

Evaluating the Renal and Splenic Protective Effects of Grape Seed Proanthocyanidin Extract in Diethylnitrosamine-Induced Male Sprague Dawley Rats

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ABSTRACT



Proanthocyanidin extract (GSPE) extracted from grape seeds is a rich and diverse source of proanthocyanidins, which have demonstrated beneficial effects on various physiological processes. The objective of this study was to assess the impact of proanthocyanidins on renal and splenic conditions induced by diethyl nitrosamine (DEN) in rats. Four groups of eight-week-old male Sprague Dawley (S.D) rats were established: the first group served as the normal control, the second group was administered DEN at a dose of 200 mg/kg intraperitoneally (IP), the third group received the same DEN dose as the second group followed by GSPE administered via intragastric lavage (300 mg/kg body weight per day), and the fourth group received 300 mg/kg body weight per day of GSPE by intragastric administration. The results revealed a significant decrease in albumin (Alb) and total protein (TP) levels in the DEN group, while treatment with GSPE led to an improvement or normalization of these parameters. In the DEN group, urea (Ur) and creatinine (Cr) levels were significantly elevated, and GSPE treatment resulted in an improvement or restoration of these values to normal levels. Significant elevations were observed in alanine aminotransferase (ALT), aspartate aminotransferase (AST), and total bilirubin (TB) levels. Treatment with GSPE showed improvement in kidney tissues upon microscopic examination. In the DEN group, there was evidence of glomerular shrinkage with a wide capsular gap and debris in the renal tubule lumen. Additionally, the spleen tissue exhibited poorly defined boundaries, congestion in the splenic artery, and reduced cellularity of the red pulp when examined microscopically in the DEN group; however, significant improvements were noted in the GSPE-treated group.

Keywords: Ameliorative effect; Antioxidant properties; DEN; Grape seed; Oxidative stress; Proanthocyanidin extract.

INTRODUCTION

Diethylnitrosamine (DEN) is a potent carcinogenic compound belonging to the nitrosamine family, known for its ability to induce cancer in various organs, particularly the liver (Mansour *et al.*, 2019). It is a direct-acting alkylating agent that can cause DNA damage through the formation of alkyl adducts, leading to mutations and carcinogenesis (Canxia and Xiaohong, 2022). DEN is widely used in research settings to induce experimental carcinogenesis in animal models, making it a valuable tool for studying cancer development and progression (Sánchez-Meza, *et al.*, 2023). Its carcinogenic effects are attributed to the production of reactive oxygen species (ROS) and the generation of DNA-damaging metabolites (Hanahan *et al.*, 2000). Apart from its carcinogenic properties, DEN is also known to induce oxidative stress, inflammation, and organ damage in tissues such as the liver, kidneys, and spleen (Fishbein *et al.*, 2021). Studies investigating the mechanisms of DEN toxicity have highlighted its role in promoting cell proliferation, inhibiting apoptosis, and disrupting cellular homeostasis. Research focusing on DEN toxicity provides valuable insights into cancer biology, toxicology, and chemoprevention, contributing to the development of novel therapeutic interventions for cancer and other related diseases (George *et al.*, 2021).

Proanthocyanidins are a subgroup of flavonoids, which are plant-based polyphenolic compounds known

for their potent antioxidant properties (Hassanpour and Doroudi, 2023). These compounds are commonly found in various plant sources, with grape seeds (Rodríguez-Pérez, 2019) pine bark, and certain fruits like berries being rich natural sources of proanthocyanidins (Park *et al.*, 201; Isaac *et al.*, 2023). Proanthocyanidins are characterized by their chemical structure, which consists of flavan-3-ol units linked together through carbon-carbon bonds (Qi *et al.*, 2023). The degree of polymerization can vary, leading to different forms of proanthocyanidins ranging from dimers to polymers. This structural diversity influences their bioavailability and biological activities.

These compounds have been studied for their antioxidant, anti-inflammatory, and cytoprotective effects. Proanthocyanidins are known to scavenge free radicals, reduce oxidative stress, and protect cells from damage caused by reactive oxygen species. Additionally, they have been associated with various health benefits, including cardiovascular protection, immune modulation, and potential anticancer properties (Huang, 2023). Research on proanthocyanidins has highlighted their role in promoting vascular health by improving endothelial function, reducing inflammation, and lowering blood pressure. These compounds have also shown promise in protecting against neurodegenerative diseases, improving skin health, and enhancing overall well-being. Proanthocyanidins are abundant in grape seeds, both in terms of number and variation. These are

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industrial byproducts made from entire grape seeds that are highly concentrated in phenolic procyanidins, flavonoids, linoleic acid, and vitamin E (Ghafoor *et al.*, 2020). In numerous animal models and cancer cell cultures, GSPE has chemopreventive properties. One of the most effective free radical scavengers, it also lowers cholesterol, hyperlipidemia, and hypertension and has cardioprotective, neuroprotective, anti-inflammatory, anti-mutagenic, anti-carcinogenic, and antineoplastic properties. It also improves working memory and lessens Alzheimer's disease symptoms.

