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Grape Juice (Vitis vinifera L.) Confers Dose-Dependent Hepatoprotection in a Model of Naproxen Sodium Toxicity

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ABSTRACT: This study investigated the prophylactic effects of freshly prepared raw grape juice (Vitis vinifera L.) against naproxen sodium-induced hepatotoxicity in male Wistar rats. Thirty rats were divided into five groups (n = 6): normal control; low- and high-dose naproxen groups (38.91 and 65.78 mg/kg); and corresponding groups receiving grape juice (27 and 54 mL/kg) with naproxen. Treatments were administered orally for 49 days. Hepatic and renal function was assessed using serum ALT, AST, ALP, BUN, creatinine, and lipid-profile parameters, while liver histopathology was evaluated microscopically. Naproxen administration significantly altered serum biochemical parameters ($p < 0.001$) and produced hepatic necrosis, fatty degeneration, and inflammatory infiltration. Grape juice co-administration significantly attenuated these biochemical and histological changes ($p < 0.05$), with the higher dose showing greater protective efficacy. Histological findings demonstrated substantial preservation of hepatic architecture. These results suggest that grape juice may provide dose-dependent protection against naproxen-associated hepatic injury, warranting further mechanistic and clinical investigation.

1. INTRODUCTION

Naproxen sodium is a common nonsteroidal anti-inflammatory drug (NSAID) that can be used to treat a variety of inflammatory conditions, including osteoarthritis, rheumatoid arthritis, acute gout, and soft tissue injuries [1]. Its primary mode of action is the inhibition of cyclooxygenase (COX) enzymes, which reduces the synthesis of prostaglandins, which are responsible for fever, pain, and inflammation [2]. Thus, naproxen sodium successfully lowers inflammatory swelling and related symptoms in both acute and chronic inflammatory diseases [3].

The parent ingredient of naproxen sodium, is categorized chemically and pharmacologically as a derivative of propionic acid (arylpropionic acid) and a member of the "profen" class of nonsteroidal anti-inflammatory medications (NSAIDs), which also includes ketoprofen and ibuprofen. Naproxen's chemical name is (+)-(S)-2-(6-methoxynaphthalen-2-yl) propanoic acid. Its structural characteristic—a chiral carbon atom next to the carboxyl group—is essential to its stereospecific pharmacological action. Its analgesic and anti-inflammatory qualities are based on this arylpropionic acid structure [4, 5]. Naproxen is a member of the propionic acid class of nonsteroidal anti-inflammatory medicines (NSAIDs), which also includes ibuprofen and

ketoprofen. It is categorized chemically as an arylpropionic acid derivative [5]. Compared to the drug's free acid form (Naprosyn), the sodium salt formulation (naproxen sodium; Anaprox) improved the drug's absorption and dissolving rate, making it a significant formulation advancement [1]. This enhancement led to a quicker onset of effect, which made naproxen sodium very helpful for treating acute inflammatory conditions—a development that became well-known in the 1980s [4, 6].

Naproxen sodium (220 mg) was granted over-the-counter (OTC) status by the U.S. Food and Drug Administration (FDA) in 1994 [7]. Its main OTC use is the short-term alleviation of mild aches and pains, such as headaches. Based on a wealth of data demonstrating naproxen sodium's safety and effectiveness for short-term, low-dose use in the treatment of mild pain problems such headaches [8]. While over-the-counter naproxen sodium is only approved for the short-term alleviation of minor aches and pains, with a maximum suggested dose of 660 mg, prescription naproxen sodium is intended for the treatment of inflammatory illnesses, including rheumatoid arthritis, with doses up to 1650 mg/day [9, 10].

Depending on their experience and the intensity of their symptoms, some people may decide to take 440 mg (two tablets) at first, then 220 mg after 12 hours. Naproxen sodium is recommended for the temporary alleviation of fever, aches, and pains brought on by the common cold as well as for ailments like back discomfort, cramping during menstruation, toothache, mild arthritis, and muscle aches. To guarantee the safe and efficient use of over-the-counter medications like naproxen sodium, it is imperative to adhere to the suggested dosage instructions and guidelines given by medical professionals or included in the medication packaging. For appropriate advice, it is advised to speak with a healthcare professional if you have any questions or concerns [11]**.** Naproxen sodium primarily works by inhibiting the activity of cyclooxygenase (COX-1 or COX-2), which stops the COX-dependent production of proinflammatory algogenic prostaglandins [12]. Reactive oxygen species (ROS) are highly reactive molecules that are generated as a byproduct of Metabolism in cells. Free radicals, which are unstable molecules with unpaired electrons, are one source of ROS production. When NSAIDs are metabolized in the body, they can generate free radicals, leading to an increased production of ROS.

Oxidative stress is a situation that can be brought on by an excess of ROS. When the equilibrium between the production of ROS and the cells' capacity to detoxify them is upset, oxidative stress results. ROS have the ability to interact with cellular constituents called DNA, proteins, and lipids, resulting in cell malfunction and damage. Oxidative discomfort has been recognized as a key mechanism in the context of NSAID toxicity. Because of its role in drug metabolism and detoxification, the liver is especially susceptible to NSAID-induced toxicity. It is thought that the underlying immunological abnormality has a role in the NSAID-induced liver damage. Oxidative stress brought on by ROS can harm cells in a number of ways. First, it can cause a process that leads to cell death. called apoptosis. Oxidative stress is one of the many factors that can cause apoptosis, a controlled type of cell death. Tissue damage and dysfunction may result from the rise in apoptosis brought on by ROS generation. Moreover, ROS can disrupt DNA repair processes, resulting in mistakes in DNA replication and repair.

This may lead to the buildup of DNA mutations and genomic instability.

