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Original Article

Hepatoprotective effect of royal jelly, grape seed extract, and Lycium barbarum against diethylnitrosamine-induced liver toxicity in rats

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Abstract

Aim: We aimed to investigate, the effects of royal jelly (RJ), grape seed extract (GSE), and Lycium barbarum extract (LBAE) against diethylnitrosamine (DEN) induced hepatotoxicity, in experimental animal model.

Material and Methods: Fifty female Sprague Dawley rats were divided into five groups (n=10): Control, DEN, DEN+RJ, DEN+GSE, DEN+LBAE. DEN administrated groups were intraperitoneally (i.p.) injected with three separate administration of DEN (200 mg/kg), on the zero, fifteenth and thirtieth treatment day. Then 100 mg/kg of RJ was given to DEN+RJ group, 100 mg/kg of GSE was given to DEN+GSE group, and 400 mg/kg LBAE was given to DEN+LBAE group with the daily drinking water from day 0 for 16 weeks. Histopathologic alterations including apoptotic changes of liver were evaluated.

Results: RJ, GSE, and LBAE treatments significantly reduced weight loss induced by DEN. DEN administrated rats significantly increases malondialdehyde (MDA) level. It also efficiently decreases glutathione (GSH) level and catalase (CAT), superoxide dismutase (SOD) activity. These results were significantly ameliorated by dietary supplements ($p<0.05$). In addition, they increased the total antioxidant status (TAS) level and decreased serum oxidative stress index (OSI), total oxidant status (TOS), alanine aminotransferase (ALT), aspartate aminotransferase (AST), and gamma glutamyl transpeptidase (GGT) levels significantly ($p<0.05$). TUNEL positive cells were extremly pervasive in the livers of DEN group.

Conclusion: Improvements were prominent in case of RJ > GSE > LBAE. Our results indicated that RJ, GSE and LBAE might be useful for prevention of hepatotoxicity induced by DEN via ameliorative effects on biochemical and oxidative stress indices.

Keywords: DEN (Diethylnitrosamine); Hepatotoxicity; Royal Jelly (RJ); Grape Seed Extract (GSE); Lycium Barbarum Extract (LBAE).

INTRODUCTION

Liver is known to be the primary organ related in the metabolism, detoxification of drugs and xenobiotics. Therefore, it is the major target of tissue damage produced by chemicals. The production of reactive oxygen species (ROS) is induced by xenobiotics, and toxicants. Thus, it generates the oxidative stress and tissue injury in liver (1). Liver is also responsible for regulation of homeostasis (2). Hence, prevention of hepatotoxic damage, and necessity of effective therapeutic agents for hepatotoxicity is of great concern. On the other hand, DEN is a powerful hepatotoxic dialkylnitrosoamine. Therefore, it is often used to induce hepatotoxicity in rodents. DEN is found in alcoholic beverages, cheddar cheese, fried meals, curd, processed food, gasoline, cosmetics, and tobacco smoke (3). Furthermore, DEN is an indirect alkylating agent that induces the generation of ROS, which damage DNA through oxidation (4). Hepatotoxicity is the limiting factor for the usage of food and beverages containing DEN. Therefore, search for finding compounds or drugs to be used as complementary therapy alongside the DEN administration is helpful to reduce the DEN-induced hepatotoxicity.

Chemoprevention is a strategy which includes natural products and chemicals to decrease the risk of

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hepatotoxicity (5). Antioxidant agents perform a critical role in preventing the damage of oxidative stress by neutralizing the effect of free radicals on cellular components (6). Some natural products as RJ, GSE, and LBAE were reported to have a protective role against oxidative damages. In addition, these products have antioxidative capability and scavenging capacity against free radicals (7-9). RJ has been reported to possess various bioactive components which reduce liver damage and induce proliferation of hepatocytes. RJ is composed of vitamins, proteins, lipids, carbohydrates, minerals, and water. Also, RJ has demonstrated affirmative effects such as antioxidant, antiviral, anti-inflammatory, antimicrobial, anti-allergic, and wound healing activities (10). GSE has been proposed as a promising immunomodulator agent, particularly due to its proanthocyanidin content, and is a naturally occuring polyphenolic compound obtained from seeds of Vitis vinifera. GSE is consisting of polyphenols, catechin, epicatechin and procyanidin. Furthermore, GSE has a plenty of favorable effects such as antioxidants, anti-mutagenic, cardioprotective, and anti-inflammatory (11). LBAE, a renowned functional food and medicinal plant from Southeast Asia, exhibits hepatoprotective properties. Also, it has been demonstrated that dry fruit of LBAE has various functions including antioxidative, anti aging, antitumor, anti-inflammatory, and hepatoprotective activities (12).