The adverse effect profile of GSPE is still unclear, despite the fact that it has demonstrated potential effects toward disease prevention and protective effects against several cancers. One member of the nitrosamine family, diethylnitrosamine (DEN) finds application in the manufacturing of gasoline, lubricant additives, stabilizers in plastics, solvents in the fiber industry, tobacco smoke, ground water, cured and fried foods, cheddar cheese, alcoholic beverages, pharmaceutical products, agricultural chemicals, occupational settings, and cosmetics. It can also be obtained through the metabolism of certain medicinal drugs (Gupta *et al.*, 2010).

Animals exposed to DEN, both as neonates and as adults, developed cancer. The main organs affected were the liver, kidney, respiratory system, and digestive tract. It produced malignancies at many tissue sites, in multiple experimental animal species, and through multiple exposure methods (Shirakami *et al.*, 2012). Rats who received DEN orally, intravenously, intraperitoneally, or during pregnancy had kidney and spleen tumors (Poornima and Gopalakrishnan, 2014). The evaluation of the impact of proanthocyanidin extract from grape seed on male Sprague Dawley rats' kidneys and spleens injected with Diethylnitrosamine (DEN) presents a compelling avenue for research in the field of preventive medicine and pharmacology. Diethylnitrosamine (DEN) is a known carcinogen that induces oxidative stress and cellular damage, particularly in vital organs such as the kidneys and spleen. Therefore, this study was conducted to investigate whether proanthocyanidin extract from grape seed can exert protective effects against DEN-induced toxicity in the kidneys and spleens of male Sprague Dawley rats. The study also will highlight the therapeutic potential of proanthocyanidin extract from grape seed in combating chemically induced organ damage.

MATERIALS AND METHODS

Experimental animals

Twenty-four male 8-week-old Sprague Dawley (S.D.) rats were divided into 4 groups for this investigation and were procured from the King Fahd Center for Medical Research at King Abdulaziz University in Jeddah, Saudi Arabia. The rats were housed in a room maintained at a temperature of 22 ± 2 °C with a 12-hour light/dark cycle and a humidity level of 55%. They were provided ad libitum access to food

and water throughout the duration of the trial. All aspects of the experiment were conducted in accordance with the guidelines outlined by the National Committee for Bioethics under No. (HAO-02-T-105) at Taif University, Ministry of Education, Saudi Arabia.

Rats groups and treatment techniques

These experimental groups were designed to assess the impact of proanthocyanidin extract from grape seed on the kidneys and spleens of male Sprague Dawley rats in the presence of Diethylnitrosamine (DEN) exposure. The experimental groups for the study were structured as follows: Group I (Normal Control): Rats receiving no treatment, serving as the control group. Group II (DEN Only): Rats injected with Diethylnitrosamine (DEN) at a dose of 200 mg/kg body weight intraperitoneally (IP) as a single dose at the beginning of the experiment. Group III (DEN + GSPE): Rats injected with Diethylnitrosamine (DEN) at 200 mg/kg body weight (IP). and treated with Grape Seed Proanthocyanidin extract (GSPE) at a dose of 300 mg/kg body weight intragastrically (Ig). Group IV (GSPE Only): Rats treated with Grape Seed Proanthocyanidin Extract (GSPE) at a dose of 300 mg/kg body weight (Ig) twice per week.

Chemicals and Doses Used in the Study

Ultra pure Diethylnitrosamine (DEN) (purchased from Sigma-Aldrich, USA) was used. Diethylnitrosamine (DEN), purchased from Sigma-Aldrich, was used. Rats were given an intraperitoneal (IP) dose of 200 mg/kg b.wt. of DEN dissolved in normal saline solution (0.9%) as described by Solt & Farber (1976). Grape Seed Proanthocyanidin Extract (GSPE) with the chemical formula $C_{30}H_{12}O_6$, CAS No. 84929-27-1, Arab Company for Pharmaceuticals & Medicinal Plants (MEPACO MEDIFOOD, Enshas ElRaml-Sharkeya-Egypt) provided highly refined GSPE for commercial application. From two days following DEN injection, animals were given 300 mg/kg b.wt. of GSPE dissolved in physiological 0.9% saline twice a week.

