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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), obtained from grape seeds, is a rich and diverse source of proanthocyanidins, which have been demonstrated to exhibit beneficial effects on a variety of physiological processes. The objective of this study was to investigate the renoprotective and plenoprotective effects of grape seed proanthocyanidin against toxicity induced by Diethy-lnitrosamine (DEN) in rats. Twenty-four adult male Sprague Dawley rats were randomly assigned to four groups, with six rats per group. The first group served as the normal control and received intraperitoneal (IP) injections of physiological saline. The second group was administered DEN at a dose of 200 mg/kg/body weight. The third group received the same dose of DEN as the second group, followed by daily intragastric lavage administration of GSPE (300 mg/kg body weight). Lastly, the fourth group received daily intragastric administration of GSPE at a dose of 300 mg/kg body weight. The results of this study revealed a significant decrease in serum albumin and total protein levels in the DEN-treated group, while treatment with GSPE led to an improvement or normalization of these biochemical parameters. In the DEN-only group, serum urea and creatinine levels were found to be significantly elevated, and GSPE treatment resulted in an improvement with restoration of these renal function markers to normal levels. Additionally, there were significant elevations observed in the activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST), as well as total bilirubin (TB) levels in the DEN-treated group. Notably, GSPE treatment exhibited a protective effect, showing improvements in these hepatic function indicators. Microscopic examination of the kidney tissues revealed evidence of glomerular shrinkage with a wide capsular gap and debris in the renal tubule lumen in the DEN-treated group, while GSPE treatment showed improvement in the renal histological architecture. Additionally, microscopic examination of the spleen tissue revealed poorly defined boundaries, congestion in the splenic artery, and reduced cellularity of the red pulp in the DEN-treated group. However, the group receiving GSPE treatment exhibited significant improvements in the splenic histological architecture, with a restoration of the normal splenic tissue organization and vasculature when compared to the DEN-only group.

Keywords: Ameliorative effect; Antioxidants; Diethylnitrosamine; Flavonoids; Grape seed extract; Oxidative stress.

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 proanth-ocyanidins (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.

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Additionally, they have been associated with various health benefits, including cardiovascular protection, immune modulation, and potential anticancer properties (Huang, 2023). Research on proantho-cyanidins 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 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, even though it has demonstrated potential effects toward disease prevention and protective effects against several cancers. One member of the nitrosamine family, (DEN) finds application in the manufacturing of gasoline, lubricant additives, and stabilizers in plastics, solvents in the fiber industry, tobacco smoke, groundwater, 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 examined 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 obtained from Vaccera in Egypt's Helwan City and then 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 with No. (HAO-02-T-105) at Taif University, application No.45-296, Ministry of Education, Saudi Arabia.

Animal groups and experimental design

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 treated with DEN. Rats were divided into four groups: 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 (Liao *et al*., 2001) 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 proanth-ocyanidin extract (GSPE) at a dose of 300 mg/kg body weight (Cheung *et al*., 2014) 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

Altra pure Diethylnitrosamine (DEN) (purached from Sigma-Aldrich, USA) was used. Diethylnitro-samine (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 and 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-Shar-keya-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.

Samples collection

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 ºC 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.

Evaluation of 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 Haemoglobin 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 following the protocol described by Yoon *et al.* (2001). Tissue samples from the kidneys and spleens of experimental rats underwent meticulous microscopic examination to identify any structural alterations, anomalies, or pathological findings. The fixation process involved immersing the tissue samples in 10% formalin at room temperature for 24-48 hrs. Subsequently, the tissues were dehydrated in ascending concentrations of ethanol and then cleared in xylene. Following dehydration, the tissues were embedded in paraffin blocks. Sections, with a thickness of 5 µm, were then cut from these blocks. Finally, the sections were stained using Harris' hematoxylin and eosin. To assess and identify any structural alterations, anomalies, or pathological findings in the kidneys and spleens of the experimental rats, a comprehensive microscopic examination of mounted tissue samples was conducted.

