Special Issue:

Veterinary Medicine between Sustainable Development and Public Health to Confront Global Changes

Protective Effect of Grape Seed Extract on Meloxicam-Induced Hepato-Renal Toxicities in Rats with relation to their Biochemical, Histological, and Immunohistochemical Confirmation

Adham Omar Mohamad Sallam[1*], Ashraf Abd El-Hakem Ahmed El-Komy[1], Enas Abdulrahman Hasan Farag[2], Samar Saber Ibrahim[3]

[1]Department of Pharmacology, Faculty of Veterinary Medicine, Benha University, Egypt; [2]Department of Pharmacology, Animal Health Research Institute (AHRI), Agriculture Research Center (ARC), Egypt; [3]Department of Forensic Medicine and Toxicology, Faculty of Veterinary Medicine, Benha University, Egypt.

Abstract | Nonsteroidal anti-inflammatory drugs (NSAIDs), including Meloxicam, are associated with significant hepato-renal toxicity due to their inhibition of cyclooxygenase enzymes. This study hypothesizes that grape seed extract (GSE), rich in potent antioxidants and anti-inflammatory flavonoids, mitigates the hepato-renal toxicity induced by Meloxicam. Fifty albino rats were divided into five groups: A control, vehicle control, Meloxicam-treated, GSE-treated, and a combination treatment group. The study evaluated biochemical parameters, oxidative stress markers, and histological changes in liver and kidney tissues. This study addresses a significant gap in understanding how GSE can counteract NSAID toxicity, with potential clinical implications for safer long-term NSAID use. GSE administration significantly reversed the elevated levels of serum alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, urea, and creatinine caused by Meloxicam toxicity. Additionally, oxidative stress markers such as malondialdehyde (MDA) were significantly reduced, while antioxidant enzymes like superoxide dismutase (SOD) and catalase (CAT) showed marked improvement in the GSE-treated groups ($p \leq 0.05$). Histological and immunohistochemical analysis confirmed these findings, showing reduced tissue damage and apoptosis. In conclusion, the obtained results suggest that GSE protects against Meloxicam-induced hepato-renal toxicity, with a marked reduction in oxidative stress and inflammation, supporting its potential therapeutic role.

Keywords: Meloxicam, GSE, SOD, CAT, MDA, Histopathological. Immunohistochemical

Received | August 18, 2024; **Accepted** | September 24, 2024; **Published** | October 07, 2024

***Correspondence** | Adham Omar Mohamad Sallam, Department of Pharmacology, Faculty of Veterinary Medicine, Benha University, Egypt; **Email:** adham.sallam@fvtm.bu.edu.eg

Citation | Sallam AO , El-Komy A , Farag E A , Ibrahim SS (2024). Protective effect of grape seed extract on meloxicam-induced hepato-renal toxicities in rats with relation to their biochemical, histological, and immunohistochemical confirmation. Adv. Anim. Vet. Sci. 12(s1): 186-197.

DOI | https://dx.doi.org/10.17582/journal.aavs/2024/12.s1.186.197

ISSN (Online) | 2307-8316; **ISSN (Print)** | 2309-3331

INTRODUCTION

All over the world, nonsteroidal anti-inflammatory drugs (NSAIDs) are among the most frequently prescribed drugs. A major purpose of prescribing NSAIDs is to limit suffering, preserve tissue structure, and restore normal organ function through the pharmacological control of pain and inflammation (Ungprasert *et al.*, 2015; Rivera-Velez *et al.*, 2019a). Despite their importance, their usage was restricted because they have several notorious adverse

side effects because these drugs were proven to possibly cause gastrointestinal, renal, hepatic, and reproductive toxicity (Burukoglu, 2016).

NSAIDs exhibits its anti-inflammatory effects by competitive non-selective/ selective inhibition of cyclooxygenase (COX) activity and subsequently blocks the biosynthesis of prostaglandins in lesion sites. The level of prostaglandin production mainly depends on the expression of cyclooxygenases (COXs) in inflammatory tissues, especially cyclooxygenases-2. PGs are hormone-like lipid compounds and are involved in many physiological reactions and play a key role in the generation of inflammatory responses. In general, PGs exert their effects by mediating the body's responses to tissue injury or inflammation. Among them, PGE2 is the dominated prostaglandin that induces typical symptoms of inflammation, such as pain, fever, tumor, and anaphylactic reaction (Ferrer *et al*., 2009; Zhiran *et al*., 2022).

COX-1 and COX-2enzymes are membrane-anchored isoforms of the cyclooxygenase enzyme, are inhibited by NSAIDs. While COX-1 is constitutively expressed and seen in a variety of tissues, COX-2 is inducible in pathological conditions and seen primarily in monocytes, macrophages, and endothelial cells. The induction of COX-2 occurs mainly during tissue damage or inflammation in response to cytokines, reactive oxygen intermediates (ROS) and transforming growth factor-β (Honma *et al*., 2014; Uzun *et al*., 2015). Previously known NSAIDs acts by inhibiting both COX-2 isoforms, whereas newly discovered specific COX-2 inhibitors are much more specific to COX-2, preserving the anti-inflammatory properties of COX-2 inhibition while theoretically reducing the adverse effects of COX-1 inhibition (Jyothi *et al*., 2023).

Considering the frequent side effects caused by non-selective drugs that target cyclooxygenase-1 (COX-1) in particular, the need for selective drugs targeting COX-2 became apparent. There is a new NSAID called Meloxicam that is widely used in humans, cattle, buffalo, goats, and dogs and has anti-inflammatory, analgesic, and antipyretic properties. Having a greater affinity for inhibiting the activity of cyclooxygenase (COX)-2 than COX-1, it exerts its effect by selective inhibition of COX-2 (Burukoglu, 2016).