Cancerous cell development may become more likely as a result of these mutagenesis processes [12]. Prostaglandins are produced from arachidonic acid by the cyclooxygenase (COX) enzyme. COX-1 and COX-2 are the two primary isoforms of the enzyme. Although most tissues contain COX-1, it has been demonstrated that COX-2 levels rise during tissue-level inflammatory processes. Cells of the stomach mucosa are protected by the kidney's production of prostacyclin (PGI2) and prostaglandin E2 (PGE2). Additionally, the COX-1 enzyme aids in the production of prostacyclin (PGI2). thromboxane A2 (TXA2) and prostaglandin E2 (PGE2). 2 In contrast, the COX-2 enzyme contributes to the synthesis of pro-inflammatory and mutagenic cytokines, bacterial endotoxins, interleukins, and tumor necrosis factor. Previous studies have demonstrated the importance of prostaglandins and COX enzymes for human reproduction, as well as their influence on the inflammatory process. Despite the fact that prostaglandins and COX enzymes are known to have several important functions [13].

Naproxen Sodium and Liver: The diagnosis of drug-induced liver disease can be confidently made when the recurrence of the anomaly is observed upon re-exposure to the suspected causal agent. In most cases, retesting is not feasible, so the diagnosis relies on the temporal relation between drug exposure and the development and resolution of the liver condition. Additionally, other obvious causes for the liver condition must be ruled out. While histological abnormalities in the liver caused by drugs are not commonly observed, there have been conclusive cases of liver illness associated with NSAIDs However, it is frequently inferred rather than conclusively shown that NSAIDs cause liver damage. We examined the information that Value Added

Medical Products (VAMP) Health gave to general practitioners (GPs) on their work computers on NSAID-induced liver damage. We used this information to determine the frequency and degree of clinically noticeable liver impairment in a group of more than 100,000 people who took naproxen and other NSAIDs [14].

Hepatoprotective role of herbal plants: Herbal plants are growing in popularity outside of India as well; in India's alternative medical system, about 1200 plants are utilized to cure a range of ailments [15]. Modern medication may not be as successful as it could be, and there aren't many alternatives for treating common liver disorders. For instance, there are differences in the effectiveness of treatments that include interferons and corticosteroids. involves the potential for negative results and is often too costly. Therapeutic medications that are effective and rarely have side effects are therefore needed. This class, which has historically been used as hepatoprotective medications, may comprise plants. Interest in them was sparked by the easy accessibility of herbal remedies without the need for laborious pharmaceutical manufacture [16].

Vitis vinifera L: In India, *Vitis vinifera L.* (grapes) (Family: Vitaceae) is also known as Angur. The well-known *Vitis vinifera* grape variety is a member of the Vitis genus's Vitaceae family. Vitis vinifera comes in red, black, and white variants, as well as seedless and seeded types. Because the Vitis vinifera species outnumbers all other species by 90%, they are easy to find. Grapes originated throughout southern Europe and Western Asia [17]. Therefore, the objective of the present study was to evaluate the hepatoprotective potential of *Vitis vinifera L.* against Naproxen Sodium-induced hepatotoxicity.

2. MATERIALS AND METHODS

The present study employed analytical-grade chemicals and standard laboratory glassware. Dimethyl sulfoxide (DMSO) was procured from Central Drug House; chloroform and chloral hydrate from RFCL Ltd. (Ranken); normal saline from Aculife Healthcare; formaldehyde from Thermo Fisher Scientific; and naproxen sodium from S.S. Drugs, Budhana, Muzaffarnagar. The glassware used included glass rods, volumetric flasks, graduated pipettes, funnels with stands, and filter papers, all utilized in accordance with standard laboratory procedures. Wistar albino rats of either sex, weighing between 150–190 g, were used for the study. The animals were housed in the animal facility of the School of Pharmacy, Bharat Institute of Technology, Meerut, under standard laboratory conditions with free access to water and a standard pellet diet, and maintained under a 12-hour light–dark cycle to regulate their circadian rhythm. The rats were acclimatized to the laboratory environment before the experiment. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) of the School of Pharmacy, Bharat Institute of Technology, Meerut (Approval No.: 1147/ab/07/CPCSEA), and all procedures were performed in accordance with the Committee for the Control and Supervision of Experiments on Animals (CCSEA), Government of India, ensuring ethical animal care. Fresh grape fruits *(Vitis vinifera L.)* were purchased from a local market in Meerut, Uttar Pradesh, India, from a single supplier, and authenticated macroscopically by Dr. Vijay Malik, Department of Botany, Chaudhary Charan Singh University, Meerut. For juice extraction, the grapes were separated from bunches, thoroughly washed, and processed using two methods: a High-Speed Centrifugal (HSC) juicer (Baja juicer) and a Low-Speed Masticating (LSM) juicer. Equal proportions of water and grapes (1:1 w/w) were blended or crushed for 5–10 minutes to obtain the juice, while grape flesh (GF) was prepared by manually removing the skins and seeds from the washed grapes (Kim et al., 2018). Based on an extensive literature survey, the experimental doses of naproxen sodium were selected as 38.91 mg/kg (low dose) and 65.78 mg/kg (high dose), as per [12]. The drug solutions were freshly prepared prior to administration.

2.1 Experimental protocol

A total of thirty *Wistar albino rats* were used in the present study. The animals were randomly divided into five groups, each comprising six rats. Group I (Control group) received *Dimethyl sulfoxide* (DMSO, 10%) orally for 14 consecutive days and served as the vehicle control, as described by Mir Hilal Ahmad *et al.* (2018). Group II (Negative control group I) was administered *Naproxen sodium* at a dose of 38.91 mg/kg orally for 14 days along with DMSO, following the method of Mir Hilal Ahmad *et al.* (2018). Group III (Negative control group II) received a higher dose of *Naproxen sodium* (65.78 mg/kg, p.o.) for 14 days in combination with DMSO, as reported by the same authors. Group IV (Test group I) was treated with grape juice at a dose of 27 ml/kg in combination with *Naproxen sodium* (38.91 mg/kg, p.o.), according to the procedures [12]. Group V (Test group II) received grape juice at a higher dose of 54 ml/kg along with *Naproxen sodium* (65.78 mg/kg, p.o.) for 42 consecutive days, as described [12]. The total duration of the experimental protocol was 49 days.