Therefore, this study purposed to determine the hepatoprotective effects and antioxidant profile of dietary supplementation of RJ, GSE and LBAE against DEN, based on biochemical parameters, oxidative stress, and histopathologic alterations.

MATERIAL and METHODS

Chemicals and samples

DEN was purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). All other chemicals were purchased from commercial suppliers and were of the highest grade. RJ was obtained from Macahel Apiculture Co. Ltd. (Artvin, Turkey). The RJ has 67% of humidity and 33% of dry matter, of these, 14.19% are crude protein, 2.01% are lipids, 0.87% are ash and 15.93% are carbohydrates. GSE was purchased from Berkem SA, Gardonne, France. It was provided in a form of standardised extract including +90% oligomeric proanthocyanidin. Dried LBAE was purchased in raw powder form (acquired from Beijing Tongren Tang Co., Ltd., Beijing, China). The constituents were as follows: 9.2% total sugar, 1.9% reducing sugar, and 9.4% total protein.

Experimental animals

In this experimental study, 50 female Sprague Dawley rats aged about 10-12 wk (230-260 g) were used for our study. They were housed under standard environmental conditions of light (12:12 h light-dark cycle), temperature (24 ± 2 °C) and humidity (60% ± 10%), and were fed with a standard rat chow and water. The protocol of this study was approved by Experimental Animal Research Center of Inonu University (Malatya, Turkey). Additionally, all procedures involving the animals were in agreement with the guidelines of the Ethical Committee for research on laboratory animals at Inonu University (No: 2013/A-67).

Experimental design

Fifty female Sprague Dawley rats were divided into five experimental groups as follows: group 1, Control; group 2, DEN; group 3, DEN+RJ; group 4, DEN+GSE; group 5, DEN+LBAE. All the DEN administrated groups were i.p. injected with three separate administration of DEN (200 mg/kg body weight, dissolved in olive oil), on the zero, fifteenth and thirtieth treatment day of the 16-week experimental period (13). DEN+RJ group fed with water supplemented with 100 mg/kg of RJ (14), DEN+GSE group fed with water supplemented with 100 mg/kg of GSE (15, 16), DEN+LBAE group fed with water supplemented with 400 mg/kg of LBAE (17, 18) from day 0 for 16 weeks. RJ, GSE and LBAE were added to the daily drinking water. The treatment course lasted 16 weeks for all groups. All rats were sacrificed under anesthesia using ethyl ether at the end of the 16th week. Blood samples were collected; liver tissues were removed by dissection, and preserved at -80 °C until analysis.

Biochemical analysis and oxidant-antioxidant parameters

The CAT activity was assessed using the Aebi's method. Hydrogen peroxide gives maximum absorbance at 240 nm. This absorbance is commensurate to the CAT enzyme activity (19). The SOD activity was examined using the Sun's method (20) with a minor modification (21). The amount of MDA was determined by the method of Draper and Hadley (22). The amount of protein in the homogenized samples was carried out by using the Lowry method. GSH content was determined using the Ellman's method by measuring the absorbance of reaction at 412 nm using a spectrophotometer (23).

Serum activities of standard liver function tests; ALT, AST, and GGT were measured by colorimetric kits (Wiesbaden, Germany) using spectrophotometer (UNICO Instruments C., Model 1200, USA).

TAS, TOS, and OSI determination

TAS, TOS, and OSI levels were measured spectrophotometrically using Erel method. The Rel Assay Diagnostics Test Kit was used to determine the levels of TAS, TOS, and OSI in serum samples (Mega Tıp San. ve Tic. Ltd. Şti., Gaziantep, Turkey). Measurement of OSI was calculated as follows: OSI (arbitrary unit, AU) = [(TOS, μmol H2O2Eq./l)/(TAS, μmol TroloxEq./l)]x100 (24-26).

