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RESEARCH ARTICLE

Modulation of Energy Drink-Induced Hepato-renal and Testicular Toxicity by Pomegranate Peel Extract: Insights from Histopathological and Immunohistochemical Analyses

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ABSTRACT

Energy drink consumption, particularly among teenagers and young adults, has experienced a significant rise in recent years. However, mounting research points to the potential for chronic energy drink use to cause biochemical and histological abnormalities in vital organs such as the liver, kidneys, and reproductive system. To investigate whether pomegranate peel extract could offer protection against these toxic effects, this study was conducted on adult male albino rats divided into five groups: a control group, a pomegranate peel extract group, an energy drink group administered Red Bull®, a group pre-treated with pomegranate peel extract before energy drink consumption, and a group given energy drink followed by pomegranate peel extract. Over a 12-week treatment period, serum and tissue samples were collected to analyze liver and kidney function markers, lipid profile, oxidative stress levels, and histological changes. Rats those consumed energy drinks for 12 weeks exhibited elevated liver enzymes, impaired kidney function, disrupted lipid profiles, increased oxidative stress, and noticeable morphological changes in liver, kidney, and testicular tissues. In contrast, rats those received pomegranate peel extract either before or after energy drink consumption showed significant improvements in these parameters, demonstrating the protective effects of pomegranate peel extract in attenuating biochemical abnormalities and restoring tissue histology. These findings suggest that pomegranate peel extract has both therapeutic and preventive potential against the toxicity induced by chronic energy drink consumption, offering valuable insights that could inform strategies to reduce the adverse health effects associated with the regular use of energy drinks.

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INTRODUCTION

In a world where instant energy has become a cultural lifeline, energy drinks have exploded from a niche athletic supplement to a global phenomenon that captured a staggering USD 53 billion market in 2019, with an anticipated 7% annual growth (Grand View Research, 2020). A striking 74% of young people aged 12 to 24

have been swept up in this caffeinated revolution (Reid *et al.,* 2017), transforming these potent beverages from a mere performance enhancer to a ubiquitous lifestyle elixir promising superhuman alertness, concentration, and vitality (Visram *et al.,* 2016). Packed with a volatile cocktail of stimulants caffeine, sugar, taurine, ginseng, and guarana these drinks deliver a powerful neurochemical punch, with caffeine levels ranging from a mild 50 to a heart-racing 500 mg per can (Heckman *et al.*, 2010). This primary ingredient acts like a molecular maestro, swiftly infiltrating the body and brain, antagonizing adenosine receptors and amplifying dopamine transmission to create an electrifying surge of energy (McLellan *et al.,* 2016). Beyond basic stimulation, high doses of caffeine trigger a biochemical cascade, promoting catecholamine release, dilating blood vessels, and accelerating fat metabolism (Bühler *et al.,* 2014). Yet, beneath the marketing hype, taurine often touted as a cognitive enhancer remains scientifically unproven, lacking conclusive evidence of its purported benefits (Giles *et al.,* 2012). Even more alarming, energy drink manufacturers deftly sidestep FDA regulations by classifying their products as dietary supplements, enabling unregulated caffeine levels that potentially mask significant health risks (Manchester *et al.,* 2017). The dark side of this energy addiction becomes stark: excessive consumption has been linked to a terrifying spectrum of health consequences, from cardiovascular disruptions and sleep disorders to potential substance dependence (Somers and Svatikova, 2020), with extreme cases resulting in fatal caffeine toxicity, heart arrhythmias, hypertension, and psychological disturbances that transform these seemingly innocuous beverages into silent physiological time bombs (Kumar *et al.,* 2014; Berger and Alford, 2009).

Emerging preclinical works have also revealed that sustained consumption of caffeinated energy drinks can produce toxic manifestations in vital organs like the liver and kidneys (Oyenihi *et al.,* 2016, Mojoyinola *et al.,* 2023). However, the underlying mechanisms of energy drink-induced organ damage have not been fully elucidated. Oxidative stress, inflammation, and impaired tissue perfusion are hypothesized as potential contributing factors (El-Mas *et al.,* 2019). Furthermore, the potential remedial strategies for energy drink toxicity remain unexplored. Several studies note hepatoprotective and nephroprotective effects of pomegranate against drug-induced organ damage (Karwasra *et al.,* 2016).

We, therefore, aimed to comprehensively evaluate the adverse biochemical and histological alterations produced by chronic energy drink administration in a rat model and assess the ameliorative potential of pomegranate peel extract supplementation against such toxicity.

MATERIALS AND METHODS

Pomegranate Peel Extract: Pomegranate (*Punica granatum L.*) peels were obtained, sliced, and dried in a dark, ventilated area before being stored in moisture-proof bags. To prepare the extract, the dried peels were heated at 40°C for 10 hours, ground into powder, and extracted with 70% methanol at a ratio of 25ml per gram of powder using a magnetic stirrer overnight. This extraction was repeated for three days to enhance yield. The filtrates were evaporated under vacuum, yielding a reddish-brown residue (15% w/w), which was stored at 4°C for further analysis (Abo-Saif *et al.*, 2023).

Energy Drink: Energy Drink (Red Bull®) was purchased from a local store in its sealed original container. Each 100ml contained taurine (400mg), glucose (5gm), caffeine (32mg), B vitamins and proprietary blend of herbs and other ingredients.

Experimental Animals and Design: Fifty male adult Sprague Dawley albino rats (180–200g) were housed in polypropylene cages under controlled conditions (12-hour light/dark cycle, 50±5% humidity, 23±2°C) with *ad libitum* access to water and standard pellet feed. The rats were divided into five groups (n=10 per group) and treated daily for four weeks: Group I (Control) received distilled water; Group II (Pomegranate) received pomegranate peel extract (150mg/kg); Group III (Energy Drink) was administered (1.1 ml/100g body weight, approximating a human intake of 770 ml/day) (Mansy *et al.*, 2017); Group IV (Pomegranate before Energy Drink) received pomegranate extract followed by the energy drink two hours later; and Group V (Energy Drink before Pomegranate) received the energy drink for six weeks followed by pomegranate extract for another six weeks. All treatments were given via oral gavage between 9:00 and 10:00 AM, with weekly dose adjustments based on body weight. Afterwards, the rats were fasted overnight, anesthetized, and euthanized via cervical dislocation. Blood was collected through retro-orbital sinus puncture, and serum was separated by centrifugation at 2500 rpm for 15 minutes. The kidneys, liver, and testes were excised, cleaned, and preserved in 10% neutral buffered formalin for histological analysis.