2.2 Specimen Collection and Processing

The experimental subjects were venipunctured appropriately to obtain blood samples, which were then placed in simple vacutainer tubes (for serum). After letting the samples clot for half an hour at room temperature, they were centrifuged for fifteen minutes at 3000 rpm. Before being examined for biochemical characteristics, the separated serum was divided into aliquots and kept at -20°C. Tissue samples (liver, kidney, etc.) were promptly fixed in 10% neutral buffered formalin for histopathology.

2.3 Biochemical Assays

A semi-automated clinical chemistry analyzer was used to examine all serum biochemical parameters. The manufacturers' directions were strictly followed while using commercial diagnostic reagent kits. Before every run, calibration was carried out using standard solutions, and control sera (both normal and pathological) were added for quality control.

2.4 Renal Function Tests

2.4.1 Blood Urea Nitrogen (BUN)

The concentration of Blood Urea Nitrogen in serum samples was determined using a standardized urease-Berthelot method [18]. Briefly, urea was hydrolyzed to ammonia and carbon dioxide using urease enzyme. The ammonia then reacts with hypochlorite and salicylate in the presence of sodium nitroprusside to form a green-colored indophenol complex, measured photometrically. One mL of functioning urease reagent was mixed with 10 µL of serum. After gently mixing the mixture, it was incubated for five minutes at 37 °C. Following incubation, a reagent blank was used as a reference to measure the absorbance at 546 nm. A standard calibration curve was used to calculate the blood urea nitrogen (BUN) concentration, which was then represented as mg/dL.

2.4.2 Serum Creatinine

The modified Jaffe's alkaline picrate technique was used to quantify serum creatinine [19]. This process creates an orange-red complex by reacting serum creatinine with picric acid in an alkaline media. The concentration of creatinine is directly correlated with the color's intensity. One mL of alkaline picrate reagent and 100 µL of serum were combined and thoroughly mixed. After 30 seconds, the absorbance was measured at 510 nm (A1). At sixty seconds, a second absorbance measurement was made (A2). Analysis was done using the change in absorbance ($\Delta A = A2 - A1$). The sample absorbance was compared to a recognized creatinine standard to determine the serum creatinine concentration, which was then represented in mg/dL.

2.5 Aspartate Aminotransferase (AST):

The activity is based on the principle of the International Federation of Clinical Chemistry (IFCC) method without pyridoxal phosphate activation [20]. Aspartate Aminotransferase (AST): The IFCC-recommended UV-kinetic method was used to evaluate serum AST activity. L-aspartate and α-ketoglutarate are converted into oxaloacetate and L-glutamate by AST. Malate dehydrogenase (MDH) converts the oxaloacetate to malate while oxidizing NADH to NAD^+. At 340 nm, the reduction in absorbance brought on by NADH oxidation was seen. The experiment involved adding 100 µL of serum to 1.0 mL of working reagent that had been preheated to 37°C. The absorbance change was then monitored for three minutes. The unit of measurement for enzyme activity was U/L.

2.6 Alanine Aminotransferase (ALT)

To quantify serum ALT activity, the IFCC-recommended UV-kinetic technique was used [20]. ALT enzymes converted L-alanine and α-ketoglutarate to pyruvate and L-glutamate. Lactate dehydrogenase (LDH) converts the generated pyruvate to lactate while simultaneously oxidizing NADH to NAD^+. ALT activity is directly correlated with the pace at which absorbance at 340 nm decreases. For the experiment, 100 µL of serum was added to 1.0 mL of working reagent at 37°C, and absorbance was measured for three minutes. Results were expressed in U/L.

2.7 Alkaline Phosphatase (ALP):

Serum ALP activity was measured using a kinetic colorimetric method with p-nitrophenyl phosphate (pNPP) as the substrate. For the test, 1.0 mL of AMP buffer (pH 10.4) containing pNPP and magnesium ions was pre-incubated at 37°C with 20 µL of serum. Enzyme activity was measured in U/L and the increase in absorbance was noted for three minutes. ALP hydrolyzes

pNPP into phosphate and p-nitrophenol in an alkaline environment. The resulting yellow-colored p-nitrophenol was detected at 405 nm.

2.8 Lipid Profile

2.8.1 Total Cholesterol

The enzymatic colorimetric CHOD-PAP technique was used to quantify serum total cholesterol. Cholesterol esterase first hydrolyzes cholesterol esters to release cholesterol. Following the oxidation of cholesterol by cholesterol oxidase, hydrogen peroxide is produced. In the presence of peroxidase, this peroxide combines with phenol and 4-aminoantipyrine to form a red quinoneimine dye. For the experiment, 1.0 mL of working reagent and 10 µL of serum were combined, and the mixture was incubated for 10 minutes at 37°C. At 500 nm, absorbance was measured, and the results were reported in mg/dL.

2.8.2 High-Density Lipoprotein Cholesterol (HDL-C):

A precipitation technique and enzymatic estimation were used to assess HDL cholesterol [21]. Using a phosphotungstic acid/$MgCl_2$ reagent, apolipoprotein B-containing lipoproteins (VLDL and LDL) were precipitated, leaving HDL in the supernatant. 500 µL of precipitating reagent and 200 µL of serum were combined, let to stand for ten minutes, and then centrifuged for fifteen minutes at 4000 rpm. The CHOD-PAP technique was utilized to estimate cholesterol from the clear supernatant, and HDL-C levels were reported as mg/dL.

2.8.3 Low-Density Lipoprotein Cholesterol (LDL-C):

The Friedewald formula was used to determine the LDL-cholesterol concentration [22]:

LDL-C (mg/dL) = Total Cholesterol − HDL-C − (Triglycerides/5).

As advised, this formula was only used when serum triglyceride levels were less than 400 mg/dL.