Terminal deoxynucleotidyl transferase-mediated deoxy-uridine-triphosphate (dUTP) nick end labeling (TUNEL) assay.

Cross sections from paraffin blocks (5-μm) were taken on poly-L-lysine coated slides. Cells entering apoptosis were determined by Apop-Tag plus Peroxidase in Situ Apoptosis Detection Kit (Chemicon, cat no: S7101, USA). In the assessment of TUNEL staining, cells with blue nuclei were normal, while brown nuclear staining was admitted as indicating apoptotic cells. Percentages of TUNEL-positive cells were quantified by counting 400 cells from ten random microscopic fields. Apoptotic index was calculated as follows: a ratio of the TUNEL positive cell number to the total cell number (normal + apoptotic cells) (27).

Statistical Analysis

The SPSS software for Windows, version 20.0 was used for statistical analyses. Body weight gain/loss data were analyzed by paired-samples T-test. All groups were compared with the paired-samples T-test at the initial of study and final of the study. Statistical analyses for biochemical parameters were evaluated using ANOVA and post-hoc LSD tests to assess significance. The results were given as mean ± SEM. Histopathological analysis was presented as mean + standard deviation (SD).
Differences in measured parameters among the groups were analyzed with a nonparametric test (Kruskal-Wallis). Dual comparisons between groups exhibiting significant values were evaluated with Mann-Whitney U test. The significance was acceptable to a level of $p \leq 0.05$.

RESULTS

Body weight measurements showed that during the 16 weeks animals grew from 237.71 g at day one to 256.57 g for the control group, 239.28 g at day one to 229.42 g for the DEN group, 236.00 g at day one to 245.14 g for the DEN+RJ group, 233.57 g at day one to 248.28 g for the DEN+GSE group and 236.28 g at day one to 252.42 g for the DEN+LBAE group at the last day of application (Table 1: paired-samples T-test for the body weight at the last day of application, p=0.010, p=0.028, p=0.022, p=0.001, and p=0.030, respectively). There was a significantly increased total body weight gain in the control, DEN+RJ, DEN+GSE, and DEN+LBAE treatment groups ($p<0.05$). However, DEN group was observed to have a significantly decreased weight gain (p=0.028) (Table 1).

Table 1. Body weights (g) of animals during treatment

Design of treatment	Control	DEN	DEN+RJ	DEN+GSE	DEN+LBAE
Initial of Study	237.71±6.98	239.28±5.51	236.00±3.36	233.57±1.10	236.28±6.69
Final of Study	256.57±4.81	229.42±4.96	245.14±5.13	248.28±1.41	252.42±4.49
Statistical comparison (Initial of study vs final of study)			(p)		
Control			0.010		
DEN			0.028		
DEN+RJ			0.022		
DEN+GSE			0.001		
DEN+LBAE			0.030		

Changes in the body weight of experimental rats. Values are expressed as mean ± SEM of ten animals. The groups were compared with the paired-samples T-test at initial and final of the treatment. p≤0.05. Abbreviations: DEN, diethylnitrosamine; GSE, grape seed extract; RJ, royal jelly; LBAE, lycium barbarum extract; DEN+RJ: 200 mg/kg DEN+100 mg/kg RJ; DEN+GSE: 200 mg/kg DEN+100 mg/kg GSE; DEN+LBAE: 200 mg/kg DEN+400 mg/kg LBAE

The results as shown in Tables 2, DEN group showed a significantly higher level of MDA (p=0.009) and significantly lower levels of GSH (p=0.047), SOD (p=0.007), and CAT (p=0.013) activities when compared to the control group (all, $p<0.05$).

RJ, GSE and LBAE treatments produced a significant decrease in the DEN-stimulated MDA level (all, $p<0.05$). These dietary supplements treatment produced a significant increase in the DEN-reduced GSH, SOD, and CAT activities (all, $p<0.05$).

MDA level decreased significantly in the DEN+RJ (p=0.002), DEN+GSE (p=0.003), and DEN+LBAE (p=0.012) groups. SOD activity increased significantly in the DEN+RJ (p=0.005), DEN+GSE (p=0.004), and DEN+LBAE (p=0.003) groups.