Serum Biochemical Analysis: Serum biochemical parameters were analyzed using standard diagnostic kits (Bio-diagnostics Company (Giza, Egypt) and a semi-autoanalyzer (Model: Chem-7, Erba Mannheim). The parameters assessed included liver function tests (AST, ALT, and ALP), kidney function tests (creatinine and BUN), lipid profile (triglycerides and total cholesterol), and markers of oxidative stress MAD and GSH.

Histological and Immunohistochemical Analysis: Formalin-fixed liver, kidney, and testis samples were processed, sectioned at 5 µm, and stained with hematoxylin and eosin (H&E). A pathologist evaluated the histological abnormalities using standardized scoring criteria.

The liver was evaluated using the Nonalcoholic Fatty Liver Disease (NAFLD) scoring system (Brunt *et al.*, 1999), focusing on ballooning degeneration, steatosis, lobular inflammation, and portal fibrosis. Renal alterations were analyzed using a semiquantitative scoring method adapted from Schick *et al.* (2014), which examined interstitial congestion, edema, tubular epithelium detachment, and vacuolization, with scores ranging from 0 (no alterations) to 3 (severe alterations).

For testicular tissues, a scoring system similar to that of Sherif *et al.* (2020) was utilized to assess seminiferous epithelial damage, interstitial edema, tubular necrosis, and

congestion, also employing a scale from 0 (normal) to 3 (severe).

For immunohistochemistry, paraffin sections were deparaffinized and rehydrated on positive charged glass slides, and autoclaved with antigen retrieval citrate buffer (pH 6.8), followed by thorough washing with phosphate-buffered saline (PBS). Sections were then incubated overnight at 4°C with primary antibodies, including rabbit polyclonal anti-caspase-3 and anti-TNF-alpha (Abcam, Cambridge, USA), at dilutions of 1:100 and 1:150, respectively. After rinsing, sections were treated with a goat anti-rabbit secondary antibody, followed by incubation in diaminobenzidine (DAB) solution (Sigma Chemical Co, USA) as the chromogen. Finally, the sections were counterstained with hematoxylin and examined under a light microscope.

Furthermore, Caspase 3 and TNF-α expression in liver, kidney, and testis sections were quantified at a magnification of 200X using Image J software, with results expressed as mean ± SEM of the positive area percentage (El-Mosbah *et al.*, 2022).

Statistical Analysis: The GraphPad Prism version 8.0 was used to analyze the data using one-way ANOVA and Tukey's post-hoc test. All results are reported as mean±SD. P-values <0.05 were considered statistically significant.

RESULTS

Effect on Liver Enzymes: As shown in Fig. 1, exposure to energy drinks significantly elevated liver enzyme levels, with AST, ALT, and ALP increasing by 1.74, 1.45, and 2.2 times, respectively, compared to the control. Pomegranate peel extract supplementation in the ‘pomegranate before energy drink’ group significantly mitigated the increase in AST, ALT and ALP by 1.05, 1.16, 1.47-fold, respectively compared to sole energy drink administration. The ‘energy drink before pomegranate’ group showed similar ameliorative effects, lowering AST, ALT and ALP by 1.21, 1.13 and 1.52-fold versus the energy drink alone group.

Effect on Kidney Function Markers: Exposure to energy drinks significantly increased serum creatinine levels by 1.69 fold compared to control. Blood urea nitrogen (BUN) also exhibited a marked elevation with energy drink treatment, with a 1.94-fold higher mean BUN than control. The energy drink group showed a 1.61-fold rise in serum uric acid levels compared to the control group. Pomegranate peel extract intervention in the ‘pomegranate before energy drink’ group significantly lowered creatinine by 1.23 fold, BUN by 1.17 fold, and uric acid by 1.19 fold compared to the energy drink alone group. Comparable nephroprotective effects were seen when pomegranate peel extract was given after energy drink exposure, reducing creatinine by 1.15-fold, BUN by 1.13-fold and uric acid by 1.14 fold versus the sole energy drink group (Fig. 1).

Effect on Lipid Profile: As illustrated in Fig. 1, exposure to energy drinks significantly altered the lipid profile, with the energy drink group showing a 1.41-fold increase in total cholesterol (TC) and a 1.49-fold increase in triglyceride (TG) levels compared to the control group. Pomegranate peel extract supplementation demonstrated a notable protective effect against these lipid alterations. In the 'pomegranate before energy drink' group TC levels were reduced by 1.08-fold and TG by 1.52-fold compared to the energy drink alone group. The 'energy drink before pomegranate' group showed similar ameliorative effects, lowering TC by 1.03-fold and TG by 1.31-fold versus the energy drink alone group.

Effect on Oxidative Stress Markers: After 12 weeks, the energy drink group's increased MDA and reduced GSH activity compared to the control group (Fig. 1).

Histopathological and Immunohistochemical Analysis of the Liver, Kidney, and Testis

Histopathological Evaluation: Histopathological examination of liver sections revealed distinct changes across different treatment groups. In the control group, polygonal hepatocytes had ovoid vesicular nuclei, intact central veins, and normal portal regions and sinusoids (Fig. 2A1). The pomegranate group showed no significant pathological changes, with hepatic lobules retaining their structure (Fig. 2B1). Conversely, the energy drink group exhibited significant alterations, including increased hepatocyte size, rarefied cytoplasm, vacuolations, pyknotic nuclei, lipid droplet infiltration, and vascular congestion. Mild-to-moderate mononuclear cell infiltration, predominantly consisting of lymphocytes and macrophages. The inflammatory pattern appeared chronic, affecting portal region with early signs of fibrosis (Fig. 2C1, D1). The combined pomegranate and energy drink groups displayed intermediate changes, suggesting a mitigating effect of pomegranate (Fig. 2 E1, F1).