2.9 Histopathological Examination

2.9.1 Histopathological Analysis (Hematoxylin and Eosin Staining)

Tissue samples were sectioned at a thickness of 4–5 µm using a rotary microtome after being fixed in 10% neutral buffered formalin and treated using standard paraffin embedding. After deparaffinizing the sections in xylene and rehydrating them with graded alcohols, they were stained with hematoxylin to see the nuclei and then eosin to see the cytoplasm. Slides were mounted and checked for histological alterations under a light microscope following dehydration and xylene clearing [23].

3. RESULTS

3.1 Blood Urea Nitrogen (BUN) and Serum Creatinine

Chronic administration of naproxen sodium resulted in significant nephrotoxicity, as evidenced by a marked increase in renal function biomarkers. Groups II (LDNS) and III (HDNS) exhibited a highly significant ($p<0.001$) elevation in Blood Urea Nitrogen (BUN) and serum creatinine levels compared to the normal control Group I (Figure 1-2). The increase in BUN was more pronounced in the high-dose naproxen group (III), suggesting a dose-dependent impairment of renal excretory function. Pre-treatment with grape juice (GJ) significantly mitigated this renal damage. Group V (HDGJ) demonstrated BUN and creatinine levels that were restored to values statistically indistinguishable from the normal control group ($p>0.05$). Group IV (LDGJ) also showed a significant ($p<0.01$) reduction in these parameters compared to the corresponding naproxen-only group, though levels remained slightly elevated relative to Group I.

3.2 Effect of Pretreatment of Grape Juice (Low & High Dose) on Blood Urea Nitrogen

The result revealed that the alkaline phosphate level was significantly higher ($p<0.01$) in group LDNS (24.4±0.6) and HDNS (29.0±0.9) when compared to the Normal group. Whereas there was no significant ($p>0.05$) in the group LDGJ (20.2±0.4) and HDGJ (19.1±0.4) when compared to the normal group (18.7±0.5).

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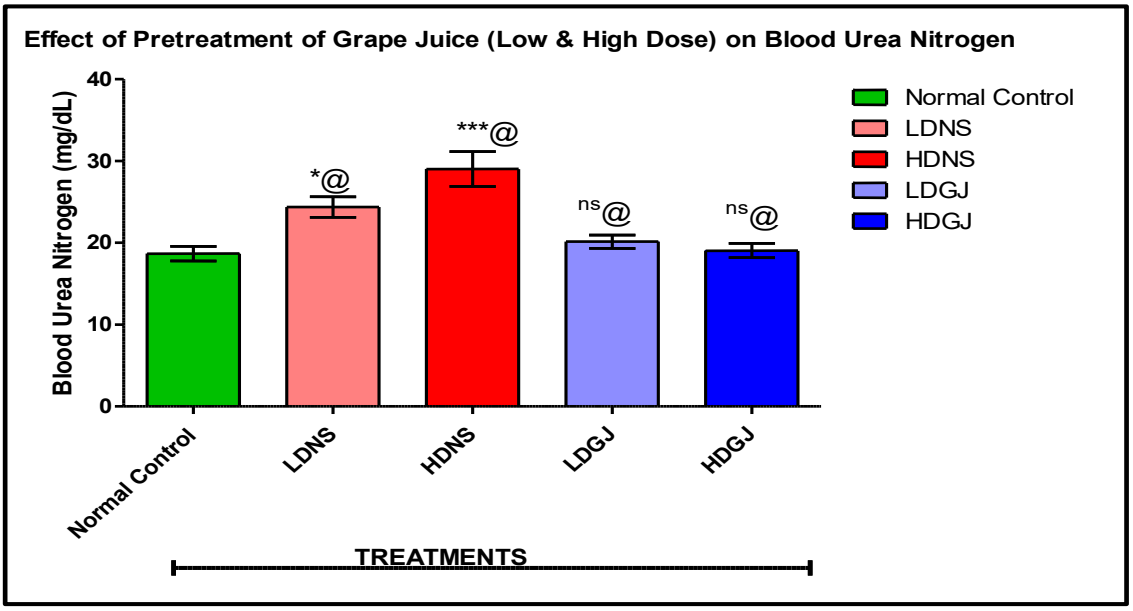

Figure 1: Represent the mean± SEM of six animals' BUN level compared by one way ANOVA followed by Dunnett's test. ***@p<0.001, *@p<0.05, ns@p>0.05 verses normal control.

3.3 Effect of Pretreatment of Grape Juice (Low & High Dose) on Serum Creatinine

The result revealed that the alkaline phosphate level was significantly higher (p<0.01) in group LDNS (2.3±0.6) and HDNS (3.6±0.4) when compared to the Normal group. Whereas there was no significant (p>0.05) in the group LDGJ (1.1±0.2) and HDGJ (0.7±0.2) when compared to the normal group (0.6±0.4).

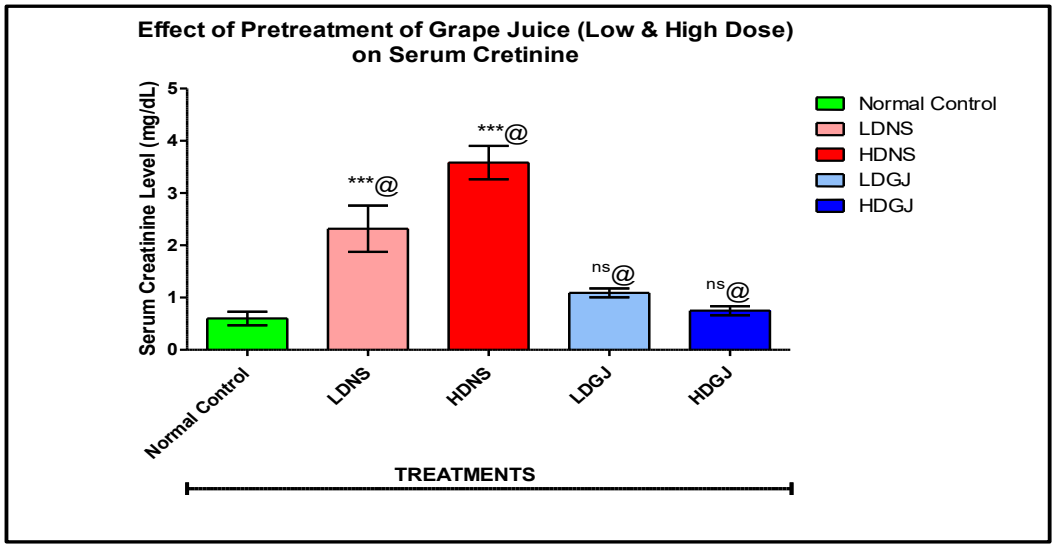

Figure 2: Represent the mean± SEM of six animals Serum creatinine level compared by one-way ANOVA followed by Dunnett's test. ***@p<1.001 , *@p<0.05 , ns@p>0.05 verses normal control.