Sample collection after treatment

After an overnight fast, diethyl ether was used to anesthetize all the experimental animals. Blood samples were collected from the experimental rats using standard venipuncture techniques. To acquire plasma and serum, each rat's abdominal aorta was harvested and its blood placed in non-EDTA glass tubes. Centrifugation was used to separate the serum for ten minutes at 3000 rpm. The obtained serum was kept cold at -18 °C until it was subjected to biochemical examination. The kidney and spleen were instantly removed and washed in cold saline before being sectioned and preserved in 10% buffered formalin for histological examinations.

Hematological Parameters

The collected blood samples were subjected to hematological analysis to evaluate various parameters including Hemoglobin (Hb), Red Blood Cell Count (RBC), Platelet Count, Hematocrit red blood cell volume (HCT), mean Cellular Volume (MCV), Cellular Hemoglobin Concentration (MCHC), Lymphocytes (LYM), Monocytes (MON) and Granulocytes (GR). Hematological parameters were analyzed using automated hematology analyzers to ensure accuracy and reliability of the results.

Histopathological examination

A histopathological investigation was performed using the protocol described by Yoon *et al.* (2001). In order to evaluate

and define any structural alterations, anomalies, or pathological findings within the kidneys and spleens of the experimental rats, a thorough microscopic examination of tissue samples was conducted. The objective of the histopathological examination was to offer significant information regarding the morphological changes and any histological damage caused by the experimental treatments. This information was then utilized to assess the overall health of the organs and the effects of the treatments throughout the study.

Biochemical analyses:

The levels of aspartate (AST) and alanine (ALT) aminotransferases were determined following the protocol outlined by Schumann and Klauke (2003). Total bilirubin (TB) was assessed using the procedures described by Abd Elhalem *et al.* (2016). Total protein (TP) was quantified using the Biuret method developed by Doumas in 1975. Albumin (Alb) levels were measured using the method established by Doumas *et al.* (1997). Urea (Ur) levels were determined in accordance with the procedure outlined by Mohamed and Ashour (2019), while creatinine (Cr) concentrations were measured following the method described by Slot (1965).

Data analysis

The data obtained were replicated and recorded as means \pm SE. One-way analysis of variance (ANOVA) was utilized to assess the impact of proanthocyanidin extract (GSPE) from grape seed in ameliorating the effects of Diethylnitrosamine-induced male Sprague Dawley rats. Additionally, the Duncan Multiple Range Test was employed to compare the various treatments.

RESULTS

Data represent in Table (1), provides a comprehensive overview of the hematological parameters measured across different study groups. Here are some observations based on the data presented:

White Blood Cell Count (WBC)

Group G2 (DEN) shows a significant ($p \leq 0.05$) decrease (5.9 ± 0.29 , $10^3 / \mu\text{L}$) compared to the negative control (G1; 7.8 ± 1.05 , $10^3 / \mu\text{L}$), possibly indicating an impact of DEN exposure on WBC count. Group G3 (DEN+GSPE) shows a recovery in WBC count, recorded 7.1 ± 0.33 , $10^3 / \mu\text{L}$; compared to the DEN group, suggesting a potential protective effect of GSPE. However, GSPE group recorded the highest value of 8.5 ± 0.09 $10^3 / \mu\text{L}$ as a protective agent.

Red Blood Cell Count (RBC), Hemoglobin (HGB), and Hematocrit (HCT)

Group G2 (DEN) exhibits a notable decrease in RBC count, HGB, and HCT compared to the negative control (G1), indicating potential adverse effects of DEN. Group G3 (DEN+GSPE) shows improvements in RBC count, HGB, and HCT compared to the DEN group, suggesting a significant ($p \leq 0.05$) beneficial impact of GSPE supplementation.

Platelet Count (PLT)

Group G2 (DEN) exhibits a significant ($p \leq 0.05$) decrease in platelet count compared to the negative control (G1), and recorded 206.7 ± 15.35 and $345 \pm 16.7 \times 10^3 / \mu\text{L}$, respectively. This results potentially indicate a negative effect of DEN on platelets. On the

other hand, Group G3 (DEN+GSPE) demonstrates a partial recovery in platelet count ($315 \pm 23.67 \times 10^3 / \mu\text{L}$) compared to the DEN group, suggesting a protective effect of GSPE. Meanwhile, G4 recorded the highest PLT but no significant difference than control group (G1).

Lymphocytes (LYM), Monocytes (MON), and Granulocytes (GR)

Some minor fluctuations are observed across these cell types, but no significant deviations are noted between the groups. Overall, the table (1) suggests that DEN exposure may lead to hematological alterations, while GSPE supplementation appears to ameliorate some of these effects. Further analysis and interpretation of these findings in the context of the study's objectives and hypotheses would be valuable.

Biochemical analyses:

The table (2) presents the biochemical parameters including Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Total bilirubin, Total protein, and Albumin levels in different treatment groups of rats.