Biochemical assays:

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 replicated data were recorded as means ± SE. One-way analysis of variance (ANOVA) was employed to assess the effectiveness of grape seed proanthocyanidin extract (GSPE) in mitigating Diethylnitrosamine-induced toxicity in male Sprague Dawley rats. Additionally, the Duncan Multiple Range Test was performed to compare the various treatments. Significant differences in data were recorded at a significance level of $p \leq 0.05$.

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 /µL) compared to the negative control (G1; 7.8±1.05, 10^3 /µL), 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 /µL; 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 /µL 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±16.7X10^3/µL, respectively. This results potentially indicat a negative effect of DEN on platelets. On the other hand, Group G3 (DEN+GSPE) demonstrates a partial recovery in platelet count (315±23.67X10^3/µL) 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 haematological 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 (Table 2).

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 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.

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

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

Data are presented in means ±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 ± 1.09^{c}	52.3 ± 1.69^{b}	0.32 ± 0.07^{b}	6.3 ± 0.16^{a}	4.5 ± 0.25^{ab}
G2 (DEN)	66.4 ± 10.99^{a}	87.4 ± 8.72^{a}	0.53 ± 0.15^{a}	5.2 ± 0.19^{c}	3.1 ± 0.35^{c}
G3 (DEN+GSPE)	35.7 ± 5.32^{b}	56.1 ± 8.85^{b}	0.31 ± 0.11^{b}	5.8 ± 0.37^{b}	4.2 ± 0.24^{b}
G4 (GSPE)	24.8 ± 4.36^{c}	53.2 ± 9.11^{b}	0.35 ± 0.18^{b}	6.4 ± 0.39^{a}	4.7 ± 0.68^{a}

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

Table (3): Comparative analysis of kideny 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)
Group1 (-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 $p \leq 0.05$.

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 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 conclued that seed extract of grap has cability 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 data obtained by Perrone *et al.* (1992); El-Shahat *et al.* (2012) and Singh *et al.* (2021). Meanwhile, treatment with GSPE showed an improvement of these values in approximate to the normal value. These recorded results are agreement with data achieved by Bao *et al.* (2015) and 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