3.4 Hepatic Function Parameters (AST, ALT, ALP)

Hepatocellular injury was a prominent feature of naproxen sodium intoxication. Groups II and III showed a severe (p<0.001) increase in the activities of the hepatic transaminases—alanine aminotransferase (ALT) and aspartate aminotransferase (AST)—as well as alkaline phosphatase (ALP) compared to Group I (Figure 3-5). The sharp rise in ALT and AST, enzymes localized in

the hepatocyte cytoplasm, indicates necrosis and increased membrane permeability. The concurrent elevation of ALP, a marker of biliary epithelial damage, points to a concomitant cholestatic component of the injury. Pre-treatment with grape juice conferred a potent, dose-dependent hepatoprotective effect. The biochemical profile of Group V (HDGJ) was normalized to control levels ($p>0.05$), while Group IV (LDGJ) showed intermediate but significant ($p<0.001$ vs. naproxen groups) improvement across all three enzymes.

3.5 Effects of Pretreatment of Grape Juice (Low & High Dose) on Aspartate Amino Transferase

The result revealed that the alkaline phosphate level was significantly higher ($p<0.01$) in group LDNS (162.4±0.04) and HDNS (181.3±0.4) when compared to the Normal group. Whereas LDJZ was significantly higher ($p<0.05$) when compared to the normal group and HDGJ (105.3±0.4) was not significant ($p>0.05$) when compared to the normal group (96.5±1.3).

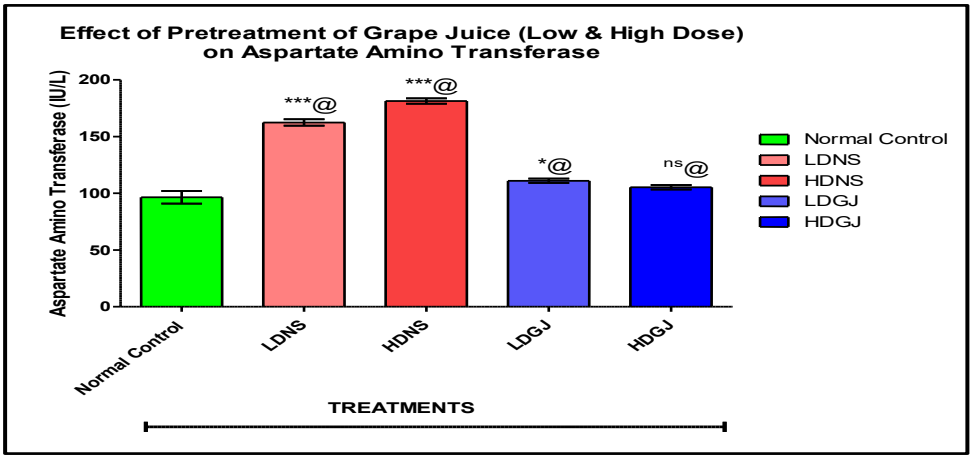

Figure 3: Represent the mean± SEM of six animals Aspartate Amino Transferase level compared by one way ANOVA followed by Dunnett's test. ***@$p<1.001$, *@$p<0.05$, ns@$p>0.05$ verses normal control.

3.6 Effects of Pretreatment of Grape Juice (Low & High Dose) on Alkaline Phosphate

The result revealed that the alkaline phosphate level was significantly higher ($p<0.01$) in group LDNS (143.9±0.8) and HDNS (116.1±0.9) when compared to the Normal group. Whereas LDGJ (102.3±0.6) was significantly higher ($p<0.05$) when compared to the normal group and HDGJ (96.0±0.5) was not significant ($p>0.05$) when compared to the normal group (85.0±1.1).

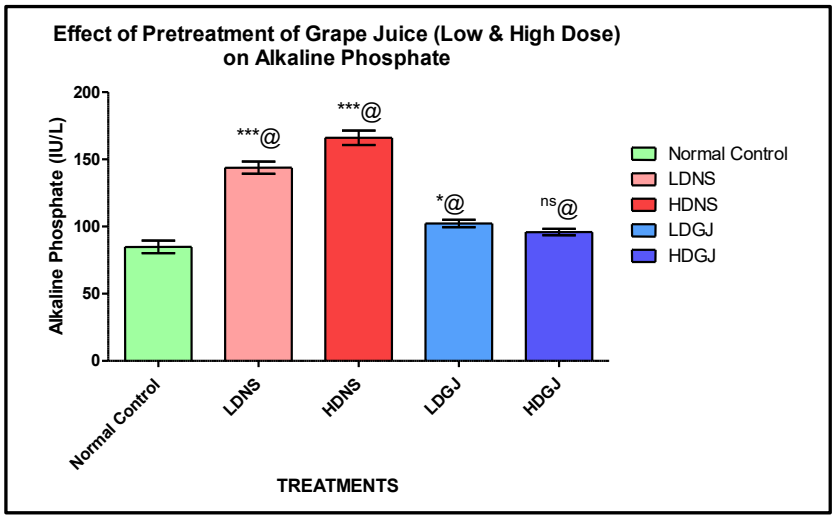

Figure 4: Represent the mean± SEM of six animals Alkaline Phosphate level compared by one way ANOVA followed by Dunnett's test. ***@p<1.001 , *@p<0.05 ns@p>0.05 verses normal control.