Alanine aminotransferase (ALT) Levels

Group G2 (DEN) showed a significant increase in ALT levels compared to Group G1 (-ve control) and Group G4 (GSPE) which recorded 66.4 ± 10.99 , 23.7 ± 1.09 and 24.8 ± 4.36 (IU/L), respectively. However, treatment with GSPE in Group G3 (DEN+GSPE) resulted in a significant ($p \leq 0.05$) decrease in ALT levels, recorded 35.7 ± 5.32 (IU/L), compared to Group G2 (DEN).

Aspartate aminotransferase (AST) Levels

Group G2 (DEN) exhibited a significant ($p \leq 0.05$) increase in AST levels compared to Group G1 (-ve control) and Group G4 (GSPE), recorded 87.4 ± 8.72 , 52.3 ± 1.69 and 53.2 ± 9.11 (IU/L), respectively. For Group G3 (DEN+GSPE) showed a significant decrease in AST levels compared to Group G2 (DEN).

Total Bilirubin Levels

There were no significant differences in total bilirubin levels among the groups (Table 2).

Total Protein Levels

Group G2 (DEN) had significantly lower total protein levels compared to Group G1 (-ve control) and Group G4 (GSPE). The recorded data were 6.3 ± 0.16 , 5.2 ± 0.19 and 6.4 ± 0.39 (g/dl) for G1, G2 and G4, respectively. Group G3 (DEN+GSPE) showed a significant increase in total protein levels, recording 5.8 ± 0.37 (g/dl), compared to Group G2 (DEN).

Albumin Levels

Group G2 (DEN) exhibited significantly lower albumin levels compared to Group G1 (-ve control) and Group G4 (GSPE). Albumin levels were 4.5 ± 0.25 , 3.1 ± 0.35 and 4.7 ± 0.68 (g/dl), respectively. However, ameliorate effect of GSPE was recorded with DEN-induced rats and recorded 4.2 ± 0.24 (g/dl) which is significantly ($p \leq 0.05$) higher than G2 (Table2).

The results indicate that Diethylnitrosamine (DEN) exposure led to liver damage as evidenced by elevated ALT and AST levels, decreased total protein, and albumin levels. Treatment with Grape seed extract GSPE

Table (1): Effects of diethylnitrosamine and grape seed proanthocyanidin extract on hematological parameters in Sprague Dawley rats

Hematological Parameters	Treatments			
	G1 (-ve control)	G2 (DEN)	G3 (DEN+GSPE)	G4 (GSPE)
WBC (X10 ³ / μ L)	7.8 \pm 1.05 ^{ab}	5.9 \pm 0.29 ^c	7.1 \pm 0.33 ^b	8.5 \pm 0.09 ^a
RBC (X10 ⁶ / μ L)	8.4 \pm 0.22 ^a	6.5 \pm 0.20 ^c	7.7 \pm 0.57 ^b	8.7 \pm 0.06 ^a
HGB (g/dl)	14.4 \pm 0.48 ^a	11.7 \pm 0.28 ^c	13.4 \pm 0.27 ^b	14.7 \pm 0.12 ^a
HCT (%)	41.7 \pm 3.35 ^a	33.5 \pm 3.85 ^c	39.7 \pm 2.43 ^{ab}	42.6 \pm 0.30 ^a
MCV (fL)	49.3 \pm 3.13 ^{ab}	51.6 \pm 6.54 ^a	51.9 \pm 4.71 ^a	48.9 \pm 0.35 ^{ab}
MCH (Pg)	17.1 \pm 0.36 ^a	17.8 \pm 0.22 ^a	17.5 \pm 1.12 ^a	16.8 \pm 0.27 ^{ab}
MCHC (g/dl)	34.8 \pm 2.26 ^a	35.1 \pm 4.49 ^a	33.7 \pm 1.73 ^a	34.4 \pm 0.51 ^a
PLT (X10 ³ / μ L)	345 \pm 16.7 ^a	206.7 \pm 15.35 ^c	315 \pm 23.67 ^b	350.0 \pm 14.48 ^a
LYM (X10 ³ / μ L)	3.4 \pm 0.35 ^a	2.5 \pm 0.26 ^c	2.9 \pm 0.43 ^b	3.5 \pm 0.15 ^a
MON (X10 ³ / μ L)	0.1 \pm 0.04 ^a	0.2 \pm 0.04 ^a	0.2 \pm 0.04 ^a	0.1 \pm 0.04 ^a
GR (X10 ³ / μ L)	0.4 \pm 0.09 ^a	0.3 \pm 0.04 ^{ab}	0.3 \pm 0.12 ^{ab}	0.4 \pm 0.01 ^a

Data are presented in means \pm SD. Raw with different superscript letter are significantly different at level $p \leq 0.05$.