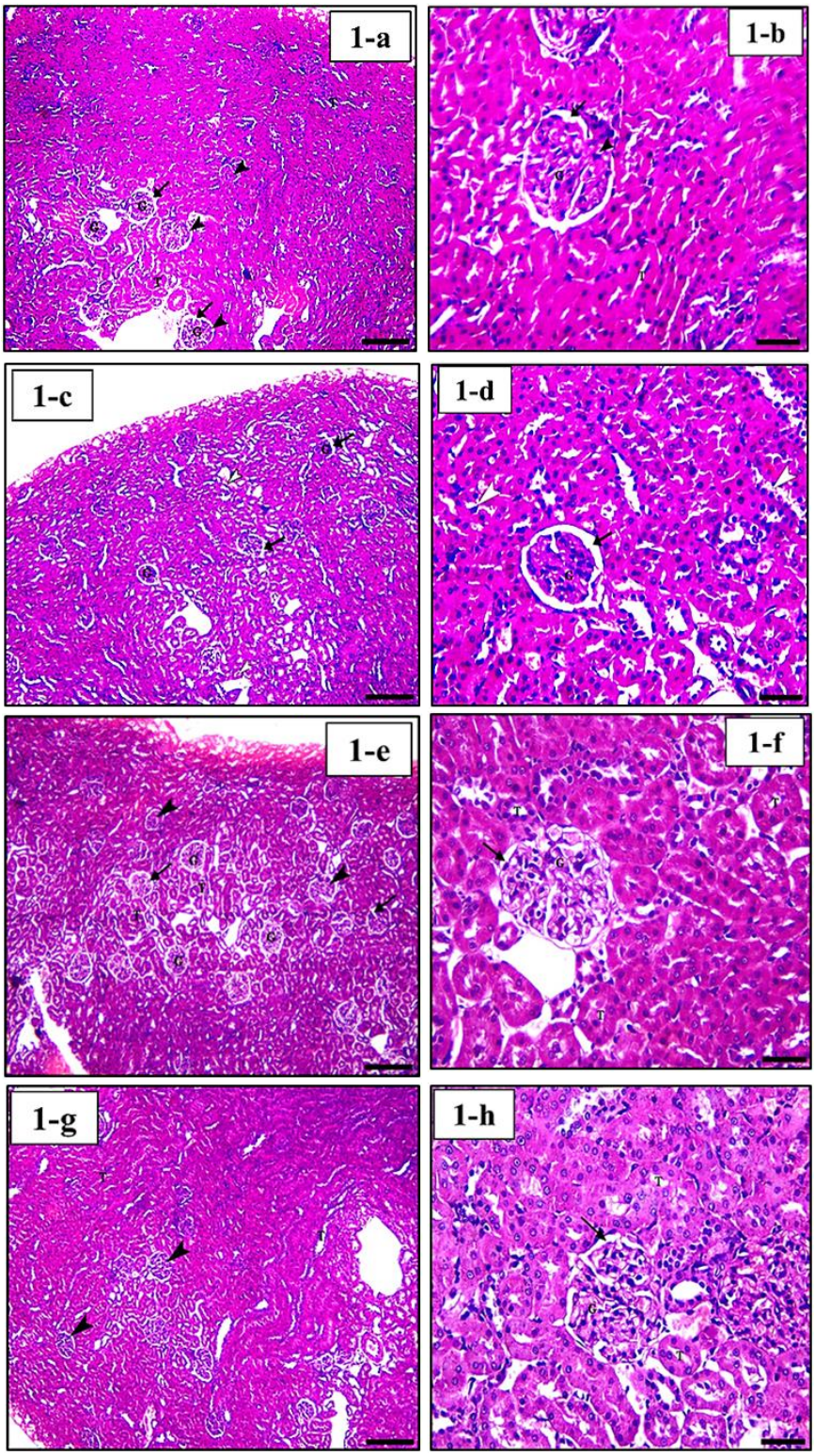

Figure (1): Histological effect of diethylnitrosamine (DEN) and grape seed proanthocyanidin extract (GSPE) on kidney Tissues. (a-b), photomicrograph of renal cortex of control group showing renal capsule intact renal corpuscles (arrow heads) with glomeruli (G) surrounded with capsular space (arrows)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