CAT activity increased significantly in the DEN+RJ (p=0.006), DEN+GSE (p=0.007), and DEN+LBAE (p=0.008) groups. GSH activity increased significantly in the DEN+RJ (p=0.009), DEN+GSE (p=0.005), and DEN+LBAE (p=0.007) groups.

Table 2. Effects of RJ, GSE and LBAE on liver lipid peroxidation and antioxidant profile in a model of DEN-induced toxicity

	STUDY GROUPS				
	Control	DEN	DEN+RJ	DEN+GSE	DEN+LBAE
Biochemical Parameters (Mean ± SEM					
MDA (nmol/g)	809.46±29.48*	926.69±15.38	772.86±30.89*	797.47±32.70*	819.86±34.02*
GSH (µmol/g)	720.02±19.23*	635.48±25.88	882.20±28.77*	813.96±27.63*	746.66±33.12*
SOD (U/g)	236.20±18.41*	160.21±22.67	352.05±17.14*	265.34±15.10*	241.17±9.67*
CAT (K/g)	209.43±21.40*	132.04±16.03	294.88±26.50*	249.81±17.27*	234.13±22.74*

Each group represents the mean ± SEM for ten rats. *p < 0.05 when compared to DEN group. Abbreviations: DEN, diethylnitrosamine; RJ, royal jelly; GSE, grape seed extract; LBAE, lycium barbarum extract; MDA, malondialdehyde; GSH, glutathione; SOD, superoxide dismutase; CAT, catalase

We measured levels of biochemical parameters in serum and the results are shown in Table 3. ALT, AST, and GGT levels were significantly increased in the DEN group compared to the control, DEN+RJ, DEN+GSE, and DEN+LBAE groups ($p<0.05$). AST, and GGT levels were significantly lower in the DEN+LBAE group compared to the DEN+RJ group, while the ALT level was increased ($p<0.05$). GGT level was significantly lower in the DEN+GSE group compared to the DEN+RJ group ($p<0.05$) (Table 3).

Ameliorative effects of RJ, GSE and LBAE treatment against DEN administration significantly increased the TAS level and decreased TOS and OSI levels ($p<0.05$). The DEN group had a significantly lower TAS level compared to the control, DEN+RJ, DEN+GSE and DEN+LBAE groups ($p<0.05$). TOS level was significantly higher in the DEN group compared to the control, DEN+RJ, DEN+GSE, and DEN+LBAE groups ($p<0.002$). OSI level was significantly higher in the DEN group compared to the control, DEN+RJ, DEN+GSE, and DEN+LBAE groups ($p<0.05$) (Table 3).

Table 3. Comparision of serum biochemical and serum oxidative stress parameters among the study population

	STUDY GROUPS				
	Control	DEN	DEN+RJ	DEN+GSE	DEN+LBAE
Biochemical Parameters (Mean ± SEM					
ALT (U/L)	24.83±3.47*	49.50±3.56	33.14±2.65*	41.50±2.47	44.71±5.83
AST (U/L)	76.16±3.99*	114.70±7.98	61.00±2.88*	68.50±3.40*	74.85±1.54*
GGT (U/L)	5.16±0.47*	8.30±0.47	4.57±0.20*	5.60±0.42*	7.42±0.20
Total Oxydant/Antioxydant (Mean ± SEM)					
TAS (mmol/L)	2.55±0.27*	1.35±0.13	3.43±0.20*	2.98±0.25*	2.72±0.24*
TOS (µmol/L)	8.80±1.02*	14.93±0.90	8.79±0.56*	9.05±0.59*	9.85±0.47*
OSI (AU)	0.67±0.15*	1.85±0.59	0.43±0.03*	0.55±0.05*	1.00±1.43*

Each group represents the mean ± SEM for ten rats. *p < 0.05 when compared to DEN group. Abbreviations: DEN, diethylnitrosamine; GSE, grape seed extract; RJ, royal jelly; LBAE, lycium barbarum extract; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyltranspeptidase; TAS, total antioxidant status; TOS, total oxidant status; OSI, Oxidative stress index; AU: Arbutrary Units

Determination of apoptosis in liver tissue

The apoptotic index results are shown in Table 4; Figure 1.