Table (2): Comparative analysis of liver function parameters in response to diethylnitrosamine and grape seed proanthocyanidin extract in treated Sprague Dawley rats.

Treatment groups	Measured parameters				
	ALT (IU/L)	AST (IU/L)	Total bilirubin (mg/dl)	Total protein (g/dl)	Albumin (g/dl)
G1 (-ve control)	23.7 \pm 1.09 ^c	52.3 \pm 1.69 ^b	0.32 \pm 0.07 ^b	6.3 \pm 0.16 ^a	4.5 \pm 0.25 ^{ab}
G2 (DEN)	66.4 \pm 10.99 ^a	87.4 \pm 8.72 ^a	0.53 \pm 0.15 ^a	5.2 \pm 0.19 ^c	3.1 \pm 0.35 ^c
G3 (DEN+GSPE)	35.7 \pm 5.32 ^b	56.1 \pm 8.85 ^b	0.31 \pm 0.11 ^b	5.8 \pm 0.37 ^b	4.2 \pm 0.24 ^b
G4 (GSPE)	24.8 \pm 4.36 ^c	53.2 \pm 9.11 ^b	0.35 \pm 0.18 ^b	6.4 \pm 0.39 ^a	4.7 \pm 0.68 ^a

Data are presented in means \pm SD. Raw with different superscript letter are significantly different at level $p \leq 0.05$.

showed protective effect by reducing ALT and AST levels and improving total protein and albumin levels in rats exposed to DEN.

Kidney function

The table (3) presents the urea and creatinine levels across different study groups which reveal the effect of seed extract on some kidney function.

Urea Levels

Based on the data provided, Urea Levels in Group II (DEN) shows a significant ($P < 0.05$) increase in urea levels compared to the negative control group (Group I), suggesting possible renal impairment induced by DEN exposure. However, Group III (DEN+GSPE) demonstrates a decrease in urea levels compared to the DEN group, indicating a potential protective effect of GSPE against DEN-induced renal damage.

Creatinine Levels

Group II (DEN) exhibits a significant ($P < 0.05$) elevation in creatinine levels compared to the negative control, indicating impaired kidney function due to DEN exposure. Group III (DEN+GSPE) shows a reduction in creatinine levels compared to the DEN group,

suggesting a beneficial effect of GSPE in mitigating DEN-induced kidney dysfunction. Generally, the data suggests that DEN exposure leads to renal dysfunction as evidenced by elevated urea and creatinine levels. However, GSPE supplementation shows potential renoprotective effects by ameliorating these alterations in urea and creatinine levels. Further analysis and comparison of these parameters in the context of renal health and treatment outcomes would provide valuable insights into the effects of DEN and GSPE on kidney function.

Histopathological analysis

Histopathological examination of renal cortex stained with H and E showed intact renal corpuscles with glomeruli surrounded with capsular space in addition to normal renal tubules in control group (Figure 1a-b), while DEN group showed shrinkage of glomeruli with wide capsular space and some debris in the lumen of renal tubules (Figure 1c-d). Treatment with GSPE after DEN showed intact renal corpuscle containing glomeruli surrounded with narrow capsular space in addition to normal renal tubules (Figure 1e-f), as well as GSPE

Table (3): Comparative analysis of kidney function parameters in response to diethylnitrosamine and grape seed proanthocyanidin extract in treated Sprague Dawley rats.

Treatment groups	Measured parameters	
	Urea (mg/dl)	Creatinine (mg/dl)
Group 1 (-ve control)	20.3±1.97 ^c	0.7±0.07 ^b
Group 2 (DEN)	29.7±1.99 ^a	0.9±0.04 ^a
Group 3 (DEN+GSPE)	26.6±1.63 ^b	0.8±0.09 ^{ab}
Group 4 (GSPE)	21.7±2.16 ^c	0.7±0.05 ^b

Data are presented in means ±SD. Raw with different superscript letter are significantly different at level $p \leq 0.05$

only groups showed normal renal cortex and tubules (Figure 1g-h).

Histological examination of spleen showed splenic follicle of white pulp, peri-arterial lymph sheath PALS and red pulp in control group (Figure 2a-b), while DEN group showed ill distinct boundaries to splenic follicle and congestion of splenic artery in addition to diminish in cellularity of red pulp (Figure 2c-d). Treatment with GSPE after DEN showed large well demarcated splenic follicle, mild congestion of splenic blood vessels in addition to normal red pulp (Figure 2e-f), as well as GSPE only groups showed normal spleen tissue (Figure 2g-h).

DISCUSSION

The study demonstrates the potential renoprotective and splenoprotective effects of GSPE in mitigating the harmful effects of DEN on kidney and spleen function in rats. The restoration of biochemical parameters and structural improvements in kidney and spleen tissues highlight the therapeutic potential of GSPE in ameliorating DEN-induced damage.