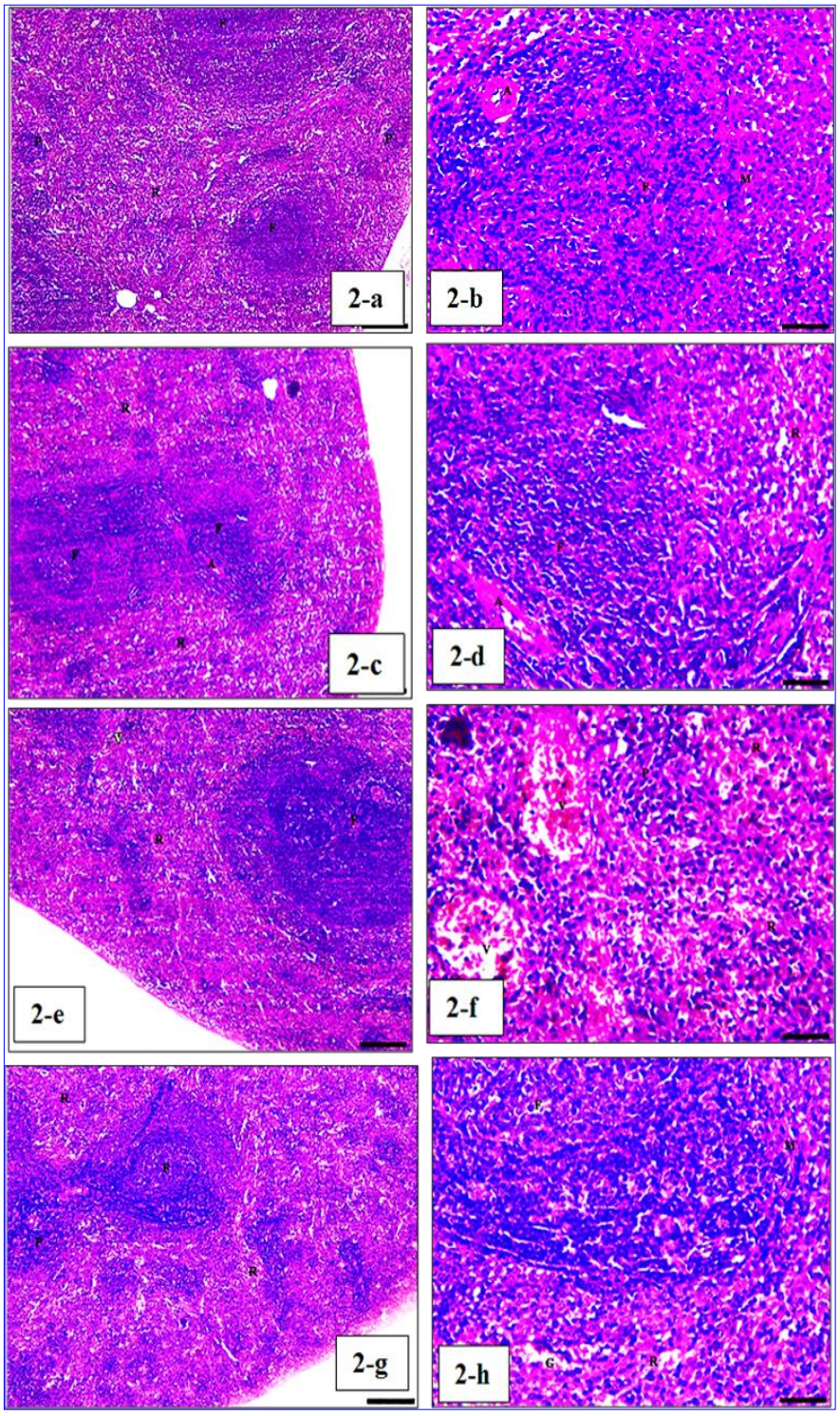

Figure (2): Histological effect of diethylnitrosamine (DEN) and grape seed proanthocyanidin extract (GSPE) on spleen Tissues. (a-b), photomicrograph of spleen of control group showing splenic follicle (F) of white pulp, peri-arterial lymph sheath PALS (P) and red pulp (R), (a) Bar=200µm, (b) Bar= 50 µm. (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 µm, (d) Bar= 50 µm. (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 µm, (f) Bar= 50 µm. (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 µm, (h) Bar= 50 µm. Stain H&E.

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)

Based on the microscopic results in the spleen, it was observed that there were ill distinct boundaries to the splenic follicle, congestion of the splenic artery, and a decrease in the cellularity of the red pulp in the DEN group. These findings align with the research conducted by Zhang *et al*. (2009) and Wang *et al*. (2002). However, the treatment with GSPE showed an improvement in the damage caused by DEN, which is consistent with the study by Alkhedaide *et al*. (2016). It's promising to see such positive results from the treatment with GSPE.

CONCLUSION

In conclusion, the study aimed to evaluate the renal and splenic protective effects of Grape Seed Proanthocyanidin Extract (GSPE) in Diethylnitro-samine (DEN)-induced male Sprague Dawley rats. Theresults 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 supplem-entation 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 impo-rtance 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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