Meloxicam inhibits COX-2 at lower doses while inhibiting COX1 at higher doses, which has well-known side effects. COX-2 inhibition is linked to meloxicam's anti-inflammatory properties via preventing prostaglandin synthesis at inflammatory sites. The drug's well-known negative side effects are linked to its suppression of COX-1 (Villalba *et al*., 2016; Jyothi *et al*., 2023). COX-2 inhibitors exert pharmacological activity through inhibition of the NF-κB pathway and thereby prohibit the production of pro-inflammatory cytokines, including NO, PGE2, IL-6, and TNF-α (Zhiran *et al*., 2022).

Through a variety of methods, long-term use of NSAIDs results in nephrotoxicity mainly due to sustained reduction in renal blood flow as a result of chronic prostaglandin inhibition. NSAIDs are also known to cause acute interstitial nephritis (AIN) with hematuria, proteinuria, flank pain, and acute tubular necrosis. Nephrotic syndrome, renal vasculitis, and acute papillary necrosis are a few of the uncommon causes resulting in glomerular injury (Ingrasciotta *et al*., 2015; Jyothi *et al*., 2023).

Because these medications block prostaglandins, which shield the stomach mucosa, long-term use of these medications raises the risk of gastrointestinal erosions, ulcers, and bleeding (Ungprasert *et al*., 2015).

NSAIDs have been related to fulminant liver failure and death in addition to elevated blood aminotransferase levels and hepatitis with jaundice as a result of Oxidative stress caused by ROS (Sriuttha *et al*., 2018).

It has been demonstrated that antioxidants guard against oxidative stress by reducing the damaging effects of free radicals. Vinifera, or grapes, are among the most well-known fruits in the world. *V. vinifera* has a wide spectrum of pharmacological effects, which are facilitated by its polyphenolic concentration in its seeds (Hasona *et al*., 2017; Sochorova *et al*., 2021).

Proanthocyanidins, one type of flavonoid found in grape seed extract, has anti-inflammatory, antioxidant, hepatoprotective, and neuroprotective qualities. Grape seed extract (GSE) not only counteracts the harmful effects of free radicals generated by prolonged usage if NSAIDs but also lessens organ damage, enhances the ratio of antioxidants to oxidants, and decreases the production of inflammatory mediators by controlling cellular oxidative damage (Hassan and Al-Rawi, 2013; Sochorova *et al*., 2021).

Since ROS can cause oxidative stress in liver and kidneys, we hypothesize that the antioxidant properties of proanthocyanidins would relieve the oxidative stress brought on by the Meloxicam-induced free radicals and subsequently prevent liver and kidney injuries.

Because of the aforementioned information, this study examines if GSE treatment shields against Meloxicam-induced hepatatorenal toxicities by examining serum biochemicals and tissue oxidative/antioxidant parameters in rats. TNF, caspase III, and bax protein expression were assessed histologically and by immunohistochemistry in all experimental groups.

MATERIALS AND METHODS

Chemicals

MEPACO Company (Inshas Elraml, El-Sharkia, Egypt) provided the grape seed extract, while Adwia Pharmaceuticals (5th Settlement, New Cairo, Egypt) provided the meloxicam. N-ethylmaleimide and polysorbate 80 NF were purchased from Sigma-Aldrich Co., USA, and derived from Arabcomed (Obour City, Egypt). Bio-diagnostics (Dokki, Giza, Egypt) provided all biochemical and antioxidant assay kits.

Experimental animals

The study involved 50 albino male rats, each weighing 145±7.3g, obtained from the Laboratory Animal Center, Faculty of Veterinary Medicine, Benha University, Egypt. A two-week acclimatization period was followed before the experiment was started. A review committee at the Faculty of Veterinary Medicine at Benha University in Egypt approved the study's experimental design (BUFVTM 14-07-23). Study animals were fed a standard laboratory diet and had access to water ad libitum.

Experimental design

Five groups of 50 albino rats were randomly numbered, and each group had ten rats. For 28 consecutive days, Group I (Control) received saline orally (vehicle for grape seed extract). For 28 days, Group II (Vehicle Control) received a 1% polysorbate 80 saline solution orally (vehicle for meloxicam). A dose of 1 mg of meloxicam per kg of body weight was administered orally once daily for 28 days (da Silva et al., 2022). The grape seed extract group (Group IV) received grape seed extract orally once daily, dissolved in saline, for 28 days (Giribabu et al., 2018). The combination treatment group (Group V) received both grape seed extract and meloxicam. During the 28-day experiment, all treatments were administered orally at 10 AM daily. Due to the distinct solubility properties of the test compounds, two different vehicles were used in this study. Saline (0.9% NaCl) was used as the vehicle for grape seed extract (GSE) due to its water solubility. A 1% polysorbate 80 solution was used as the vehicle for meloxicam, which has poor water solubility. To minimize bias, the study was conducted blindly. The individuals responsible for performing biochemical, histopathological, and immunohistochemical evaluations were not informed about the group assignments. This ensured that data collection, analysis, and interpretation remained objective and free from potential bias.

Sampling

Anesthetizing the rats with isoflurane was done at the end of the experiment, after 24 hours. The retro-orbital plexus was used for collecting blood samples from each mouse that was centrifuged for 20 minutes at 1200 g for each rat. Coagulation was permitted at room temperature for 30 minutes before centrifugation. After storing the serum at -20°C, further biochemical analysis was performed. A cervical decapitation was used to euthanize the rats after blood collection. Prior to collecting tissue samples, we excised the liver and kidneys and washed them with physiological saline. Using an electrical homogenizer, 5 ml of phosphate buffer (pH 7.4) was homogenized with a gram of each tissue. The homogenates were treated with N-ethylmaleimide to prevent GSH oxidation. We centrifuged the homogenates at 1200 rpm for 20 minutes at 4°C, and collected the supernatants to analyze for biomarkers of oxidative stress. Histological and immunohistochemical studies were conducted on the remaining liver and kidney tissues fixed in 10% neutral buffered formalin for 48 hours. Rats and tissue remnants were disposed of in a hygienically appropriate burial pit under strict control.

Analyses of serum biochemistry

Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) (Reitman and Frankel, 1957) and alkaline phosphatase (ALP) activities (Tietz *et al.*, 1983) were measured as indicators of liver injury. The liver function was assessed by measuring serum albumin and total protein, and subtracting albumin from total protein to calculate globulin levels. To determine kidney function, serum urea and creatinine levels were measured according to Coulombe and Favreau (1963) and Bartels *et al.* (1972).