Reactive oxygen species (ROS) are produced by diethylnitrosamine (DEN), a very hazardous environmental carcinogen that causes oxidative stress and cellular damage. Cytochrome P450 metabolizes DEN in the body, producing extremely reactive free radicals that trigger the lipid peroxidation process in other cell organelles. Free radicals produce oxidative damage to DNA, proteins, and lipids in cells. Thus, the antioxidants found in herbal medications such as GSPE shield the kidney, spleen, and liver-three essential organs-from the oxidative stress and nephrotoxicity caused by DEN. The goal of the current study was to determine whether GSPE's antioxidant property, which it retains, may protect the kidney and spleen from damage caused by DEN (El-Tohamy, 2012). GSPE improved liver and kidney functions changes happened by DEN to near normal values while DEN made significant changes in parameters in rats. The result showed an significant increase in Alanine aminotransaminase (ALT) Aspartate aminotransferase (AST) and Total bilirubin (TB) while Albumin (Alb) and Total protein (TP) significant decreased in DEN group when compared with normal group and this agreed with (Pradeep *et al.*, 2010) (El-Shahat *et al.*, 2012) (Sun *et al.*, 2012) while treatment with GSPE

has improved or returned these values to the normal value and these results are agreement with (Fiorani *et al.*, 2006) (Hamlaoui *et al.*, 2012).

The results of this study indicate notable changes in these biochemical parameters, reflecting potential alterations in kidney function, particularly for those DEN-induced. Interestingly, Group (III) receiving both DEN and Grape Seed Proanthocyanidin Extract (GSPE) showed a reduction in urea and creatinine levels compared to Group (II). This finding suggests that GSPE may have a protective effect against DEN-induced kidney damage, as evidenced by the lower levels of these biomarkers. These results are inconsistent with the data obtained by Ozkan *et al.* (2012) and Yusong *et al.* (2020). In their study they concluded that seed extract of grap has capability to amilorate the effect of any drug induced kidney dysfunction. It also showed an significant increase in Urea (Ur) and Creatinine (Cr) in DEN group when compared with normal group and this agreed with (Perrone *et al.*, 1992) (El-Shahat *et al.*, 2012) (Singh *et al.*, 2021). While treatment with GSPE has improved or returned these values to the normal value and these results are agreement with (Bao *et al.*, 2015) (Li *et al.*, 2017).

Histological observations of the kidney and spleen corroborate the biochemical results. The kidney's microscopic results revealed shrinkage of glomeruli with wide capsular space and some debris in the lumen of renal tubules in DEN group and these findings concur with (Singh *et al.*, 2021). While treatment with GSPE showed improved these findings concur with (Bao *et al.*, 2015) (Li *et al.*, 2017)

Microscopic results in spleen showed ill distinct boundaries to splenic follicle and congestion of splenic artery in addition to diminish in cellularity of red pulp in DEN group and these findings concur with (Zhang *et al.*, 2009) (Wang *et al.*, 2002). While treatment with GSPE showed improved and these findings concur with (Alkheddaide *et al.*, 2016).

CONCLUSION

In conclusion, the study aimed to evaluate the renal and splenic protective effects of Grape Seed Proanthocyanidin Extract (GSPE) in Diethylnitrosamine (DEN)-induced male Sprague Dawley rats. The