The apoptotic hepatic cells were defined by TUNEL assay in the liver sections; control (Figure 1A) group demonstrated only several TUNEL-positive cells.

The number of TUNEL-positive cells prominently increased in the DEN group (Figure 1B) compared with the control group ($p<0.05$). DEN+RJ (Figure 1C), DEN+GSE (Figure 1D) and DEN+LBAE (Figure 1E) groups were similar and demonstrated rare TUNEL-positive cells.

Antioxidant treatment (DEN+RJ, DEN+GSE and DEN+LBAE groups) (Figure 1C, 1D and 1E) decreaced the number of TUNEL-positive cells as compared with the DEN group ($p<0.05$).

Table 4. Effects of RJ, GSE and LBAE against DEN on apoptotic index (%) in rat liver

Groups	Apoptotic Index (%) (AI; mean ± SD)
Control	2.65±0.92*
DEN	14.60±1.20
DEN+RJ	6.80±1.20*
DEN+GSE	4.80±2.10*
DEN+LBAE	7.50±1.75*

The apoptotic index of all the groups. Values are mean ± SD for ten rats in each group. *p < 0.05 when compared to DEN group. Abbreviations: DEN, diethylnitrosamine; RJ, royal jelly; GSE, grape seed extract; LBAE, lycium barbarum extract