Detection of oxidative/antioxidant cascades

The serum concentrations of glutathione (GSH), catalase activity (CAT), and malondialdehyde (MDA) were measured using diagnosis kits from Bio-diagnostics, Egypt (Ohkawa *et al.*, 1979).

Histological examination

The liver and kidney tissues of both groups were fixed, dehydrated in graded alcohols, removed from xylene, and embedded in paraffin. Following the protocols described by Bancroft *et al.* (2013), sections of 5 mm thickness were mounted on glass slides and stained with hematoxylin and eosin (H & E). Histopathological sections were semi-quantitatively scored based on the staining intensity of tissue lesions. The scoring system used was as follows: negative (-) for no staining, weak (+) for less than 10% positive cells, moderate (++) for 10–50% positive cells, and strong (+++) for more than 50% positive cells. At least five random sections per organ were analyzed for each rat. Two blind pathologists conducted the scoring to eliminate bias and ensure objectivity in the data collection.

Immunohistochemical studies

Immunohistochemical localization of BAX, Caspase-3, and TNF-α in liver and kidney tissues was performed on 5

µm paraffin sections mounted on positively charged slides. Sections were dewaxed and rehydrated, and endogenous peroxidase activity was blocked. In order to retrieve the antigens from the sections, citrate buffer (pH6) was heated at 90°C for 30 minutes. After blocking non-specific binding with 10% bovine serum albumin for 30 minutes, specific binding was restored. We incubated sections for 1 hour with primary antibodies (rabbit monoclonal anti-BAX and anti-Caspase-3 at dilutions of 1:200 and 1:500, Abcam, Boston, USA). Following this, biotinylated donkey anti-mouse IgG (Abcam, Boston, USA) was incubated for 30 minutes at room temperature. Diaminobenzene (DAB) was used as chromogen and hematoxylin as counterstain to visualize the immunoreactions (Santa Cruz Biotech, CA, USA). Immunohistochemical staining of Caspase-3, BAX, and TNF-α in both hepatic and renal tissues was evaluated in a blinded manner across all groups. A Leica DM3000 microscope was used to examine at least five random high-power fields at 400X magnification. The immunohistochemical staining intensity for apoptotic and inflammatory markers (Caspase-3, Bax, TNF-α) was quantified based on the area percentage of positive staining. The positive staining areas were calculated as a percentage of the total tissue section area. The scoring was performed by two independent, blinded pathologists, and inter-rater reliability was ensured through consensus. At least five sections per organ per animal were analyzed, and the averages were calculated.

Statistical analysis

The statistical analysis was performed using SPSS software (Version 20.0; SPSS Inc., Chicago, IL, USA). ANOVA followed by Duncan's post hoc test was used to analyze differences between groups. Data are presented as mean ± SEM, with significance set at $P < 0.05$.

RESULTS AND DISCUSSION

Serum biochemical studies

Pharmacological Effect of GSE treatment on liver biochemical tests (Albumin, total protein, ALP, ALT, and AST) and kidney Creatinine in rats induced Meloxicam toxicity. Meloxicam administration significantly ($P \leq 0.05$) increased serum ALT, AST, and ALP activities compared with those in control rats. Similarly, Meloxicam significantly ($P \leq 0.05$) increased the levels of creatinine and urea. Conversely, serum total protein and albumin significantly ($P \leq 0.05$) decreased due to meloxicam administration compared to that in control rats. Grape seeds extract administration with Meloxicam restored these parameters towards the normal values (Table 1).

Meloxicam administration significantly ($P \leq 0.05$) increases serum cholesterol, triglycerides, and LDL levels with decrease in HDL level (Table 2). The effect of meloxicam on lipid profile can be improved by administration of GSE.

Table 1: Pharmacological Effect of GSE treatment on liver and kidney biochemical tests in rats induced Meloxicam toxicity.

Parameters	Control saline	Tween 80	Meloxicam	*GSE	Meloxicam +GSE
AST (U/L)	37.20 ± 2.46^{c}	34.80±1.80^{c}	131.80±1.46^{a}	32.00±2.10^{c}	95.20±5.55^{b}
ALT (U/L)	33.76 ± 3.84^{c}	29.07±3.56^{c}	140.33±12.14^{a}	27.68±2.55^{c}	79.26±3.77^{b}
ALP (U/L)	174.68±23.41^{c}	133.68±23^{c}	408.99±8.59^{a}	165.28±5.43^{c}	303.12±12.50^{b}
Total protein (g/dl)	10.32±0.38^{a}	10.15±0.26^{a}	6.05±0.20^{c}	10.06±0.28^{a}	8.09±0.27^{b}
Albumin (g/dl)	4.14±0.11^{a}	4.36±0.11^{a}	2.29±0.13^{c}	4.14±0.10^{a}	3.20±0.04^{b}
Creatinine (mg/dl)	0.71±0.04^{c}	0.76±0.07^{c}	2.10±0.12^{a}	0.74±0.06^{c}	1.59±0.03^{b}
Urea (mg/dl)	2.27±0.10^{c}	2.39±0.09^{c}	5.22±0.06^{a}	2.21±0.04^{a}	4.10±0.12^{b}

*The dose of Meloxicam is 1 mg/kg P.O, the dose, the dose of Grape seed extract is 200 mg/kg PO, the doses of AST, ALT, & ALP enzymes involved in the aspartate aminotransferase reaction, and the dose of GSE is 200 mg/kg PO. Data are presented as (Mean ± S.E). S.E = Standard error. Mean values with different superscript letters in the same row are significantly different at (P<0.05).

Table 2: Pharmacological Effect of GSE on serum Cholesterol, Triglycerides, HDL, and LDL in rats induced Meloxicam toxicity.