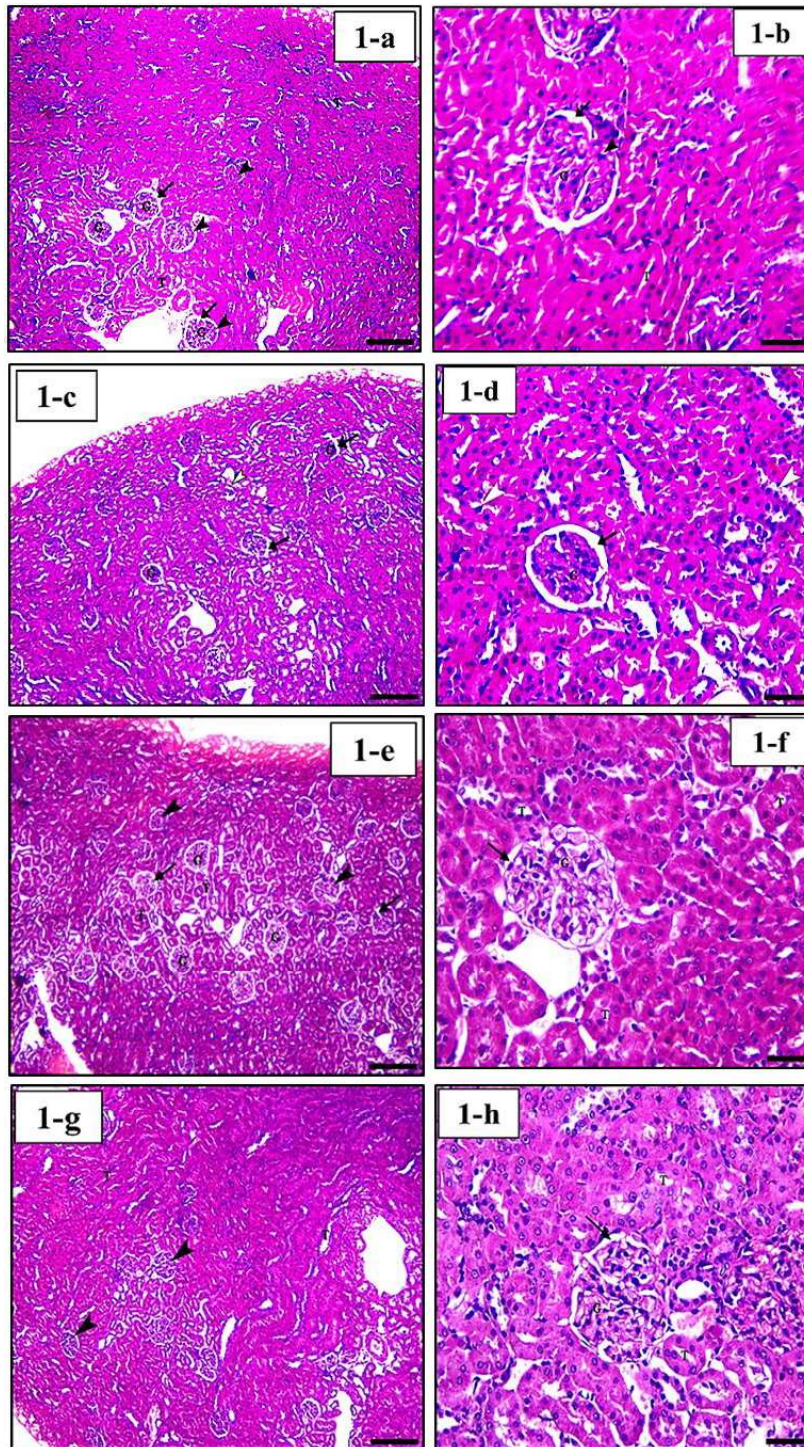


Figure (1): Histological effect of diethylnitrosamine (DEN) and grape seed proanthocyanidin extract (GSPE) on kidney Tissues. (a-b), photomicrograph of renal cortex of control group showing renal capsule intact renal corpuscles (arrow heads) with glomeruli (G) surrounded with capsular space (arrows) in addition to normal renal tubules (T), (a) Bar=200 μ m, (b) Bar= 50 μ m. (c-d) Photomicrograph of renal cortex of DEN treated group showing shrinkage of glomeruli (G) with wide capsular space (arrows) and some debris in the lumen of renal tubules (white arrow heads), (c) Bar= 200 μ m, (d) Bar= 50 μ m. (e-f) photomicrograph of renal cortex of DEN and GSPE treated group showing intact renal corpuscle (arrow heads) containing glomeruli (G) surrounded with narrow capsular space (arrows) in addition to normal renal tubules (T), (e) Bar= 200 μ m, (f) Bar= 50 μ m. (g-h) photomicrograph of renal cortex of GSPE treated group showing normal renal corpuscle (arrow heads) and intact renal tubules (T), (g) Bar= 200 μ m, (h) Bar= 50. Stain H&E.

