of benzene and its metabolites contributes to toxicity [8,9]. So in this study the toxic effects of benzene on liver and kidney tissues also investigated. It was known that exposure to benzene causes oxidative stress and damage [10]. MDA one of the known secondary products of lipid peroxidation induced by oxidative damage, may be used as an indicator of cell membrane injury [11]. Due to its large role in countering increases in lipid peroxidation which occurs during oxidative stress, the response of GSH has been frequently studied. GSH is an important antioxidant, preventing damage to cellular components caused by oxidative stress [12]. So we investigated the MDA and GSH levels and tissue pathology for determine oxidative damage of benzene on liver and kidney tissues.

The use of certain materials may help to decrease the toxicity created by benzene. Recently, biopolymer materials such as zinc, selenium and leaf extract of *Ocimum basilicum* L. have been used for decreasing the toxic effects of benzene [13,14]. Grape seed extract (GSE) is a natural extract obtained from the seed of grape [15]. Grapes and grape products are good sources of dietary flavonoids, which are powerful antioxidant compounds [16]. GSE is a complex mixture of polyphenols containing dimers, trimers, and other oligomers (procyanidins) of catechin and epicatechin [17]. Several experimental studies have demonstrated that GSE is highly bioavailable and provides significantly greater protection against free radicals [18]. In order to overcome the potential harmful effect of benzene, GSE was tried as a toxicity-limited agent. For determine the effects of GSE, different GSE doses were orally given to the animals through benzene treatment. The aim of this study is to investigate the toxicity of benzene on liver and kidney tissues specifically out of MDA and GSH level measurements and the beneficial effects of GSE against benzene induced toxicity in an animal model. Recently, genomic data has been added to techniques to make close comparisons between species and determine relatedness. Humans share about 90% of genome with mice [19]. Scientists have been able to take advantage of these similarities in generating experimental and predictive models of human disease. So in this study the toxicity of benzene was investigated in an animal-mice model.

Materials and Methods

Animals and GSE

GSE (formerly Grape Seed PCO Phytosome 50–120 tabs) was purchased from Health Genesis Corp. (Bay Harbor Island, FL, USA). A total number of 36 adult male *Mus musculus* var. *albino* mice weighing 25-30 g were used in the current study. Healthy mice were obtained from the Animal Research Center of the Refik Saydam Hifzissiha Institute (Ankara, Turkey). All animals were housed in 26x15x50 cm stainless steel cages and kept under controlled laboratory conditions of 22±3°C and 55±5% relative humidity with a 12-hour light–dark cycle throughout the experiment. The animals were allowed to acclimatize for 1 week before the planned experimental test and fed a standard pellet diet (Samsun Food Industry, Samsun, Turkey). In this study, the methods and techniques applied to mice were carried out according to the guidelines set by the World Health Organization (Geneva, Switzerland) and the ethical standards of the local ethical committee for animal experiments at Giresun University (B.30.2.GRE.0.28.00.00/370-178).

Experimental protocol

The animals were divided into six groups each containing six mice. Distilled water was administered to mice in Group I and served as control [20]. Mice in Group II and III were fed orally with two different doses of GSE as 50 mg/kg and 150 mg/kg of body weight, respectively. Group IV was fed orally by using feeding cannula with 250 mg/kg of body weight benzene; Group V was treated with 50 mg/kg of body weight GSE + 250 mg/kg of body

weight benzene. Group VI was treated with 150 mg/kg of body weight GSE + 250 mg/kg of body weight benzene. The dose of benzene used in this study was determined to be 250 mg/kg. This dose was chosen because it induced an increase in the frequency of toxicity that was essential to determine the beneficial role of GSE [21,22]. In this study the experimental period was applied for 50 days to determine the long effect of benzene in accordance with literature. In some studies the toxicity of benzene was investigated for 3 days [23] or 6 days [24] for determine the short term effect; also in some studies the toxicity was studied for 30 days [25] or 50 days [26] to determine the long term effect. GSE doses were chosen that were comparable to those daily consumption amounts recommended by practitioners of nutritional medicine to support optimal health, and 50 and 150 mg/kg of body weight were effective doses for protection by GSE [27].