Parameters	Control saline	Tween 80	Meloxicam	GSE	Meloxicam + GSE
Cholesterol (mg/dl)	138.76±7.40^{c}	144.54±1.53^{c}	231.54±6.43^{a}	136.30±5.72^{a}	188.06±3.69^{b}
Triglycerides (mg/dl)	146.68±13.66^{c}	146.48±11.00^{c}	334.68±9.01^{a}	150.10±7.98^{a}	226.66±12.31^{b}
HDL (mg/dl)	56.94±2.85^{a}	53.28±3.29^{a}	23.00±1.16^{c}	54.34±3.98^{a}	37.85±1.71^{b}
LDL (mg/dl)	52.49±7.98^{c}	61.96±2.73^{c}	141.60±6.17^{a}	51.94±7.91^{c}	104.88±3.00^{b}

Meloxicam at dose of 1 mg/Kg. PO; GSE, Grape seed extract at dose of 200 mg/Kg PO; HDL, High density lipoprotein; LDL, Low density lipoprotein. Data are presented as (Mean ± S.E). S.E = Standard error. Mean values with different superscript letters in the same row are significantly different at (P<0.05).

Table 3: Effects of GSE treatment on oxidative stress markers in liver and kidney tissues in rats induced meloxicam toxicity.

Parameters	Organ	Control saline	Tween 80	Meloxicam	GSE	Meloxicam + GSE
CAT (U/gm)	Liver	531.15±16.93[a]	531.50±20.68[a]	229.67±16.46[c]	514.17±13.30[a]	414.81±16.59[b]
SOD (U/gm)	Liver	294.91±15.21[a]	283.92±11.73[a]	111.84±7.63[c]	282.80±14.61[a]	193.51±17.18[b]
MDA (nmol/gm)	Liver	129.94±10.05[c]	130.46±8.85[c]	362.93±20.78[a]	129.73±8.24[c]	238.33±13.31[b]
CAT (U/gm)	Kidney	540.81±7.56[a]	551.31±5.96[a]	343.98±6.12[c]	521.87±11.88[a]	425.87±21.23[b]
SOD (U/gm)	Kidney	219.44±11.58[a]	222.45±8.86[a]	99.39±4.77[c]	232.90±6.45[a]	152.90±9.39[b]
MDA (nmol/gm)	Kidney	156.86±12.83[c]	161.56±6.68[c]	318.60±7.68[a]	161.99±11.12[c]	245.04±7.45[b]

Meloxicam at dose of 1 mg/Kg. PO; GSE, Grape seed extract at dose of 200 mg/Kg PO; MDA, malondialdehyde; GSH, reduced glutathione; CAT, catalase. Data are presented as (Mean ± S.E). S.E = Standard error. Mean values with different superscript letters in the same column are significantly different at ($P<0.05$).

Detection of oxidative/antioxidant cascades

It was found that the liver and kidney tissues of rats intoxicated by meloxicam displayed substantial increases in MDA levels as well as dramatic decreases in SOD and CAT levels. In contrast to the meloxicam-treated group, the Meloxicam + GSE group showed a decrease in MDA levels and an increase in GSH and CAT levels in hepatic and renal tissues (Table 3).

Histopathological results

The histopathological analysis of the liver and kidney tissues across the different groups revealed distinct changes (Figure 1). In the Untreated Control group, the liver tissues exhibited normal histological architecture with well-preserved hepatocytes, clear sinusoids, and central veins, with no signs of inflammation, necrosis, or fibrosis. The Vehicle Control group showed a similar histological appearance to the untreated control, with no significant pathological changes, and hepatocytes appeared normal, without any signs of inflammation or necrosis. The Grape Seed Extract (GSE) Control group presented with also preserved hepatic architecture. In contrast, the Meloxicam-treated group displayed marked hepatocellular damage, characterized by centrilobular necrosis, inflammatory cell infiltration, and vacuolization, along with evidence of congested central veins and sinusoidal dilation.

However, the Combination Treatment (Meloxicam + GSE) group showed significant improvement in liver histology, with reduced necrosis, inflammation, and sinusoidal congestion, and the hepatocytes were better preserved compared to the Meloxicam-only group, indicating a protective effect of grape seed extract. Similarly, in the kidney tissues, the Untreated Control group exhibited normal histological features with well-defined glomeruli, intact tubular structures, and no signs of inflammation or necrosis. The Vehicle Control group demonstrated similar normal histology, with no significant alterations in glomeruli or tubules. The Grape Seed Extract (GSE) Control group showed no observable alteration in histological architecture, with no tubular necrosis or interstitial inflammation. The glomeruli appeared more intact, and there was no fibrosis observed. Conversely, the Meloxicam-treated group exhibited severe tubular damage (d), including tubular necrosis, interstitial inflammation, and glomerular shrinkage, with evidence of interstitial mononuclear cell infiltration (in). The Combination Treatment (Meloxicam + GSE) group displayed significant amelioration of Meloxicam-induced damage, with improved tubular integrity, reduced necrosis, and minimal interstitial inflammation, demonstrating the protective effect of grape seed extract in preserving renal tissue structure.

Immunohistochemical results

Immunohistochemical analysis revealed distinct patterns in the expression of Caspase-3, Bax, and TNF-alpha (Figure 2-4) across different treatment groups. In the untreated Control group, staining for Caspase-3, Bax, and TNF-alpha was minimal, indicating low levels of apoptosis and inflammatory cytokines in both liver and kidney tissues. Similarly, the Vehicle Control group displayed minimal expression of these markers, reflecting an absence of significant apoptotic or inflammatory activity. In the Grape Seed Extract Control group, immunohistochemical analysis showed minimal expression of Caspase-3, Bax, and TNF-alpha, suggesting that grape seed extract does not induce apoptotic and inflammatory responses. In contrast, Meloxicam treatment resulted in a marked increase in Caspase-3 and Bax staining, particularly in areas of tissue damage, which indicated heightened apoptotic activity. Additionally, TNF-alpha expression was elevated, reflecting increased inflammatory responses in both liver and kidney tissues. However, the combination of Meloxicam and Grape Seed Extract led to further reductions in the staining of Caspase-3, Bax, and TNF-alpha compared to the Meloxicam group, demonstrating a significant protective effect of grape seed extract against Meloxicam-induced apoptosis and inflammation in both liver and kidney tissues.