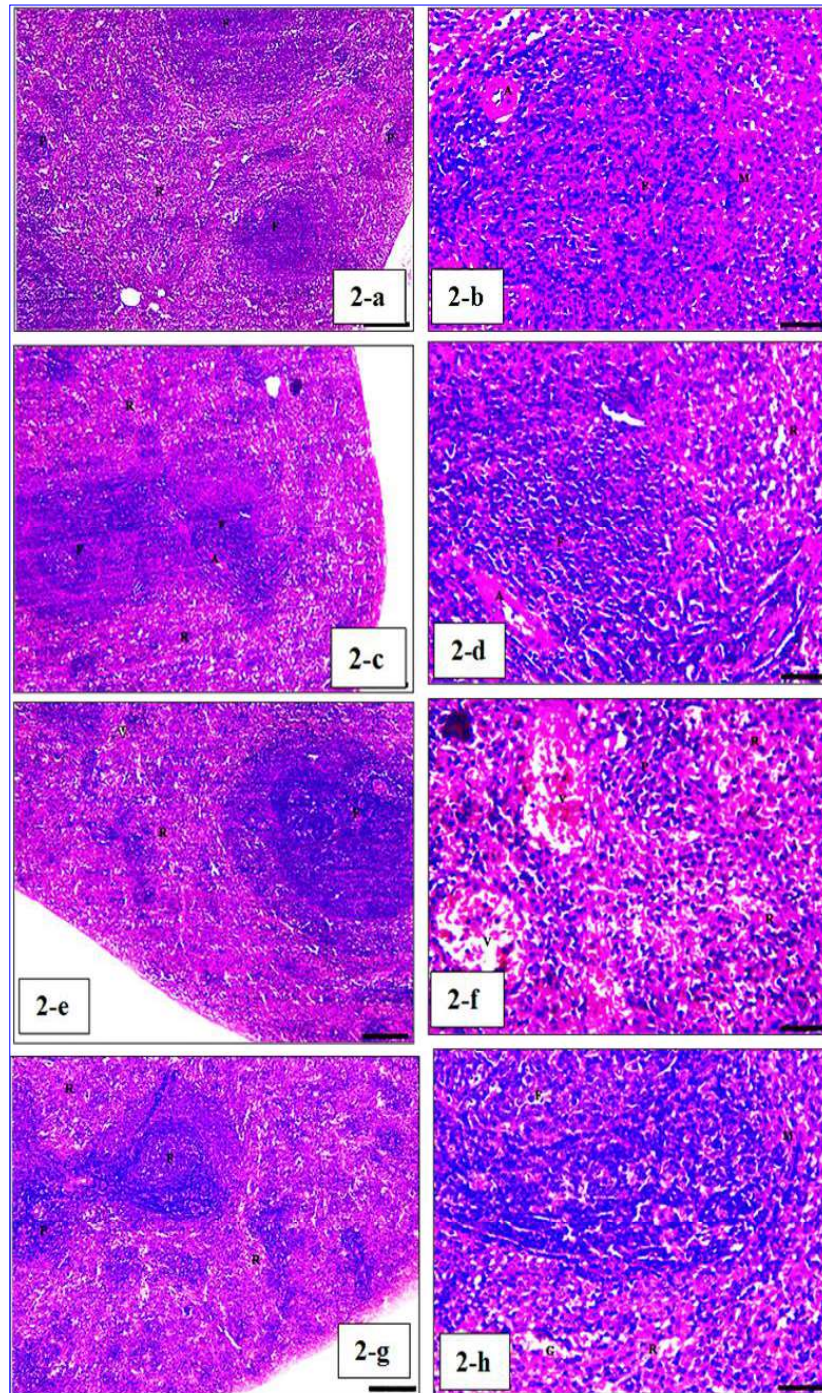


Figure (2): Histological effect of diethylnitrosamine (DEN) and grape seed proanthocyanidin extract (GSPE) on spleen Tissues. (a-b), photomicrograph of spleen of control group showing splenic follicle (F) of white pulp, peri-arterial lymph sheath PALS (P) and red pulp (R), (a) Bar=200 μ m, (b) Bar= 50 μ m. (c-d) photomicrograph of spleen of DEN treated group showing ill distinct boundaries to splenic follicle (F) and congestion of splenic artery (A) in addition to diminish in cellularity of red pulp (R), (c) Bar= 200 μ m, (d) Bar= 50 μ m. (e-f) photomicrograph of spleen of DEN and GSPE treated group showing large well demarcated splenic follicle (F), mild congestion of splenic blood vessels (V) in addition to normal red pulp(R), (e) Bar= 200 μ m, (f) Bar= 50 μ m. (g-h) photomicrograph of spleen of GSPE treated group showing whit pulp contained splenic follicle (F) and PALS (P) in addition to red pulp (R) and splenic capsule (C), (g) Bar= 200 μ m, (h) Bar= 50 μ m. Stain H&E.

results indicate that DEN exposure led to significant alterations in urea and creatinine levels, suggesting potential renal impairment. However, supplementation with GSPE showed promising effects in mitigating these changes, hinting at a protective role against renal damage induced by DEN. Moreover, the study revealed that GSPE supplementation not only attenuated the adverse effects on renal parameters but also showed partial recovery in platelet count, indicating potential splenic protective effects. These findings highlight the therapeutic potential of GSPE in ameliorating DEN-induced renal and splenic dysfunction in male Sprague Dawley rats. Further research is warranted to elucidate the underlying mechanisms of GSPE's protective effects on renal and splenic function. Additionally, exploring the long-term implications and dose-dependent responses of GSPE supplementation in similar experimental models could provide valuable insights for potential clinical applications in combating renal and splenic disorders. Overall, the study underscores the importance of GSPE as a promising natural agent for protecting against renal and splenic damage induced by toxic substances like DEN, paving the way for future investigations in the field of renal and splenic protection.

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