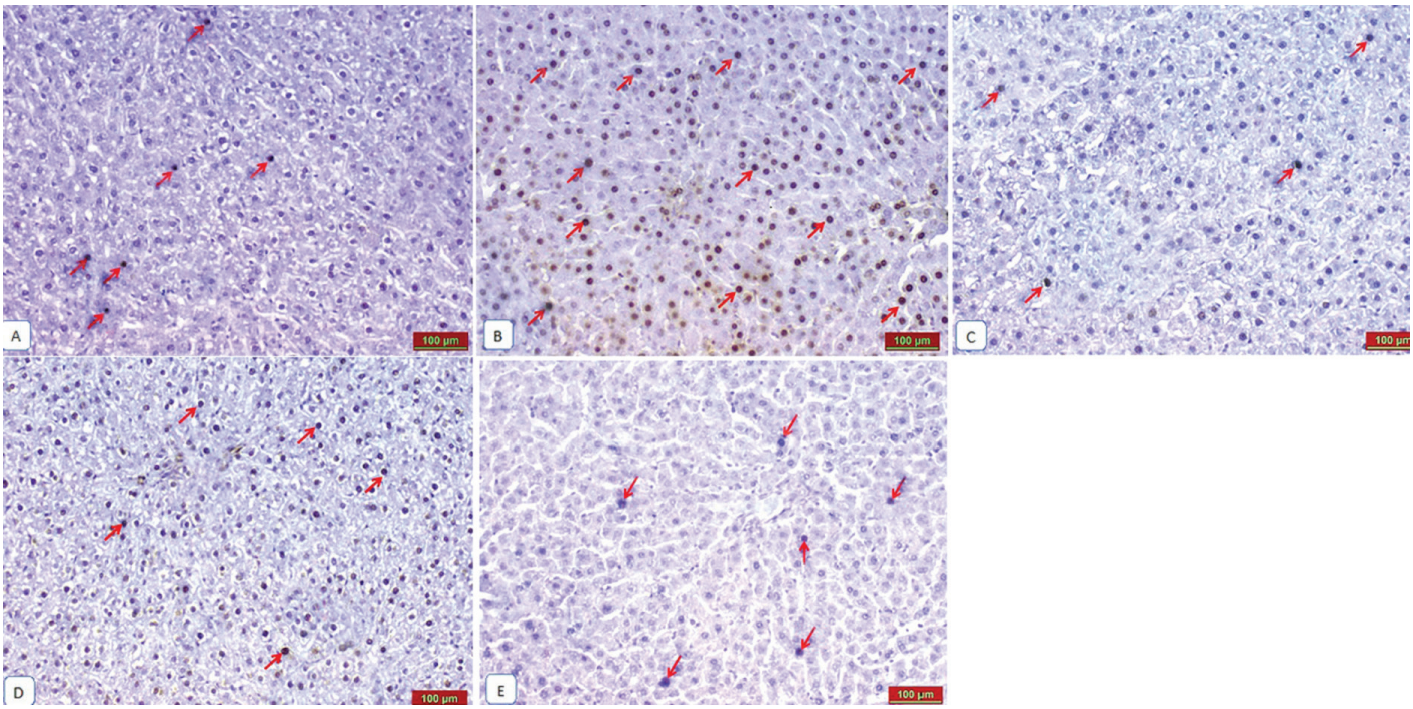

Figure 1. Representative photomicrographs of TUNEL staining in all five groups (scale bars=100 µm), showing: (A) Group 1 (control) only few TUNEL-positive cells (arrow); (B) Group 2 (DEN) a lot of TUNEL-positive cells (arrows); (C) Group 3 (DEN+RJ), (D) Group 4 (DEN+GSE) and (E) Group 5 (DEN+LBAE) similarly rare TUNEL-positive cells (arrows). This analysis was exerted in at least eight areas of each liver section (two sections/animal), and the sections were analyzed at 400× magnification. The evaluation of TUNEL staining was exerted based on the extent of the staining of apoptotic cells. The extent of TUNEL staining was scored semiquantitatively as 0 (no), 1 (light), 2 (medium), and 3 (intense)

DISCUSSION

This study was performed to investigate, the role of RJ, GSE, and LBAE in decreasing liver damage and oxidative stress state produced as a result of DEN-induced liver toxicity in rats. The herbal medications have been used effectively to prevent and treat diseases, including hepatotoxicity for centuries. Hence, RJ has been widespreadly supported as a useful therapeutic, and a health food in the world (28). In the recent years there has been an increased interest in the research of isoflavones and proanthocyanidin-rich GSE. Therefore, it appears that GSE can be used as a promising agent for preventing liver damage (29). In support of traditional properties, the latest studies have documented that LBAE fruit have a series of bioactive effects, such as antioxidant properties, antitumor activity and cytoprotection (30). On the other hand, DEN has been associated with liver damage in rats, and the mechanisms of DEN-induced hepatotoxicity are less elucidated. Furthermore, administration of DEN could cause DNA damage and hepatocytes cell death, resulting in hepatotoxicity (31).

Chemical-induced liver damage is related with alterations in oxygen radical metabolism of liver. The results of our study demonstrated that administration of DEN caused significant elevation of MDA and decreased activities of SOD, CAT, and level of GSH that were approved by histopathological findings (Figure 1). These biochemical changes were significantly ameliorated after RJ, GSE, and LBAE treatment to DEN-induced toxicity (Figure 1; Table 2). These favorable changes were considerable in case of RJ > GSE > LBAE.

Lipid peroxidation is considered as an end product determined and a widely used indicator of oxidative stress. It has been reported that administration of DEN causes increased lipid peroxidation (32). Accordingly, in our study following DEN administration there was a significant increase in the level of lipid peroxidation. Elevated MDA level considered an increased lipid peroxidation leading to liver injury and depletion of antioxidant protection mechanisms. Thus RJ, GSE, and LBAE treatment provide protective effect against DEN-induced liver damage in terms of preventing lipid peroxide formation and blocking oxidative chain reaction. This investigation shows that these dietary supplements, to some extent, might cause hepatoprotection by decreasing the oxidative stress.

Major studies have demonstrated that DEN diminished activities of antioxidative enzymes (33). In this study, significant reduction in antioxidant enzyme (SOD, CAT) activities showed oxidative stress of the liver by DEN. We reported that these dietary supplements considerably increased the levels of SOD and CAT, and preserved the liver from DEN, demonstrating that prevention of the oxidative cascading stress was one of the main mechanisms in DEN-induced liver damage. It is reported that RJ, GSE, and LBAE enhanced synthesis of the endogenous antioxidant enzymes (34). These results denoted that these dietary supplements have been evidenced to have ROS scavenging and antioxidant features. GSH is an intracellular and extracellular preventive antioxidant which performs a key role in cell. Our observations also showed that RJ, GSE, and LBAE supplementation prevented the

level of GSH decrease and reduced DEN-induced hepatic GSH depletion. Moreover, a decline in the levels of GSH and GSH dependent enzymes, due to DEN administration might be caused by decline in the biosynthesis of these antioxidants during hepatocellular damage or extreme reduction of the antioxidants due to their utilization during the metabolism of DEN as scavenging agents against the free radicals formed (35). These agents may preserve liver against DEN-induced toxicity by upregulation of SOD, and CAT activities and GSH levels. It is well-established that these dietary supplements play an important role as an indirect antioxidant and increases the activities of antioxidant enzymes such as SOD, CAT activities and GSH levels. Hence, it seems that pretreatment with dietary supplements may reduce the damage in DEN-induced hepatotoxicity via induction of antioxidant activity.