3.7 Effects of Pretreatment of Grape Juice (Low & High Dose) Alanine Amino Transferase

The result revealed that the alkaline phosphate level was significantly higher (p<0.01) in group LDNS (48.4±0.9) and HDNS (72.2±1.0) when compared to the Normal group. Whereas LDJZ (31.3±0.6) was significantly higher (p<0.05) when compared to the normal group and HDGJ (22.9±0.9) was not significant (p>0.05) when compared to the normal group (21.5±1.1).

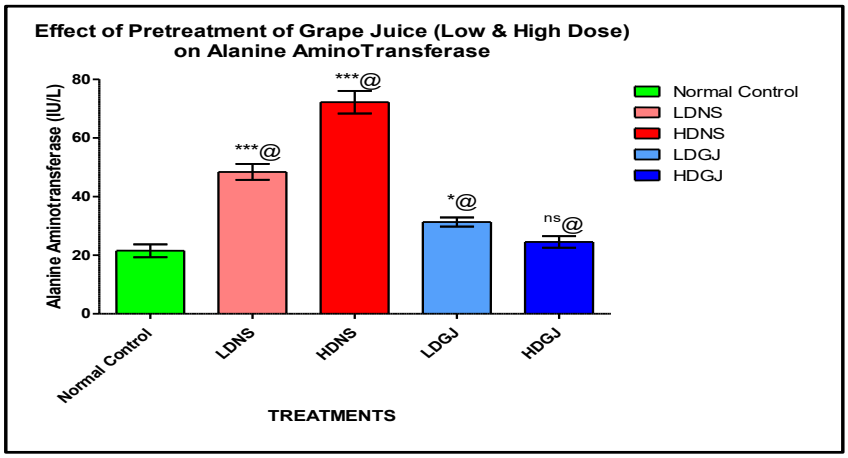

Figure 5: Represent the mean± SEM of six animals Alanine Amino Transferase level compared by one way ANOVA followed by Dunnett's test. ***@p<1.001 , *@p<0.05 , ns@p>0.05 verses normal control.

3.8 Serum Lipid Profile (Total Cholesterol, LDL-C, HDL-C)

Naproxen administration induced a state of significant dyslipidemia. Groups II and III displayed a substantial (p<0.001) increase in serum total cholesterol and low-density lipoprotein cholesterol (LDL-C) compared to Group I (Figure 6-7). Furthermore, a significant alteration in high-density lipoprotein cholesterol (HDL-C) levels was observed (Figure 8). Pre-treatment with grape juice effectively reversed this dyslipidemic state. Group V (HDGJ) exhibited a lipid profile (total cholesterol, LDL-C, HDL-C) that was not statistically different from the normal control group. Group IV (LDGJ) also showed significant amelioration, though the normalization was less complete than in the high-dose GJ group.

3.9 Effects of Pretreatment of Grape Juice (Low & High Dose) on Total cholesterol

The result revealed that the alkaline phosphate level was significantly higher (p<0.01) in group LDNS (57.9±0.7) and HDNS (67.1±1.0) when compared to the Normal group. Whereas there was no significant (p>0.05) in the group LDGJ (42.2±1.0) and HDGJ (35.8±1.0) when compared to the normal group (38.4±1.3).

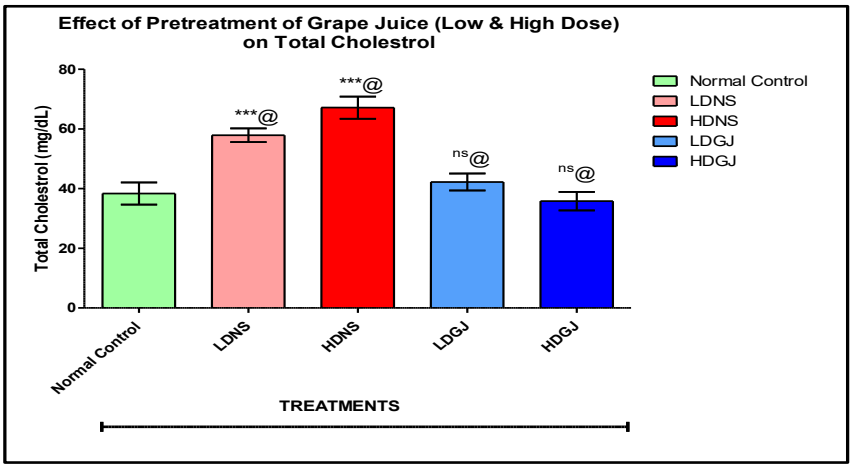

Figure 6: Represent the mean± SEM of six animals Total cholesterol level compared by one way ANOVA followed by Dunnett's test. ***@p<1.001 , *@p<0.05 , ns@p>0.05 verses normal control.

3.10 Effects of Pretreatment of Grape Juice (Low & High Dose) on HDL

The result revealed that the alkaline phosphate level was significantly higher (p<0.01) in group LDNS (64.5±0.4) and HDNS (52.6±0.2) when compared to the Normal group. Whereas LDGJ (64.6±0.2) was significantly higher (p<0.05) when compared to the normal group and HDGJ (54.9±0.4) was no significant (p>0.05) when compared to the normal group (30.2±3.4).