In liver tissue (Table 1), histopathological scores revealed significant differences among treatment groups. The E-Drink and E-Drink+Pomegranate groups exhibited higher scores for ballooning degeneration, steatosis, lobular inflammation, and portal fibrosis compared to controls and Pomegranate-treated groups. These findings suggest heightened susceptibility to liver damage induced by energy drinks, mitigated to some extent by pomegranate peel extract.

Kidney sections were evaluated using a semiquantitative scoring methodology. The control group (Fig. 3A1) as well as the pomegranate group (Fig. 3B1) showed normal renal architecture, while the energy drink group (Fig. 3C1, D1) exhibited significant renal alterations, including interstitial congestion, cellular edema, tubular epithelium detachment, and vacuolization. Combined treatment groups also showed mitigated renal damage, with the energy drink before pomegranate group (Fig. 3E1) demonstrating less severe alterations compared to the pomegranate before energy drink group (Fig. 3F1).

Similarly, in kidney tissue (Table 2), the Energy Drink group showed pronounced renal damage compared to controls. Pomegranate pre-treatment and post-treatment groups demonstrated moderate protective effects, as indicated by lower histopathological scores. This underscores pomegranate peel extract's potential to ameliorate renal tissue injury associated with energy drink consumption.

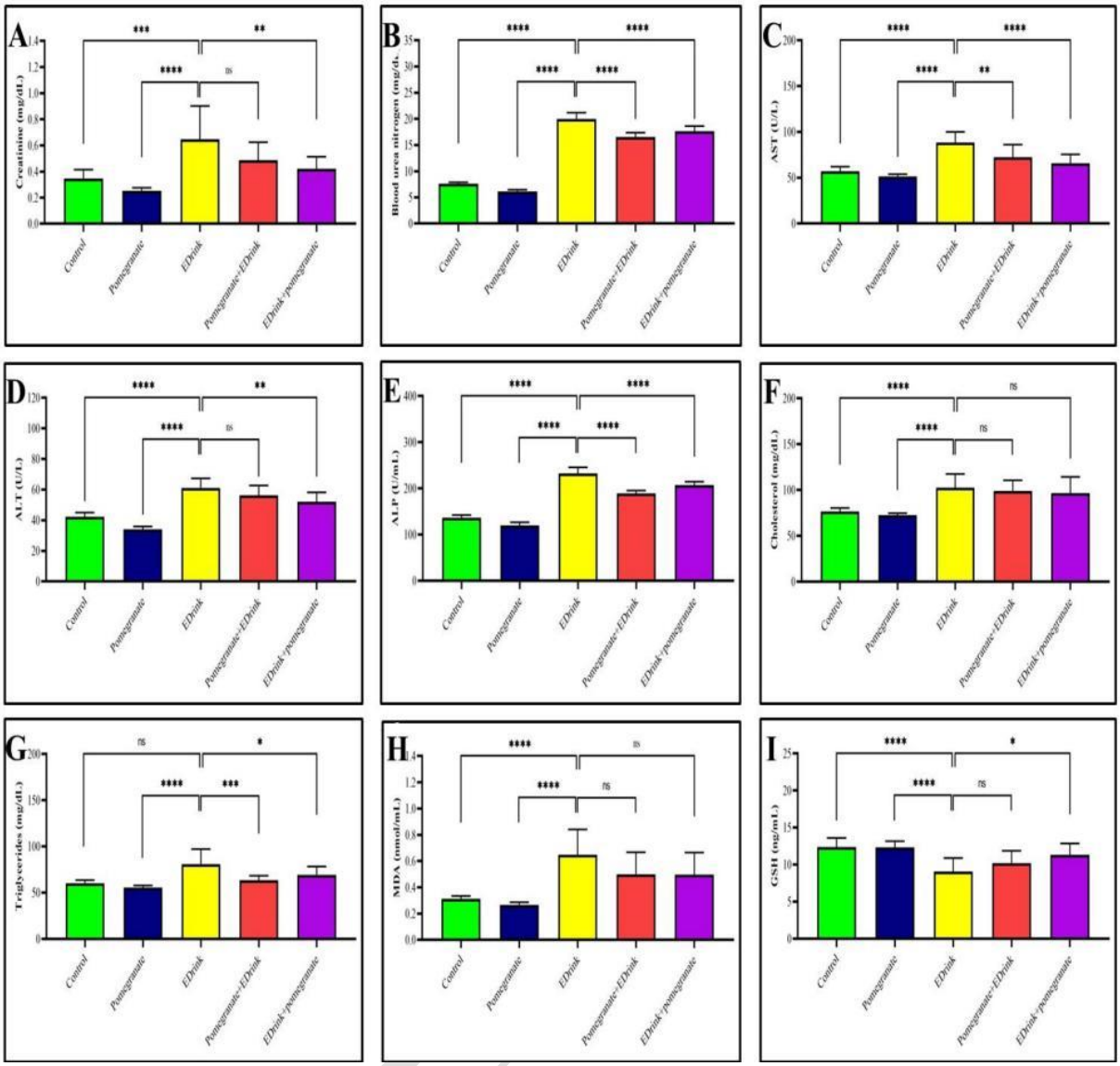

Fig. 1: Effect of Pomegranate extract and Energy drink on rats' serum biochemical parameters and antioxidant parameters.

Table 1: Statistical analysis of histopathological scores for liver tissues (Mean±SD).