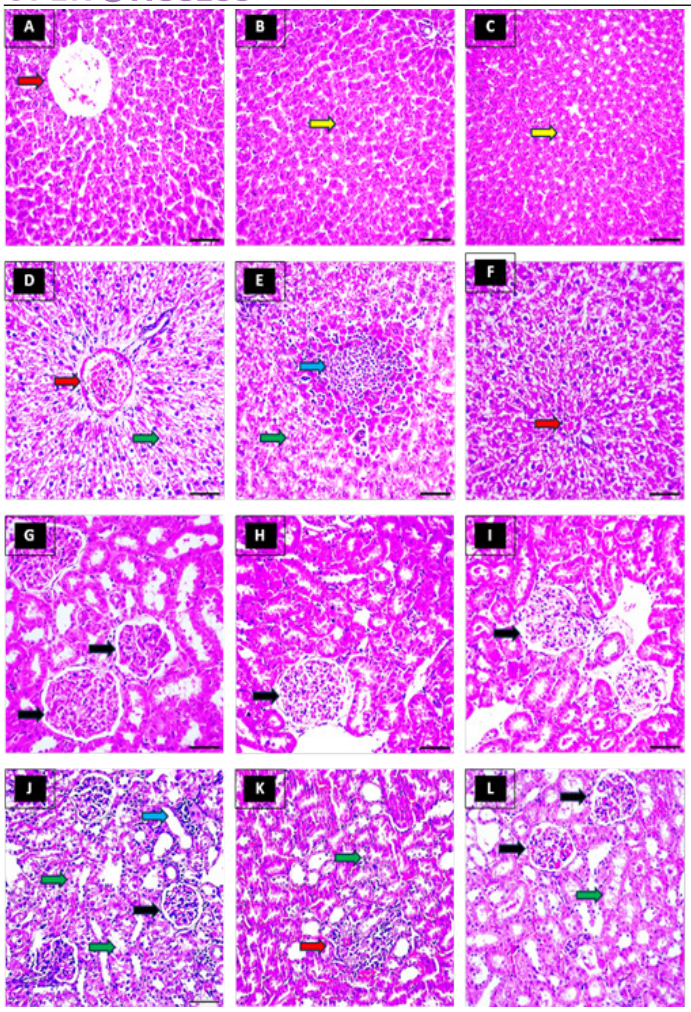

Figure 1: Histopathological analysis of liver and kidney tissues across different treatment groups. In the Untreated Control group, liver tissues (A) and kidney tissues (G) exhibit normal histological architecture, with well-preserved hepatocytes and central veins (red arrow) in the liver, and intact glomeruli (black arrow) and tubular structures in the kidneys, with no signs of inflammation, necrosis, or fibrosis. Similarly, the Vehicle Control group (B) and (H) show no significant pathological changes, with normal hepatocyte morphology (yellow arrow) and glomerular and tubular structures. The Grape Seed Extract (GSE) Control group (C) and (I) present relatively preserved hepatic architecture and glomerular integrity. In contrast, the Meloxicam-treated group (D) and (J) exhibit marked hepatocellular damage (green arrow) and severe tubular damage (green arrow), characterized by centrilobular necrosis, inflammatory cell infiltration (blue arrow), vacuolization, congested central veins, sinusoidal dilation, tubular necrosis, interstitial inflammation, glomerular shrinkage, and interstitial fibrosis. The Combination Treatment group (Meloxicam + GSE) (E) and (K) demonstrates significant improvement in liver and kidney histology, with reduced necrosis, inflammation, and sinusoidal congestion in the liver, and improved tubular integrity, reduced necrosis, and minimal interstitial inflammation in the kidneys, indicating a protective effect of grape seed extract.