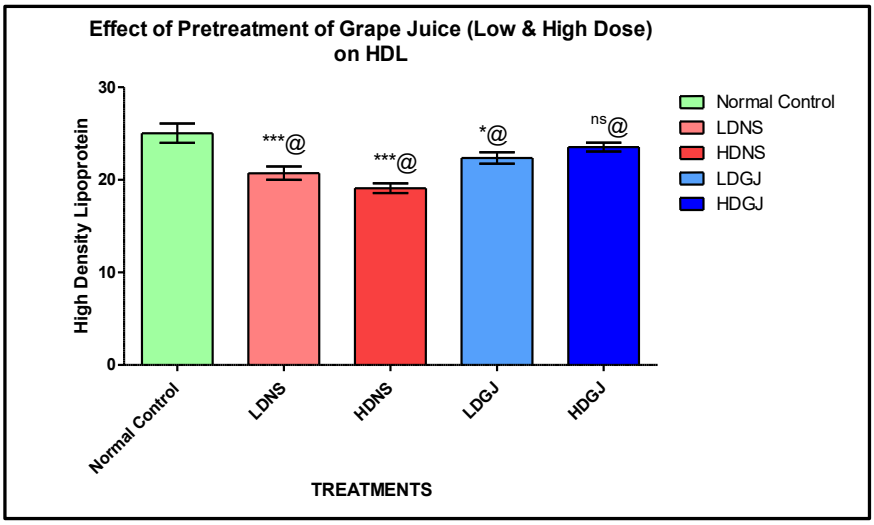

Figure 7: Represent the mean± SEM of six animals HDL level compared by one way ANOVA followed by Dunnett's test. ***@p<1.001 , *@p<0.05 , ns@p>0.05 verses normal control.

3.11 Effects of Pretreatment of Grape Juice (Low & High Dose) on LDL

The result revealed that the alkaline phosphate level was significantly higher (p<0.01) in group LDNS (29.06±0.3) and HDNS (34.7±0.4) when compared to the Normal group. Whereas there was no significant (p>0.05) in the group LDGJ (21.9±0.3) and HDGJ (21.7±0.6) when compared to the normal group (20.8±0.4).

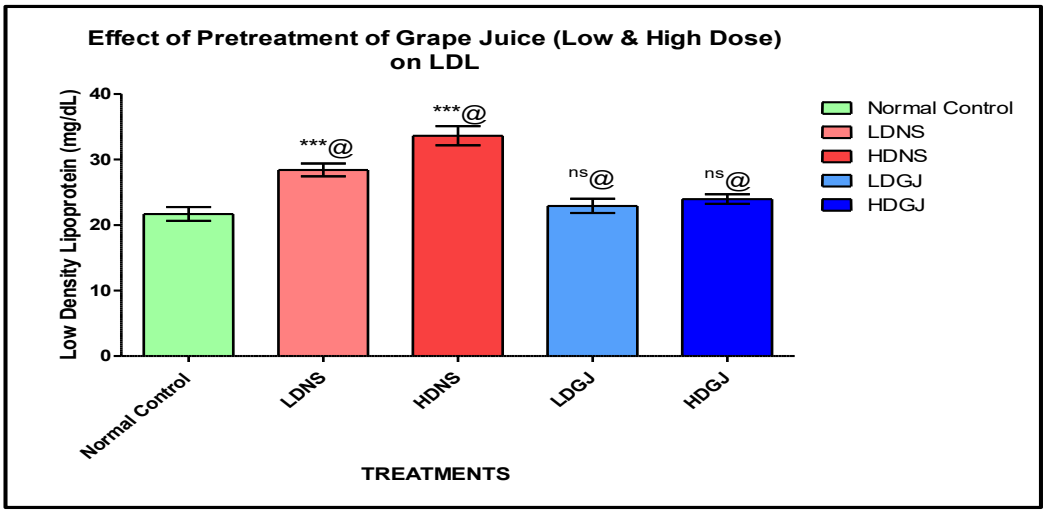

Figure 8: Represent the mean± SEM of six animals LDL level compared by one way ANOVA followed by Dunnett's test. ***@p<1.001 , *@p<0.05 , ns@p>0.05 verses normal control.

3.12 Histopathological Observations (H&E Staining)

Histological analysis of liver and kidney tissues corroborated the biochemical findings (Figure 9). Liver sections from Groups II and III (naproxen-only) revealed severe centrilobular necrosis, marked vacuolar degeneration (indicative of fatty change), inflammatory cell infiltration, and sinusoidal congestion. Kidney sections from these groups showed acute tubular necrosis, glomerular congestion, hyaline cast formation, and interstitial edema. In stark contrast, tissue architecture in Groups IV and V (grape juice pre-treated) was markedly preserved. Group V (HDGJ) showed near-normal hepatic parenchyma and renal tubules with only occasional, mild degenerative changes. Group IV (LDGJ) displayed moderate protection, with noticeable but significantly reduced histopathological lesions compared to the injury groups.