Serum analysis

For serum isolation, blood samples were collected by cardiac puncture with the animal under mild ether anesthesia. Blood samples were drawn directly into precooled vacutainer tubes (BD Vacutainer Systems, San Jose, CA, USA) for storage and centrifuged at 1.200 g for 10 minutes at 4°C. Aspartate aminotransferase (AST) (AST/GOT liquid reagent, catalog number A559-150, Teco Diagnostics, Anaheim, CA, USA), alanine aminotransferase (ALT) (ALT/GPT liquid reagent, catalog number A524-150, Teco Diagnostics) and alkaline phosphatase (ALP) (Liquid kinetic, catalog number A504-150, Teco Diagnostics) enzyme activities, blood urea nitrogen (BUN) (catalog number B549-150, Teco Diagnostics) and creatinine (catalog number C513-480, Teco Diagnostics) concentrations were measured by commercially available kits using an autoanalyzer (model 99M Chemistry Analyzer, Medispec, Germantown, MD, USA).

MDA and GSH levels

At the end of the experimental period, the mice were fasted overnight sacrificed by cardiac exsanguination under ether anesthesia. For histopathology investigation liver and kidney tissues were carefully collected from control and experimental groups at the end of 50th day. The tissues were immediately washed, dried, and processed for biochemical measurements. Sample tissues were then homogenized in 0.15 M ice-cold KCl for 3 minutes at 16.000 rpm with a homogenizer (Ultraturrax type T25-B, IKA Labortechnik, Staufen, Germany). Homogenates were centrifuged at 5.000 g at 4°C for 1 hour. The supernatants were collected and stored at – 40°C until used for analyses [28]. GSH and MDA levels in related tissues were measured spectrophotometrically (UV mini-1240, Shimadzu, Kyoto, Japan) using the colorimetric methods described by Beutler et al. [29] and of Yoshoiko et al. [30], respectively.

Histopathological examinations

For light microscopic examination, fresh tissue samples including the liver and kidneys were fixed in 10% neutral

Table 1. Effect of grape seed extract administration on selected biochemical parameters in albino mice treated with benzene

Parameter	Group I (Control)	Group II (GSE 50)	Group III (GSE 150)	Group IV (Benzene)	Group V (GSE 50+Benzene)	Group VI (GSE150+Benzene)	Statistical significance
AST (U/L)	167.38±7.28^{d}	168.15±980^{d}	167.84±11.23^{d}	258.26±8.09^{a}	245.54±11.73^{b}	232.52±13.53^{c}	d: p>0.05; a,b,c: <0.05
ALT (U/L)	46.53±4.42^{c}	46.98±2.93^{c}	48.33±3.71^{c}	66.17±5.42^{a}	59.50±5.52^{b}	51.16±6.60^{c}	c: p>0.05; a,b: <0.05
ALP (U/L)	161.77±8.04^{c}	162.29±12.25^{c}	166.75±6.13^{c}	191.99±5.45^{a}	181.42±7.72^{b}	171.73±7.64^{c}	c: p>0.05; a,b: <0.05
BUN (mg/L)	278.99±7.67^{d}	277.73±9.63^{d}	277.25±6.06^{d}	378.73±9.67^{a}	361.23±10.84^{b}	347.53±9.59^{c}	d: p>0.05; a,b,c: <0.05
Creatinine (mg/L)	5.50±0.46^{d}	5.50±0.51^{d}	5.42±0.40^{d}	9.69±0.66^{a}	8.34±0.67^{b}	7.06±0.65^{c}	d: p>0.05; a,b,c: <0.05

* Data are mean±SD values (n=6). Statistical significance between means was assessed using one-way analysis of variance followed by Duncan's test as a post-analysis of variance test and all variables were normally distributed. Within each line, means not significantly different share the same letter; means with no common letters are significantly different from each other.

buffered formalin solution for routine processing, embedded in paraffin wax, sectioned at 5 µm, and stained with hematoxylin and eosin (H-E). Histopathological changes were semiquantitatively assessed under the light microscope [31].

Statistical analysis

The statistical analysis software SPSS for Windows version 10.0 (SPSS, Inc., Chicago, IL, USA) was used for statistical data analysis. Statistically significant differences between the groups were compared using one-way analysis of variance and Duncan's test. The data are given as mean ±SD values, and values of P<.05 are considered statistically significant.