Group	Ballooning Degeneration	Steatosis	Lobular Inflammation	Portal Fibrosis
Control	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]
Pomegranate	0.20±0.42[a]	0.20±0.42[a]	0.20±0.42[a]	0.20±0.42[a]
E-Drink	0.50±0.53[b]	0.50±0.53[b]	0.50±0.53[b]	0.50±0.53[b]
Pomegranate+E-Drink	0.40±0.52[b]	0.40±0.52[b]	0.40±0.52[b]	0.40±0.52[b]
E-Drink+Pomegranate	0.60±0.52[b]	0.60±0.52[b]	0.60±0.52[b]	0.60±0.52[b]

Note: Groups sharing the same letter are not significantly different from each other (Tukey's post hoc test, P<0.05).

Table 2: Statistical analysis of histopathological scores for kidney tissues (Mean±SD).

Groups	Average Field Score
Control	0.00±0.00[a]
Pomegranate	0.25±0.42[a]
Energy Drink	2.40±0.84[c]
Pomegranate Before Energy Drink	1.58±0.79[b]
Energy Drink Before Pomegranate	1.00±0.88[b]

Note: Groups sharing the same letter are not significantly different from each other (Tukey's post hoc test, P<0.05).

Testicular histopathology was assessed using a standardized scoring system evaluating seminiferous epithelial damage, interstitial edema, tubular necrosis, and congestion. The control group (Fig. 4A1) as well as pomegranate group (Fig.4 B1) displayed normal testicular histology, while the energy drink group (Fig.4 C1, D1) exhibited severe histopathological changes. Combined treatment groups showed reduced histopathological damage, with the energy drink before pomegranate group (Fig.4 E1) showing better preservation of testicular architecture than the pomegranate before energy drink group (Fig.4 F1).

In testicular tissue (Table 3), the Energy Drink group exhibited the highest overall injury scores, reflecting significant seminiferous epithelial damage, interstitial edema, tubular necrosis, and congestion. Conversely, pomegranate-treated groups displayed markedly lower scores, indicating a protective effect against testicular damage induced by energy drinks.

Immunohistochemical Assessment: Hepatic Caspase 3 and TNF-α expression in liver sections were quantified. The control group showed minimal expression (Fig. 2A2, A3), indicating negligible apoptotic and inflammatory activity.