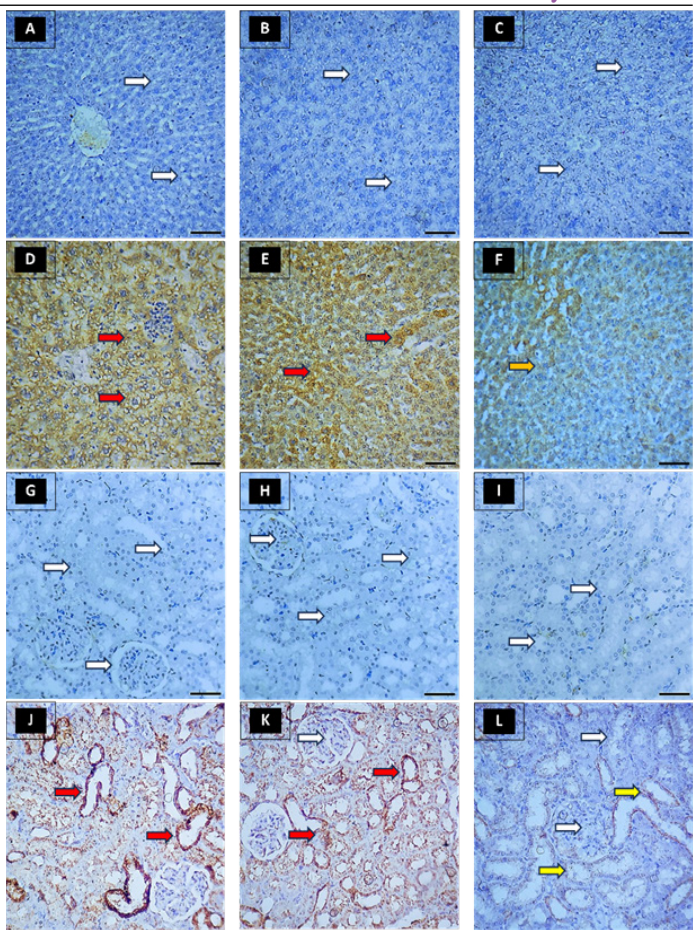

Figure 2: Immunohistochemical staining for Bax in liver and kidney tissues across different treatment groups. Panels (A) and (G) display "Negative" Bax expression in both liver and kidney tissues of the control group, indicating no detectable Bax activity. Similarly, panels (B) and (H) for the Vehicle Control group also show a "Negative" Bax expression. In the Grape Seed Extract group, panels (C) and (I) reveal "Negative" Bax expression in liver tissues, consistent with controls, with a slight increase in activity observed in one field of the kidney. Conversely, Meloxicam treatment resulted in "Strong" Bax expression (s) in both liver and kidney tissues, as shown in panels (D) and (E) for the liver and (J) and (K) for the kidney, with high activity across most fields. The combination of Meloxicam and Grape Seed Extract (F) and (L) led to a "Moderate" Bax expression (m) in liver tissues, reflecting a moderate level of activity. In contrast, kidney tissues displayed a "Weak" Bax expression (w), indicating lower activity than Meloxicam alone. Staining intensities are denoted as follows: white arrow = negative, yellow arrow = weak, orange = moderate, red arrow = strong.

Semi-quantitative analysis of apoptotic and inflammatory markers in liver and kidney tissues

The semi-quantitative analysis of caspase-3, Bax, and TNF-α expression in liver and kidney tissues across different treatment groups provides a comprehensive overview of the effects on apoptotic and inflammatory pathways.

Table 4: Semi-quantitative scoring of caspase-3 in liver and kidney tissues.

Group	Liver (5 Field scores)	Liver (Mean score)	Kidney (5 Field scores)	Kidney (Mean score)
Control	-, -, -, -, -	Negative	-, -, -, -, -	Negative
Vehicle control	-, -, -, +, -	Negative	-, -, -, -, -	Negative
Grape seed extract	-, -, -, -, -	Negative	-, +, -, -, -	Negative
Meloxicam	+++, ++, +++, ++, ++	Moderate	+++, +++, ++, +++, +++	Strong
Meloxicam + Grape seed extract	++, +, +, +, +	Week	++, +, ++, +, ++	Moderate

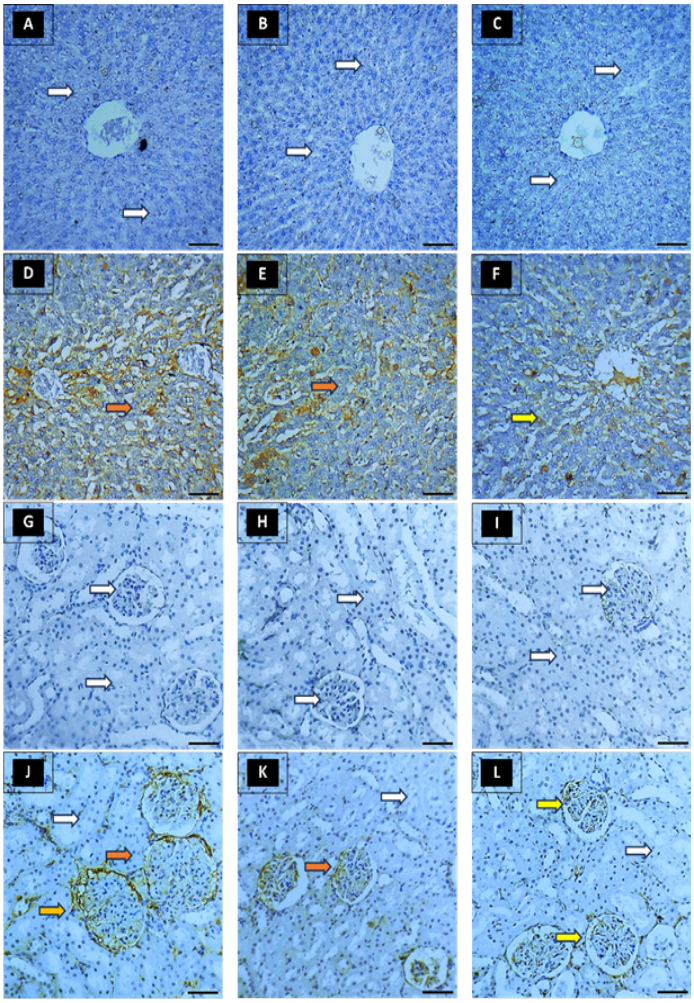

Figure 3: Immunohistochemical staining for TNF-α in liver and kidney tissues across different treatment groups. Panels (A) and (G) show "Negative" TNF-α expression in the control group, while panels (B) and (H) illustrate similar results in the Vehicle Control group, indicating no detectable TNF-α activity. In the Grape Seed Extract group (C) and (I), TNF-α expression remains "Negative," consistent with the control findings. Panels (D) and (E) for liver and (J) and (K) for kidney tissues treated with Meloxicam display a "Moderate" mean score for TNF-α, reflecting a moderate level of expression. In contrast, the combination of Meloxicam and Grape Seed Extract (F) and (L) results in a "Weak" mean score for TNF-α in both tissues, demonstrating reduced expression compared to Meloxicam alone. Staining intensities are denoted as follows: white arrow = negative, yellow arrow = weak, orange = moderate, red arrow = strong.

Caspase-3 activity: In the control group, both liver and kidney tissues exhibited no detectable caspase-3 activity, with all fields scoring as Negative. Similarly, the Vehicle Control group also showed a Negative mean score for caspase-3 in both tissues, indicating minimal or no activity. Grape Seed Extract treatment resulted in a Negative score for liver tissues, with no detectable caspase-3 activity across fields, while kidney tissues also remained Negative, although a slight increase in activity was observed in one field. In contrast, Meloxicam treatment led to a Moderate mean score for caspase-3 in liver tissues, reflecting substantial activation, with most fields showing either Strong or Moderate activity. In kidney tissues, Meloxicam produced a "Strong" mean score, indicating extensive caspase-3 activation in all fields. The combination of Meloxicam and Grape Seed Extract resulted in a Weak mean score for liver tissues, suggesting limited caspase-3 activity, whereas kidney tissues showed a "Moderate" mean score, indicating a moderate level of caspase-3 activity (Table 4).