Results

Serum enzyme parameters

Table 1 demonstrates the changes in serum AST, ALT, ALP, BUN, and creatinine levels of all treatment groups. There were no significant differences in the levels of AST, ALT, ALP, BUN and creatinine among the control group and the groups treated with GSE alone (p>.05). Serum AST, ALT, ALP, BUN, and creatinine levels significantly increased after oral benzene exposure (p<.05). Oral administration of the two different doses of GSE decreased the enzyme activities in Groups V and VI compared with Group IV, but values were still significantly higher compared to control group. Also, GSE had an effect on BUN and creatinine levels, which are sensitive markers of kidney injure. Serum levels of BUN and creatinine significantly decreased (p<.05) in Group V and VI compared to group treated with benzene alone. In Group VI, the mean level of BUN was about 1.09-fold lower and the mean level of creatinine was about 1.37-fold lower than in Group IV.

MDA and GSH levels

Table 2 shows the alterations of GSH and MDA levels in liver and kidney tissues. No significant differences were observed among the control group, Group II and III in MDA and GSH levels of the liver and kidney tissues (p>.05). GSH levels were significantly decreased, whereas MDA levels were increased in liver and kidney tissues in Group IV, V and VI compared to Group I (p<.05). However, oral administration of GSE with two doses in Group V and VI was reversed the GSH and MDA levels back to the control levels in the liver and kidney tissues (p<.05).

Pathological findings

Similar liver and kidney histology were observed in control group and group III (Fig. 1,2). Severe histopathological changes were observed in group IV and V. In these groups, hyperemia in the central veins and sinusoids, degenerative and necrotic hepatocytes in liver; hyperemia and haemorrhagia, degeneration of tubular epithelium and intratubulary haemorrhagia in kidney were detected (Fig. 3-5). In the group VI, similar changes were observed, but

Table 2. Effect of grape seed extract administration on malondialdehyde and reduced glutathione in liver and kidneys of albino mice treated with benzene

Tissue parameter	Group I (Control)	Group II (GSE 50)	Group III (GSE 150)	Group IV (Benzene)	Group V (GSE 50+Benzene)	Group VI (GSE150+Benzene)	Statistical significance
MDA_{Liver} (nmol/g)	0.26 ± 0.03^{d}	0.26 ± 0.02^{d}	0.26 ± 0.03^{d}	0.82 ± 0.07^{a}	0.58 ± 0.06^{b}	0.43 ± 0.02^{c}	d: p>0.05; a,b,c: <0.05
MDA_{Kidney} (nmol/g)	0.28 ± 0.01^{d}	0.28 ± 0.02^{d}	0.28 ± 0.02^{d}	0.88 ± 0.03^{a}	0.70 ± 0.02^{b}	0.39 ± 0.02^{c}	d: p>0.05; a,b,c: <0.05
GSH_{Liver} (mg/g)	0.55 ± 0.04^{a}	0.53 ± 0.03^{a}	0.54 ± 0.04^{a}	0.17 ± 0.02^{d}	0.26 ± 0.03^{c}	0.37 ± 0.05^{b}	a: p>0.05; b,c,d: <0.05
GSH_{Kidney} (mg/g)	0.61 ± 0.02^{a}	0.61 ± 0.02^{a}	0.61 ± 0.03^{a}	0.20 ± 0.02^{d}	0.25 ± 0.03^{c}	0.36 ± 0.02^{b}	a: p>0.05; b,c,d: <0.05

*Data are mean±SD values (n=6). Statistical significance between means was assessed using one-way analysis of variance followed by Duncan's test as a post-analysis of variance test and all variables were normally distributed. Within each line, means not significantly different share the same letter; means with no common letters are significantly different from each other.