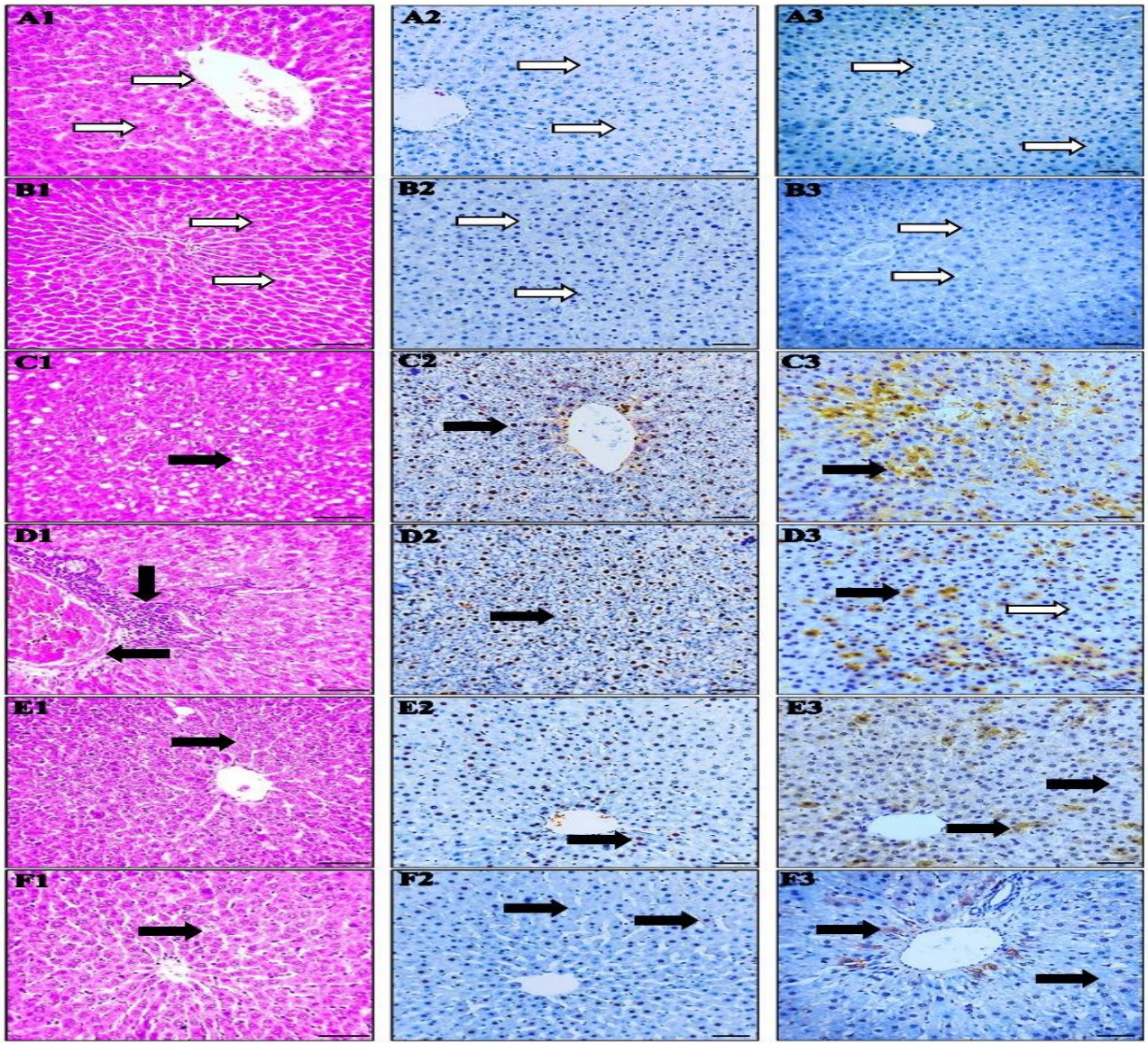

Fig. 2: Histopathological (HE) and immunohistochemical analysis (Caspase-3, TNF-α) of rat liver tissue across experimental groups. (A1-A3) Control group: Normal hepatic architecture with intact central veins and rounded vesicular nuclei. Minimal Caspase-3 and TNF-α immunoreactivity, indicating standard low apoptotic activity and inflammation. (B1-B3) Pomegranate group: Preserved liver histology comparable to control. Negligible Caspase-3 and TNF-α expression, suggesting typical defensive anti-apoptotic and anti-inflammatory effects. (C1-C3, D1-D3) Energy Drink group: Significant hepatocellular alterations, including hepatocyte enlargement, vacuolations, vascular congestion, and inflammatory cell infiltrations. Elevated Caspase-3 and TNF-α immunoreactivity are indicative of heightened apoptosis and inflammation. (E1-E3) Pomegranate Before Energy Drink group: Attenuated histopathological changes, including reduced vacuolar degeneration and vascular congestion, compared to the Energy Drink group. Decreased Caspase-3 and TNF-α expression, suggesting protective effects of pomegranate pre-treatment. (F1-F3) Energy Drink Before Pomegranate group: Minimal histopathological alterations. Reduced Caspase-3 and TNF-α immunoreactivity compared to the Energy Drink group, indicating efficient ameliorative effects of post-exposure pomegranate administration. White arrow indicate normal structure (Hepatic plate, Central vein) and negative immune expression. Black arrow indicate histopathological alteration (Vacuolation, Congestion, inflammation and degeneration) and positive immune expression.

Table 3: Statistical analysis of testicular injury scores (Mean±SD).

Group	Seminiferous Epithelial Damage	Interstitial Edema	Tubular Necrosis	Congestion	Overall Injury Score
Control	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}
Pomegranate	0.1±0.1^{a}	0.1±0.1^{a}	0.1±0.1^{a}	0.1±0.1^{a}	0.1±0.1^{a}
Energy Drink	1.8±0.2^{c}	1.7±0.2^{c}	1.6±0.2^{c}	1.8±0.2^{c}	1.7±0.09^{c}
Pomegranate Before Energy Drink	0.5±0.1^{b}	0.4±0.1^{b}	0.4±0.1^{b}	0.5±0.1^{b}	0.45±0.05^{b}
Energy Drink Before Pomegranate	0.2±0.1^{a}	0.2±0.1^{a}	0.2±0.1^{a}	0.2±0.1^{a}	0.2±0.1^{a}

Note: Groups sharing the same letter are not significantly different from each other (Tukey's post hoc test, P<0.05).

Testicular histopathology was assessed using a standardized scoring system evaluating seminiferous epithelial damage, interstitial edema, tubular necrosis, and congestion. The control group (Fig. 4A1) as well as pomegranate group (Fig.4 B1) displayed normal testicular 2C2) and TNF-α (Fig.2 C2, C3, D2, D3) expression, indicating high levels of apoptosis and inflammation. Combined treatment groups (Fig. 2E2, E3, F2, F3) showed intermediate expression levels, suggesting partial mitigation of energy drink-induced damage by pomegranate.