We assessed the serum concentrations of ALT, AST, and GGT to investigate liver functions. Also, their decreased levels in the liver tissues and increased serum levels could be owing to toxic compounds affecting the liver tissues (36). The results of our study displayed that DEN administration leads to liver injury in rats. Accordingly, serum ALT, AST, and GGT levels were markedly increased, as in preceding studies (37). On the contrary, treatment of RJ, GSE, and LBAE markedly inhibited liver injury as proved by diminished serum ALT, AST and GGT activities. These results proposed that they could be beneficial in reversing the liver toxicity induced by DEN. Also, previous studies were reported that they preserved from damage of oxidation by modulating the expression of antioxidant defense systems. The result of this study showed that after administration of dietary supplements the activities of the serum marker enzymes were restored to normal level, thus indicating that they preserved the structural integrity of hepatocellular components and protected the liver from the harmful effect of this hepatotoxin. On the other hand, it is well established that these dietary supplements exert antioxidant effects directly by scavenging free radicals or indirectly by increasing the antioxidant enzymes (38).

Oxidative stress is one of the main mechanisms of liver injury. We measured TAS, TOS, and OSI levels to appraise the oxidative stress. The results indicated that serum TAS levels increased and TOS, OSI levels decreased with RJ, GSE, and LBAE treatment as in the previous studies (39). In addition, we have observed that these dietary supplements could protect liver from DEN-induced liver injury. The useful effects of them, obtained in this study, are very likely due to their potent antioxidant features and may be associated with their constituent compounds. This implies that these dietary supplements presented a hepatoprotective activity probably due to their antioxidant capacity. The restoration of tissue antioxidant function by them may be attributed to its ability to upregulate antioxidant gene expression.

The present study indicated that DEN damaged histological structure, impaired function and inhibited antioxidant system in liver tissue. Therefore, DEN-induced liver injury causes increased ROS formation and subsequent toxic events. Accordingly, in our study RJ, GSE, and LBAE treatment of the cells against DEN exposure, the apoptotic cell injury and death were greatly rescued (Figure 1; Table 4).

The underlying mechanism of protective effect of them may be related with the prevention of apoptosis via death receptor-mediated pathways. Hence, it can be assumed that the antioxidant activity of these dietary supplementations may be due to the effect on mitochondria-independent apoptotic pathway which has to be supported with further experimentations.

In this study we measured the initial and final body weight of experimental animals. RJ, GSE, and LBAE treatment caused an increase in body weight of the animals as compared to DEN administrated rats. Hence, these dietary supplements significantly prevent DEN-induced weight loss, which might suggest a potential effectiveness on human subjects. Therefore, they are safe compounds for co-administration with hepatotoxic agents-induced weight loss without influencing their therapeutic action. In addition, these results are certain to influence the choice of protective compound when several options exist and to institute preemptive strategies for weight management (40).

This study demonstrated the biological evidences and encouraged the use of RJ, GSE, and LBAE in the treatment of chemical induced hepatotoxicity. They protect against DEN-induced hepatic damage by protecting cellular integrity, preventing oxidative stress and lipid peroxidation, improving antioxidant enzymes activities and inhibition of the damaged histological structure. It is seen that they have adequate ameliorative effects on recovery of liver damage. These favorable changes were prominent in case of RJ > GSE > LBAE. These results have shed some light on the clinical therapeutic potential of these dietary supplements against hepatotoxic agents. More studies should be performed similar to this research in order to point out a certain

CONCLUSION

Therefore, future studies are needed to unveil the cellular mechanisms responsible for the therapeutic effect of these dietary supplements.

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Ethical approval: Additionally, all procedures involving the animals were in agreement with the guidelines of the Ethical Committee for research on laboratory animals at Inonu University (No: 2013/A-67)..

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