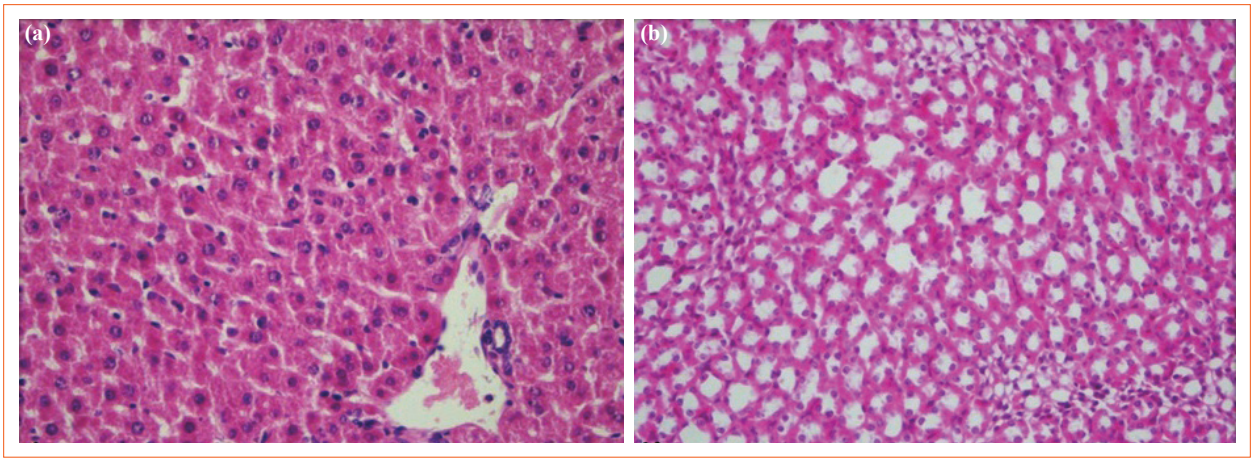

Figure 1. Group I (control group). The histological appearance of the liver **(a)** and kidney **(b)** is normal. Hematoxylin and Eosin.

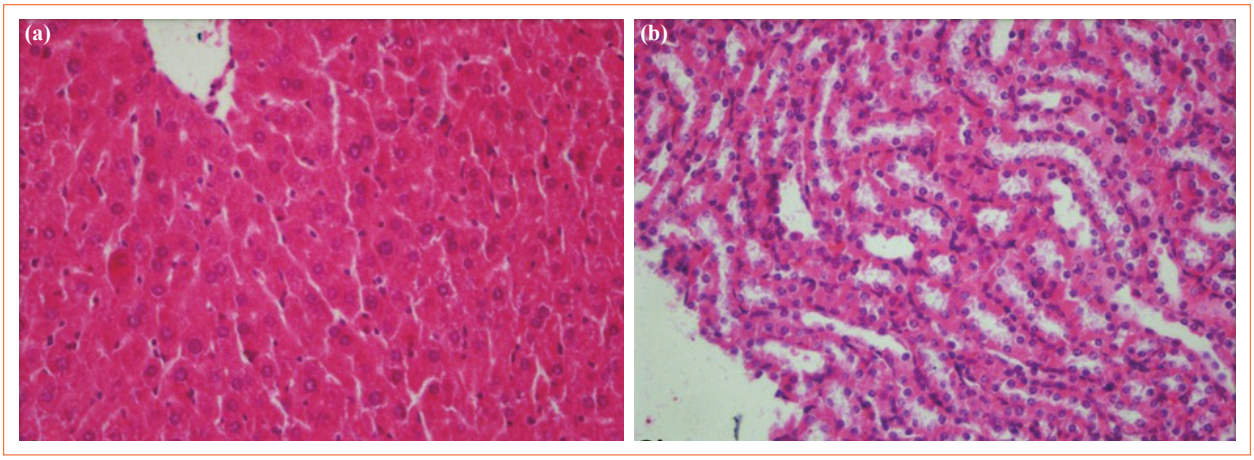

Figure 2. Group III (GSE 150). The hepatocytes **(a)** and tubules **(b)** of the liver and kidney revealed normal histological architecture. Hematoxylin and Eosin.