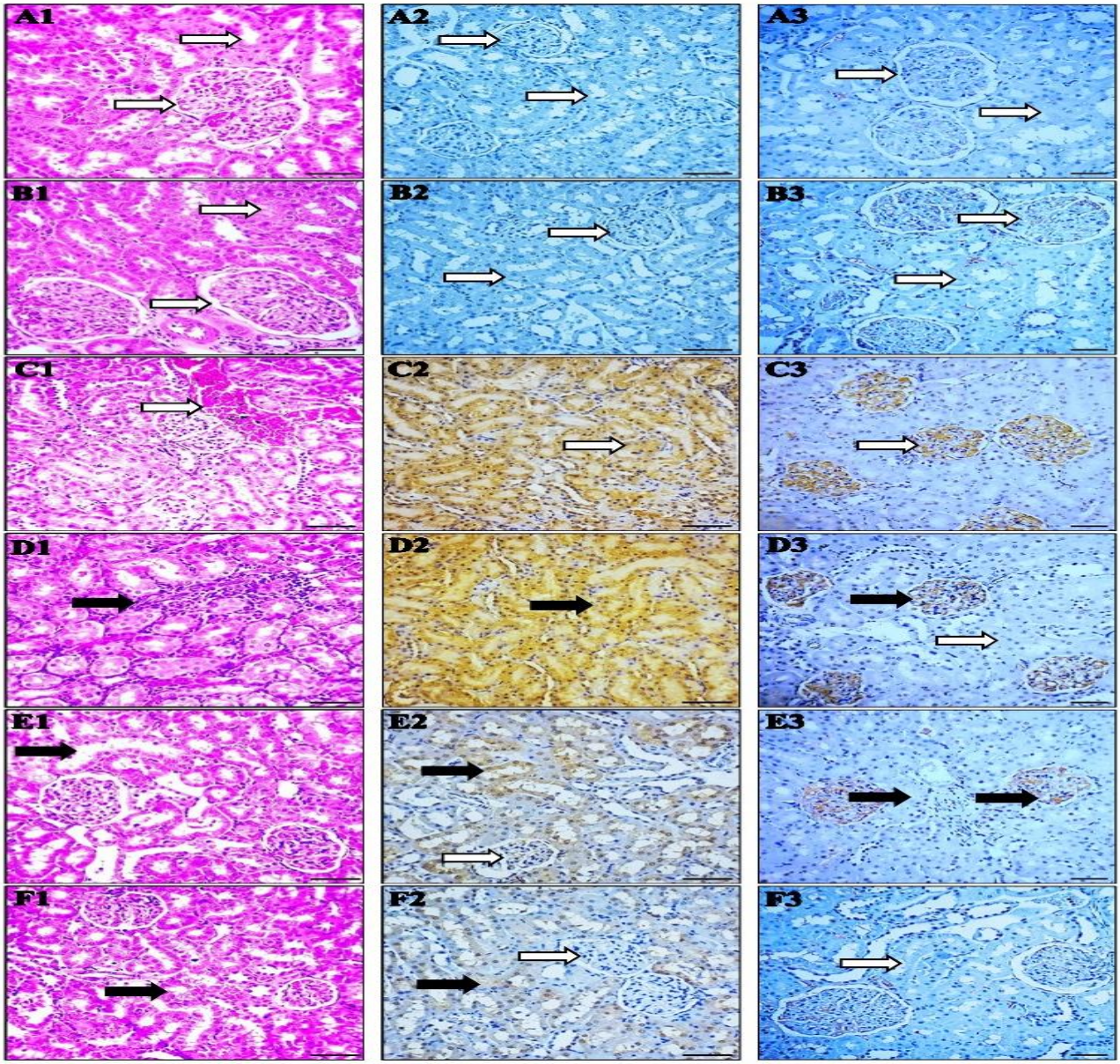

Fig. 3: Histopathological and immunohistochemical analysis of renal tissue (HE, Caspase-3, TNF-α) across experimental groups. (A1-A3) Control group: Normal renal architecture with intact glomeruli and tubules. Minimal Caspase-3 and TNF-α immunoreactivity, indicating modest apoptotic activity and inflammation. (B1-B3) Pomegranate group: Preserved renal histology comparable to control. Negligible Caspase-3 and TNF-α expression, suggesting regular anti-apoptotic and anti-inflammatory effects. (C1-C3, D1-D3) Energy Drink group: Marked pathological alterations, including glomerular atrophy, expanded urinary spaces, degenerative changes, and inflammatory cell infiltration. Elevated Caspase-3 and TNF-α immunoreactivity revealed increased apoptosis and inflammation. (E1-E3) Pomegranate Before Energy Drink group: Attenuated histopathological changes compared to Energy Drink group. Reduced Caspase-3 and TNF-α expression, suggesting protective effects of pomegranate pre-treatment. (F1-F3) Energy Drink Before Pomegranate group: Minimal histopathological alterations. Decreased Caspase-3 and TNF-α immunoreactivity compared to the Energy Drink group, demonstrating potential ameliorative effects of post-exposure pomegranate administration. White arrow indicate normal structure (Glomeruli, renal tubules) and negative immune expression. Black arrow indicate histopathological alteration (congestion, inflammation, and degeneration) and positive immune expression.

In kidney tissue, caspase-3 expression varied significantly. The control group (Fig. 3A2, A3) as well as pomegranate group (Fig. 3B2, B3) exhibited baseline expression, while the energy drink group (Fig. 3C2, C3, D2, D3) showed marked increases, indicating substantial apoptotic and inflammatory activity. Combined treatment groups also showed moderated caspase-3 levels, with pre-treatment groups (Fig. 3E2) showing less good protection than treatment groups (Fig. 3F2). TNF-α expression followed similar patterns, with the energy drink group showing significant increases, while pomegranate prevention (Fig. 3E3) and also treatment (Fig. 3F3) reduced TNF-α levels, indicating reduced inflammation.

In testicular tissue, similar patterns were observed. The control group (Fig. 4A2, A3) and pomegranate group (Fig. 4B2, B3) exhibited minimal caspase-3 and TNF-α expression. The energy drink group (Fig. 4C2, C3, D2, D3) showed substantial increases in both markers, indicating heightened apoptotic and inflammatory responses. Combined treatment groups showed intermediate levels of caspase-3 and TNF-α expression, with pre-treatment (Fig. 4E2, E3) providing less protection than treatment group (Fig. 4F2, F3) against energy drink-induced damage.

Analysis of TNF-α expression (Fig. 5) across liver, kidney, and testicular tissues revealed substantial upregulation in the Energy Drink group compared to