Bax expression: For Bax expression, the control and Vehicle Control groups in both liver and kidney tissues showed Negative scores across all fields, indicating no detectable Bax activity. Grape Seed Extract treatment also resulted in a Negative mean score for Bax in liver tissues, consistent with the controls, and only a slight increase in activity was observed in one kidney field. Conversely, Meloxicam treatment caused Strong Bax expression in both liver and kidney tissues, with high activity across most fields. The combination of Meloxicam and Grape Seed Extract led to a Moderate mean score for Bax expression in liver tissues, suggesting a moderate level of activity, while kidney tissues showed a Weak mean score, reflecting a lower level of Bax activity compared to Meloxicam alone (Table 5).

TNF-α expression: In the control (A), (G), and Vehicle Control (B), (H) groups, both liver and kidney tissues displayed "Negative" TNF-α expression, indicating no detectable activity. Grape Seed Extract treatment (C), and (I) also resulted in "Negative" mean scores for TNF-α in both liver and kidney tissues. Meloxicam treatment led (D, E), (J, K) to a "Moderate" mean score for TNF-α in both tissues, reflecting a moderate level of expression. However, the combination of Meloxicam and Grape Seed Extract (F), (L) resulted in a "Weak" mean score for TNF-α in both liver and kidney tissues, indicating reduced expression compared

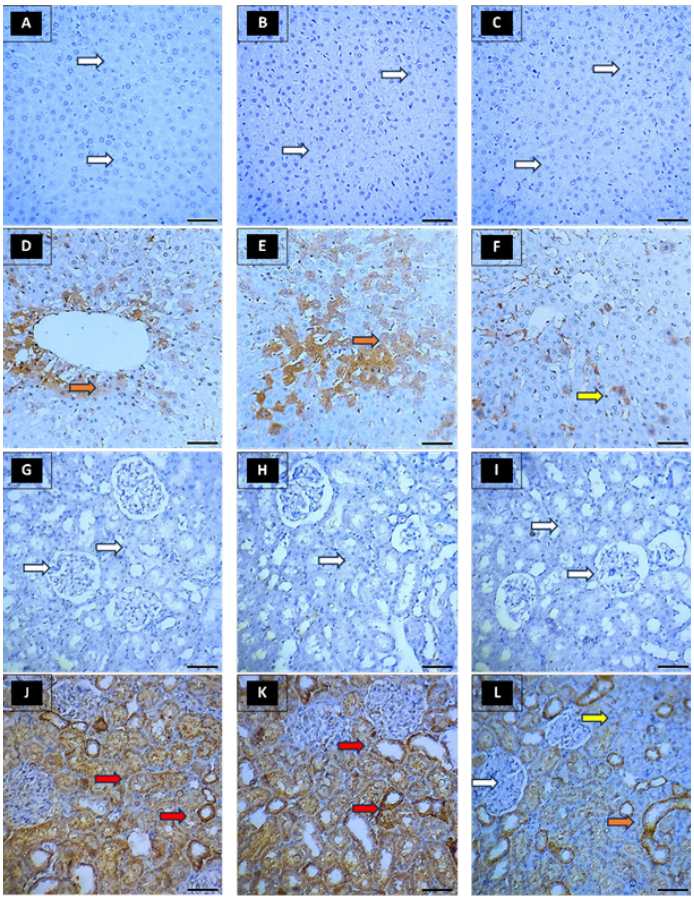

Figure 4: Immunohistochemical staining for Caspase-3 in liver and kidney tissues across different treatment groups. Panels (A) and (G) illustrate "Negative" Caspase-3 activity in both liver and kidney tissues of the control group, with all fields showing no detectable activity. Similarly, panels (B) and (H) for the Vehicle Control group also exhibit "Negative" Caspase-3 expression, indicating minimal or no activity. In the Grape Seed Extract group, panels (C) and (I) display "Negative" Caspase-3 activity in liver tissues, consistent with controls, while kidney tissues also remain "Negative," though a slight increase in activity is observed in one field. In contrast, Meloxicam treatment (D, E) and (J, K) resulted in a "Moderate" mean score for Caspase-3 in liver tissues, reflecting substantial activation with most fields showing either "Strong" (s) or "Moderate" (m) activity. Meloxicam also produced a "Strong" mean score in kidney tissues, indicating extensive Caspase-3 activation across all fields. The combination of Meloxicam and Grape Seed Extract (F) and (L) led to a "Weak" mean score for liver tissues, suggesting limited Caspase-3 activity; while kidney tissues showed a "Moderate" mean score, reflecting a moderate level of Caspase-3 activity. Staining intensities are denoted as follows: white arrow = negative, yellow arrow = weak, orange = moderate, red arrow = strong.

to Meloxicam alone (Table 6). A quantitative evaluation of liver and kidney damage and the area percentages of immunohistochemical markers, including Caspase-3, Bax, and TNF-α. These quantitative measures provide a more objective understanding of the extent of tissue damage and the protective effects of GSE. Table 7 summarizes the average histopathological scoring and area percentages of the immunohistochemical markers, giving a clearer comparison across the experimental groups. Quantitative assessment of liver and kidney tissue damage showed that Meloxicam treatment caused significant damage, with liver damage scored at 3.8 and kidney damage at 3.2. In contrast, co-administration of GSE with Meloxicam significantly reduced tissue damage, with scores of 2.2 for the liver and 2.0 for the kidney, indicating a protective effect of GSE. Control groups showed minimal damage, with scores ranging from 0.4 to 0.8. The area percentages of Caspase-3, Bax, and TNF-α expression were significantly elevated in the Meloxicam-treated group, with Caspase-3 levels reaching 16.0% in the liver and 87.5% in the kidney. Bax expression was particularly high in the liver at 94.0%, while TNF-α expression reached 13.5% in the liver and 4.0% in the kidney. Co-administration of GSE notably reduced these levels, with Caspase-3 decreasing to 4.2% in the liver and 21.5% in the kidney, Bax to 18.8% in the liver, and TNF-α to 4.0% and 1.2% in the liver and kidney, respectively. These results demonstrate the significant protective effect of GSE on apoptotic and inflammatory responses (Table 7).