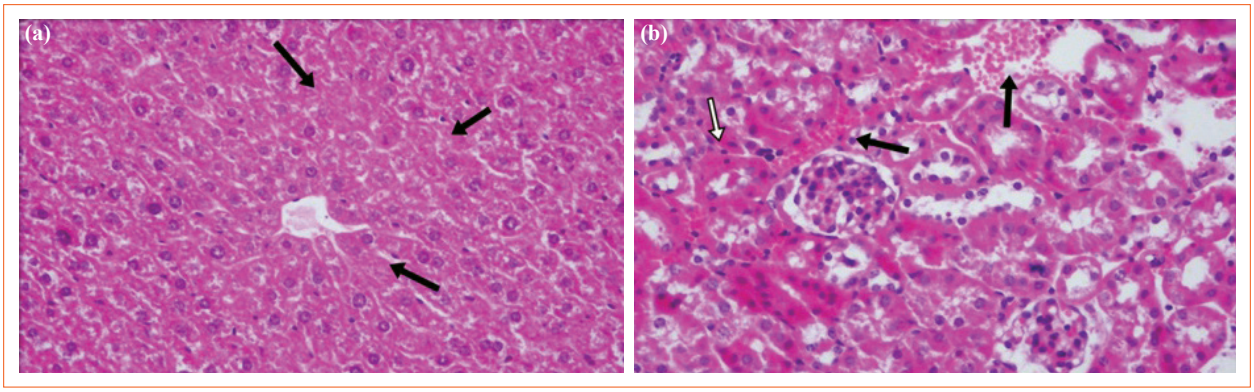

Figure 3. Group IV (Benzen). The liver showed degenerative or necrotic alterations of hepatocytes (arrows) **(a)**; the kidneys contained haemorrhagia foci (black arrows) and pyknotic nuclei of tubulary epithelium **(b)**. Hematoxylin and Eosin.

the severity of intratubular haemorrhagia was less frequently than group IV and V (Fig. 5).

Discussion

In this study the toxic effects of benzene on biochemical parameters of albino mice was investigated. AST, ALT and ALP levels of benzene treated group were increased compared to control group. This increase may be mainly due to the leakage of these enzymes from the liver cytosol into the blood stream [32]. Lipid soluble benzene is transported in the blood and absorbed by different cell membranes. It tends to accumulate in tissues and about 50% of the absorbed dose may be eliminated unchanged while the remaining is metabolized in liver [33]. In this

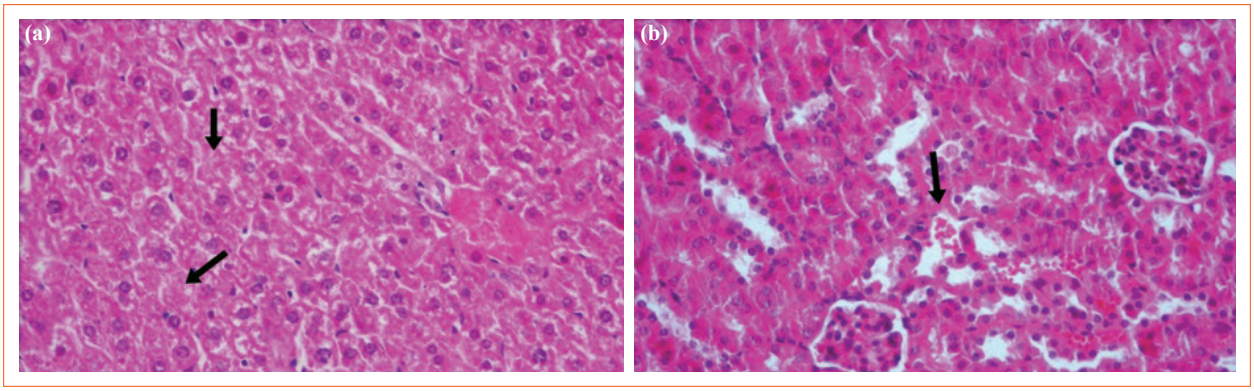

Figure 4. Group V (GSE 50+Benzen). The liver showed necrotic alterations of hepatocytes (arrows) **(a)** The kidneys contained haemorrhagia foci (black arrow) **(b)**. Hematoxylin and Eosin.