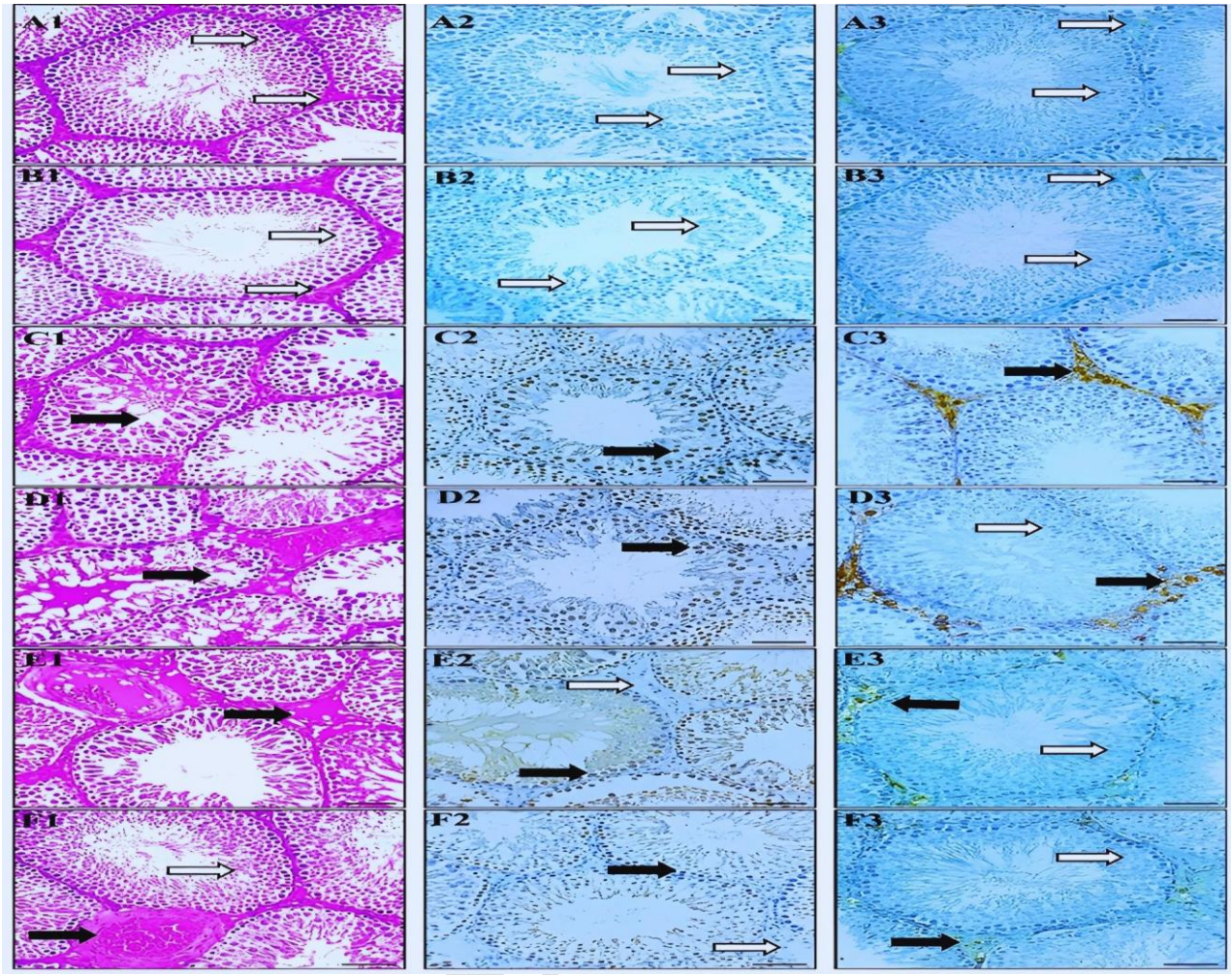

Fig. 4: Histopathological and immunohistochemical analysis of rat testicular tissue across experimental groups. (A1-A3) Control group: Normal testicular architecture with minimal Caspase-3 and TNF-α immunoreactivity, demonstrating subtle apoptotic activity and inflammation. (B1-B3) Pomegranate group: Preserved testicular histology comparable to control. There was an imperceptible expression of Caspase-3 and an insignificant elevation in TNF-α expression, suggesting standard protective mechanisms against testicular injury. (C1-C3, D1-D3) Energy Drink group: Significant testicular alterations as degeneration, vacuolation, and germinal epithelial cell sloughing accompanied by markedly elevated Caspase-3 and TNF-α immunoreactivity, indicative of increased apoptosis and inflammation. (E1-E3) Pomegranate Before Energy Drink group: Attenuated histopathological changes compared to Energy Drink group. Moderated increase in Caspase-3 and TNF-α expression, suggesting protective effects of pomegranate pre-treatment. (F1-F3) Energy Drink Before Pomegranate group: Substantial histological preservation with reduced mild Caspase-3 and TNF-α immunoreactivity compared to Energy Drink group, signifying potential ameliorative effects of post-exposure pomegranate administration. White arrow indicate normal structure (germinal epithelium, interstitial tissue) and negative immune expression. Black arrow indicate histopathological alteration (degeneration, vacuolation, and congestion) and positive immune expression.

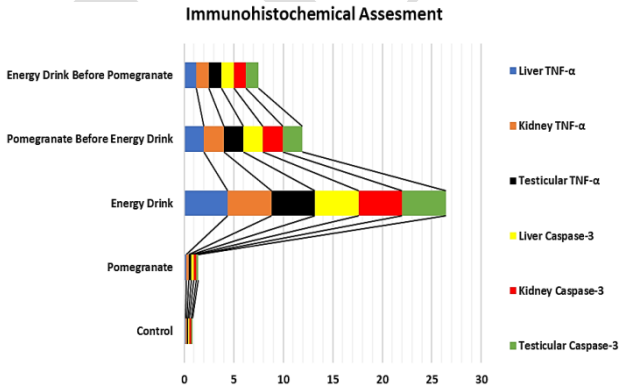

Fig. 5: TNF-α and Caspase-3 expression in liver, kidney, and testicular tissues.

controls. Pomegranate administration, either before or after energy drink consumption, significantly attenuated TNF-α expression, highlighting its anti-inflammatory properties in mitigating tissue inflammation Caspase-3 expression (Fig. 5), indicative of apoptosis, followed a similar pattern, with the Energy Drink group exhibiting elevated levels across all tissues. Pomegranate peels extract effectively reduced Caspase-3 expression, underscoring its role in mitigating apoptosis induced by energy drinks in liver, kidney, and testicular tissues.

DISCUSSION

In recent years, energy drinks have gained widespread popularity, especially among teenagers and young adults due to targeted marketing. However, recent studies suggest that consuming large amounts of these caffeinated drinks on a regular basis may have negative health effects (Goldfarb *et al.*, 2014). The present study aimed to evaluate the biochemical, oxidative stress and histological alterations of prolonged energy drink intake in a rat model. Additionally, assessing the protective potential of pomegranate peel extract against energy drink-evoked toxicity. Interestingly, pomegranate peel

extract supplementation before or after energy drink administration mitigated many of the adverse biochemical and histological changes. Pomegranate contains a rich blend of polyphenols including punicalagin, anthocyanins and ellagic acid, which impart a robust array of pharmacological benefits (Banihani *et al.,* 2013). These phytochemicals enhance endogenous antioxidant capacity, suppress inflammatory pathways, maintain membrane integrity, and confer organoprotective effects (Kazmi *et al.,* 2012, Viuda-Martos *et al.,* 2010). Our findings reaffirm the therapeutic utility of pomegranate against drug-induced liver and kidney toxicity (Bakir *et al.,* 2015). Nevertheless, additional clinical research in human subjects is warranted to evaluate its protective efficacy against energy drink toxicity.

Our findings revealed that 12 weeks of daily energy drink administration provoked marked impairments in liver function, indicated by sharp elevations in serum AST, ALT, and ALP levels. The observed hepatotoxicity is mostly due to the high caffeine level of these beverages. Earlier reports showed that sustained exposure to caffeine triggers hepatocellular injury mediated through enhanced oxidative stress, inflammation, and apoptosis (Chavez-Valdez *et al.,* 2016). Caffeine is metabolized by CYP450 enzymes in liver, resulting in the formation of reactive oxygen species (ROS) and depletion of endogenous antioxidants like glutathione. The resulting oxidative damage disturbs membrane integrity and alters liver function (Braganza *et al.,* 2017).