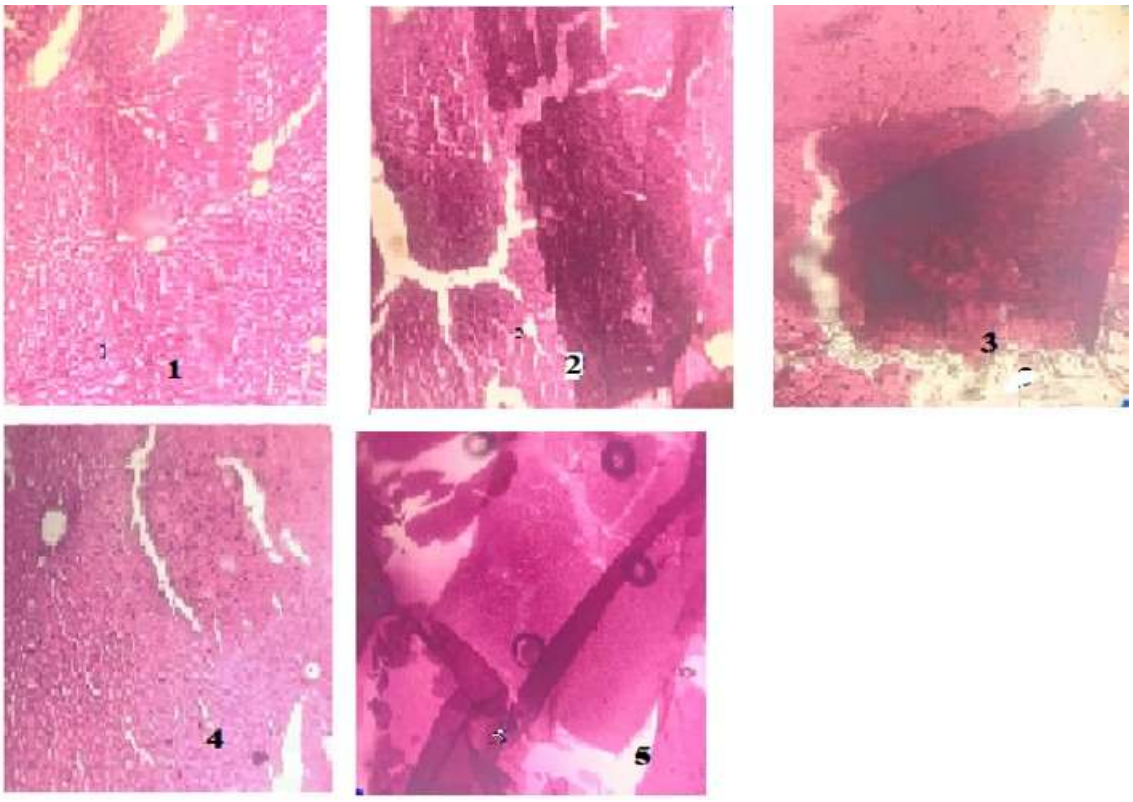

Figure 9: Showing Histological examination.

4. DISCUSSION

The present study demonstrates that chronic naproxen sodium administration induces severe, concurrent hepatorenal toxicity and dyslipidemia in the experimental model. The results affirm the protective efficacy of grape juice pre-treatment, likely mediated through its rich repertoire of antioxidant and anti-inflammatory polyphenols.

The observed elevation in serum creatinine and BUN is a hallmark of impaired glomerular filtration rate (GFR). This nephrotoxicity is a well-documented adverse effect of non-selective NSAIDs like naproxen, arising from the inhibition of renal cyclooxygenase (COX)-derived prostaglandins (PGs), particularly PGE2 and PGI2, which are crucial for maintaining renal blood flow and glomerular filtration, especially under physiological stress [24]. The dose-dependent nature of the renal impairment aligns with studies by [25], who reported that NSAID-induced renal dysfunction is often related to dosage and duration of exposure [25]. The hepatotoxicity, evidenced by the sharp rise in ALT, AST, and ALP, indicates a mixed pattern of hepatocellular necrosis and cholestasis. Naproxen is metabolized primarily in the liver, and its reactive metabolites can deplete hepatic glutathione, induce mitochondrial dysfunction, and trigger oxidative stress and apoptotic pathways [26]. The significant co-elevation of transaminases and ALP is consistent with case reports and studies on NSAID-induced liver injury, which can range from asymptomatic enzyme elevation to fulminant hepatitis [27].

The induction of dyslipidemia (elevated total cholesterol, LDL-C, and altered HDL-C) by naproxen is a notable finding. While NSAIDs are not classically considered major dyslipidemic agents, chronic inflammation and oxidative stress can profoundly

disrupt lipid metabolism. Oxidative stress can modify LDL particles, making them more atherogenic, and impair the function of lecithin-cholesterol acyltransferase (LCAT), an enzyme critical for HDL maturation and reverse cholesterol transport [28]. The observed lipid abnormalities may therefore represent a secondary metabolic consequence of sustained hepatorenal injury and systemic oxidative stress induced by naproxen.

The dose-dependent protective effect of grape juice against all measured parameters can be robustly attributed to its high content of bioactive compounds, notably polyphenols like resveratrol, flavonoids (e.g., quercetin), and anthocyanins. These compounds exert their effects via multiple, synergistic mechanisms such as antioxidant activity and anti-inflammatory action**.** Antioxidant activity and anti-inflammatory action of the Grape juice are due to presence of polyphenols which are potent scavengers of free radicals (ROS/RNS) generated by naproxen metabolism serve as antioxidant. They enhance the endogenous antioxidant defense system by upregulating enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) [29]. This directly mitigates lipid peroxidation of cellular membranes in hepatocytes and renal tubular cells. Polyphenols inhibit key pro-inflammatory signaling pathways, including NF-ϰB and MAPK, leading to reduced production of cytokines like TNF-α and IL-6 [30] and serve as anti-inflammatory. This attenuates the inflammatory cascade that exacerbates tissue injury following the initial insult. Whereas anti-apoptotic effects in Grape juice are due to presence of compounds like resveratrol which have shown to preserve mitochondrial membrane potential, inhibit cytochrome c release, and modulate Bcl-2 family proteins, thereby protecting cells from apoptotic death [31]. The improvement in the lipid profile is likely due to polyphenols' ability to modulate the activity of hepatic enzymes involved in lipid synthesis (e.g., HMG-CoA reductase) and to promote cholesterol efflux via upregulation of transporters like ABCA1 [32].

The histopathological findings provide crucial morphological validation of the biochemical data. The severe necrosis and inflammation in the naproxen-only groups and the marked architectural preservation in the grape juice pre-treated groups offer direct visual evidence of tissue-level protection. This correlates with studies where plant-derived antioxidants have been shown to prevent histopathological lesions in drug-induced organ toxicity models [33].

4. CONCLUSION

Chronic naproxen sodium administration causes significant hepatorenal dysfunction and dyslipidemia, primarily driven by oxidative stress and inflammation. Pre-treatment with grape juice, rich in polyphenolic antioxidants, effectively attenuates these toxicities in a dose-dependent manner, normalizing biochemical markers, restoring lipid homeostasis, and preserving tissue architecture. This underscores the potential of dietary polyphenols as adjunctive protective agents against the adverse effects of chronic NSAID therapy.

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AUTHOR CONTRIBUTIONS

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ETHICS STATEMENT

The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) of the School of Pharmacy, Bharat Institute of Technology, Meerut (Approval No.: 1147/ab/07/CPCSEA).

DATA AVAILABILITY STATEMENT

All the data generated in this study have been included in the manuscript.

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