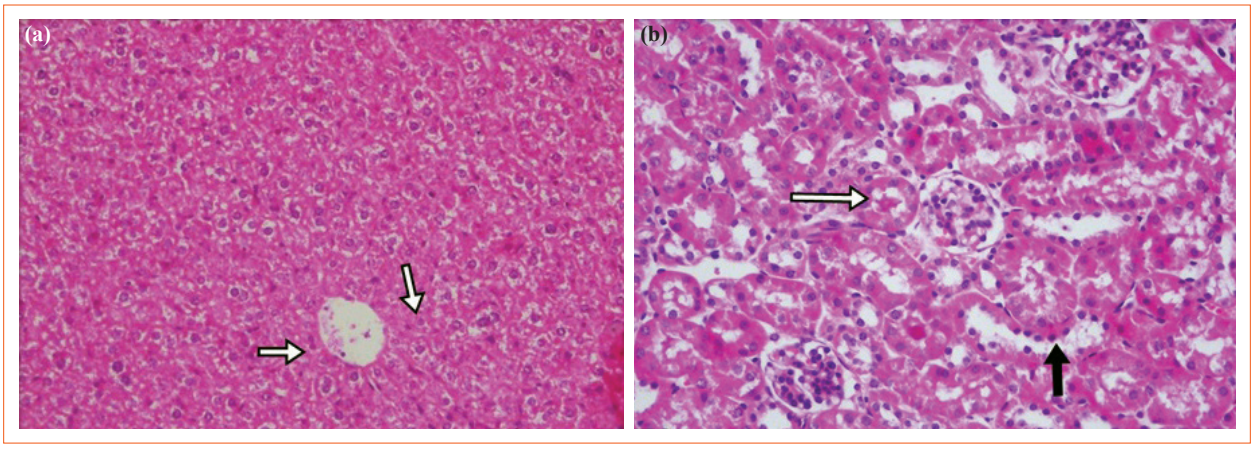

Figure 5. Group VI (GSE 150+Benzen). Hepatocytes had pyknotic nuclei (arrows) **(a)** and there was intratubular hyaline accumulation (black arrows) in the kidneys, but there was no haemorrhagia **(b)**. Hematoxylin and Eosin.

pathway liver cell membranes were selectively damaged by benzene although to different extents. When liver cells are damaged, AST, ALT and ALP enzymes released into the bloodstream [34,35] and the levels of these enzymes were increased. BUN and creatinine are nitrogenous waste products that are eliminated by kidneys, when excretion is suppressed in renal insufficiency [36]. The effect of benzene on BUN and creatinine levels has also been described by other authors [4,25]. BUN and creatinine levels in benzene treated groups were higher than in control groups.

Administration of two different doses of GSE significantly decreased the levels of AST, ALT, ALP, BUN, and creatinine in Groups V and VI compared with Group IV. Similarly, Yalcin et al. [27] reported that GSE treatment reversed the doxorubicin-induced increase in serum AST, ALT, BUN, and creatinine levels in albino mice. It was concluded that benzene administration resulted a significant increase in serum creatinine and BUN indicating significant damage to the kidney accompanied by degeneration of tubular epithelium and intratubulary haemorrhagia in kidney. The pathological changes caused kidney failure were verified from histopathology test results.

Benzene and its metabolites are known to produce oxidized species and reactive oxygen indicating the increased risk of cell membrane damage [37]. MDA is one of the most known secondary products of lipid peroxidation, and it can be used as a marker of cell membrane injury [38]. In present study, we found that the application of benzene led to a significant increase in MDA levels, which is in agreement with similar studies [39,40]. GSH and its metabolizing enzymes provide the major antioxidant defense against reactive oxygen species–induced cellular damage [41,42]. In contrast to MDA, GSH levels profoundly decreased in kidney and liver tissues. Benzene and its metabolites are known to produce oxidized species indicating the increased risk of cell membrane damage [37]. The decrease in GSH level and increase in MDA level were associated with production of radicals and increased lipid peroxidation. GSE treatment in Group V and VI had a beneficial role in benzene toxicity and the levels of MDA and GSH were neared to control levels in these groups. The potent protective effect of GSE as antioxidant in suppressing lipid peroxidation was reported before [43,44]. GSE showed potent antioxidant activity by inhibiting free radical formation, delaying lipid oxidation, and reducing the concentration of H_2O_2 produced by the oxidative stress [45]. GSE is effective in preventing

the oxidative stress associated loss of membrane surface charge which maintains the membrane integrity and function [44]. As a result, it is concluded that, the findings of the present study demonstrated the beneficial role of GSE against benzene toxicity via its antioxidant capacity. Therefore, the antioxidant role of GSE may be used as a ''toxicity-limiting agent'' to reduce effects on human health of chemical agents in the near future.

Conflict of Interest

There are no conflicts of interest among the authors.

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