Kidney function was also severely compromised following chronic energy drink intake, as demonstrated by higher blood creatinine, BUN, and uric acid levels in rats. Excessive caffeine consumption is known to impair renal function by stimulating renin release and disrupting glomerular filtration (Verrotti *et al.,* 2015). Caffeine further potentiates kidney injury by promoting inflammation, renal cell apoptosis, and collagen deposition through adenosine receptor interactions (O'Neill *et al.,* 2014). Taurine, another major constituent of energy drinks, has likewise been reported to cause dose-dependent toxicity in renal tubular cells in animal models (Menzie *et al.,* 2014).

Energy drink consumption led to significant alterations in lipid profiles, including increased total cholesterol and triglyceride levels. These changes are associated with the high sugar content and stimulants in energy drinks, which can disrupt lipid metabolism (Al-Muslhi and Ali, 2024). Pomegranate peel extract supplementation effectively modulated these lipid alterations, reducing cholesterol and triglyceride levels. This effect is likely due to the ability of pomegranate polyphenols to improve lipid metabolism by enhancing antioxidant defenses and reducing oxidative stress (Barghchi *et al.,* 2023; Singh *et al.,* 2023). Such modulation of lipid profiles suggests that pomegranate peel extract could serve as a natural therapeutic agent for managing dyslipidemia induced by energy drink consumption.

The results of this study suggest a potential role of oxidative stress as a mechanism in energy drink consumption and organ responses. Oxidative stress plays a significant role in the process of tissue damage (Soliman *et al*., 2022, Aboubakr *et al*., 2023a, Aboubakr *et al*., 2023b, Elsayed *et al*., 2024, Soliman *et al*., 2024). Chronic consumption of energy drinks has been shown to disturb antioxidant enzymes such as GSH, which aligns with previous findings linking high caffeine intake to oxidative damage (Alsunni, 2015). The administration of pomegranate peel extract, rich in polyphenolic compounds like ellagic acid and punicalagin, effectively counteracted these oxidative effects. These compounds are known for their potent antioxidant properties, enhancing endogenous antioxidant capacity and reducing oxidative stress (Singh *et al.,* 2023). This is consistent with studies demonstrating the ability of pomegranate polyphenols to upregulate antioxidant defenses and protect against cellular damage (Wang *et al.,* 2023).

Oxidative stress emerged as a key mechanism underlying energy drink-mediated toxicity. The treated rats exhibited a significant increase in MDA and declined GSH activity, denoting depleted antioxidant defenses. Caffeine metabolism triggers ROS overproduction while simultaneously downregulating the expression of cytoprotective enzymes (Ullah *et al.,* 2018). Excessive ROS inflicts cellular damage by reacting with lipids, proteins, and nucleic acids. Our findings align with earlier studies demonstrating caffeine-induced oxidative stress in hepatic, renal, and neurological tissue (Ullah *et al.,* 2018).

Histological observations further confirmed the detrimental impact of prolonged energy drink administration. Liver sections revealed distorted architecture, dilated sinusoids, lymphocytes infiltration, and widespread hepatocellular necrosis, corresponding to the extent of biochemical liver damage. Kidney sections similarly displayed structural anomalies, including glomerular shrinkage, tubular atrophy, and extensive multifocal, mononuclear interstitial infiltration. Comparable histopathological abnormalities following caffeine or energy drink exposure have been documented across multiple animal toxicity studies (Martinello *et al.,* 2017).

The study also underscores the anti-inflammatory potential of pomegranate peel extract in mitigating energy drink-induced tissue damage. Histopathological analysis revealed significant reductions in inflammatory markers such as TNF-α and caspase-3 in liver, kidney, and testicular tissues upon treatment with pomegranate peel extract. This supports existing literature that attributes the anti-inflammatory effects of pomegranate peel to its high content of flavonoids and tannins, which inhibit pro-inflammatory pathways and cytokine production (Singh *et al.,* 2023). These findings suggest that pomegranate peel extract prevents oxidative damage and modulates inflammatory responses, offering comprehensive organ protection. Caspase-3 is a crucial executioner enzyme in the process of apoptosis, playing a significant role in cellular homeostasis and the elimination of damaged cells. The study findings reveal chronic energy drink consumption significantly elevates caspase-3 expression in the liver, kidney, and testicular tissues, indicating increased apoptotic activity. This aligns with existing literature suggesting that excessive caffeine and other stimulants in energy drinks can induce apoptosis through oxidative stress and mitochondrial dysfunction mechanisms (Kusmardi *et al.,* 2021). The administration of pomegranate peel extract demonstrated a notable reduction in caspase-3 expression across these tissues.

This effect is attributed to the high concentration of polyphenolic compounds in pomegranate peel, such as ellagic acid and punicalagin, which possess potent antioxidant properties that mitigate oxidative stress and inhibit apoptotic pathways (Jebur *et al.,* 2023). These findings are consistent with previous studies that have shown the ability of pomegranate extracts to downregulate caspase-3 expression and protect against apoptosis in various pathological conditions (Abo-Saif *et al.,* 2023).

Conclusions: The current work elucidates clear evidence regarding the hepatotoxic and nephrotoxic impact of long-term energy drink consumption in a rat model. Oxidative stress was identified as a significant pathogenic mechanism underlying the organ damage. Pomegranate peel extract mitigated these harmful effects, validating its potential as a supplementary functional beverage and energy drink. Our results raise serious health concerns regarding uncontrolled energy drink intake and highlight the need for stringent safety regulations on caffeinated beverages.

Ethical Statement: This study complied with the regulations and procedures approved by the Ethics Committee at the Faculty of Medicine, Benha University (Approval No. RC-8-2-2024).

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Authors contribution: MA, MA, and MMSG (methodology). MA, AS, MA, MMSG, SS design experimental protocol. MMSG, MA, and MS performed histopathology and IHC. OA, SE, MFA, KM, FWH, AF and SS original draft preparation, review, and editing. All authors read, revised, and approved the final manuscript.

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