One of the most commonly prescribed medicines in the world are the non-steroidal anti-inflammatory drugs (NSAIDs) by controlling pain and inflammation pharmacologically (Ungprasert *et al*., 2015; Rivera-Velez, 2019b). It appears that meloxicam suppresses inflammatory through its selective COX-2 inhibition and subsequent reduction in pro-inflammatory prostaglandin production.

After oral administration meloxicam treatment significantly reduced paw swelling and joint inflammation adjuvant-induced arthritis (Crofford *et al*., 2000). In addition, meloxicam orally showed potent anti-inflammatory effects, reducing joint swelling and pain-related behavior in a model of monosodium urate crystal-induced joint inflammation (Nishida *et al*., 2004). Furthermore, meloxicam significantly reduced paw edema after oral administration in a carrageenan-induced paw edema model (Gupta *et al*., 2007). Meloxicam treatment significantly attenuated the lipopolysaccharide-induced increase in inflammatory markers in rat models (Ávila-Vázquez *et al*., 2011). Despite Meloxicam importance its usage was restricted because it possess several notorious adverse side effects because these drugs were proven to possibly cause gastrointestinal, renal, hepatic toxicity (Burukoglu, 2016; Ungprasert *et al*., 2015).

At lower doses, meloxicam inhibits COX-2, while at higher doses, it inhibits COX1, resulting in notorious adverse effects. COX-2 inhibition is related to meloxicam's anti-inflammatory effects by inhibiting prostaglandin synthesis

Table 5: Semi-quantitative scoring of Bax in liver and kidney tissues

Group	Liver (5 field scores)	Liver (mean score)	Kidney (5 field scores)	Kidney (mean score)
Control	-, -, -, -, -	Negative	-, -, -, -, -	Negative
Vehicle control	-, -, -, -, -	Negative	-, -, -, -, -	Negative
Grape seed extract	-, -, -, -, -	Negative	-, -, -, -,+	Negative
Meloxicam	+++, +++, +++, ++, +++	Strong	+++, ++, ++, +++, +++	Strong
Meloxicam + Grape seed extract	++, ++, ++, +, +	Moderate	++, +, +, +, +	Week

Table 6: Semi-quantitative scoring of TNF-α in liver and kidney tissues.

Group	Liver (5 field scores)	Liver (mean score)	Kidney (5 field scores)	Kidney (mean score)
Control	-, -, -, -, -	Negative	-, -, -, -, -	Negative
Vehicle control	-, -, -, -, -	Negative	-, -, -, -, -	Negative
Grape seed extract	-, -, -, -, -	Negative	-, -, -, -,-	Negative
Meloxicam	++, +, ++, ++, +	Moderate	++, +, +, ++, +	Moderate
Meloxicam + Grape seed extract	+, +, +, -, +	Week	+, +, -, +, -	Week

Table 7: A quantitative evaluation of the effects of GSE and meloxicam on Liver and Kidney damages, and immunohistochemical markers in the present study.

Group	Liver damage	Kidney damage	Caspase-3 (Liver)	Caspase-3 (Kidney)	Bax (Liver)	Bax (Kidney)	TNF-α (Liver)	TNF-α (Kidney)
Control	0.4	0.6	0.2%	0.3%	0.1%	0.2%	0.3%	0.4%
Vehicle control	0.6	0.8	0.3%	0.4%	0.2%	0.3%	0.4%	0.5%
GSE control	0.4	0.4	0.1%	0.2%	0.05%	0.1%	0.2%	0.3%
Meloxicam	3.8	3.2	16.0%	87.5%	94.0%	28.0%	13.5%	4.0%
Meloxicam + GSE	2.2	2.0	4.2%	21.5%	18.8%	2.5%	4.0%	1.2%

at inflamed areas. COX-1 inhibition is associated with its notorious adverse side effects (Villalba *et al*., 2016; Jyothi *et al*, 2023). In addition to causing nephrotoxicity through multiple mechanisms, such as, hematuria, proteinuria, flank pain, and acute tubular necrosis as a result of sustained prostaglandin inhibition during long-term treatment. It is possible to develop acute interstitial nephritis (AIN) caused by NSAIDs. Among the rare mechanisms that cause glomerular damage are acute papillary necrosis, renal vasculitis, and nephrotic syndrome (Ingrasciotta *et al*., 2015; Jyothi *et al*, 2023).

Due to its inhibition of prostaglandins, which provide protection to gastric mucosa, meloxicam can cause erosions, ulcers, and bleeding in the gastrointestinal tract over time (Burukoglu, 2016; Ungprasert *et al*., 2015).

The antioxidant properties of *Vitis vinifera* (Grape) have been recognized as beneficial against oxidative stress since they can neutralize the adverse effects of free radicals. Since it contains numerous polyphenol ingredients, *V. vinifera* has a wide range of pharmacological activities. It also contains flavonoids, which are potent antioxidants, anti-inflammatory, and hepatoprotectors (Hasona *et al*., 2017; Sochorova *et al*., 2021).

Meloxicam treatment was found to significantly ($P < 0.05$) raise serum levels of ALT, AST, ALP, urea, creatinine, cholesterol, triglycerides, and LDL. On the other hand, it significantly ($P \leq 0.05$) reduced serum total protein, albumin, and HDL in comparison to the control group. These values reverted to normal after GSE was administered with Meloxicam. Rats that were inebriated with meloxicam also showed elevated MDA and decreased levels of CAT and SOD. The liver and kidney tissues of the Meloxicam + GSE group had higher levels of GSH and CAT and lower MDA levels than the Meloxicam-treated group. These biochemical and antioxidative alterations were validated by immunohistochemical and histopathological analyses.

In reference to our findings regarding meloxicam toxicity, Ahmed *et al*. (2015) reported that meloxicam produced a hepatotoxic effect by elevating tissue levels of AST, ALT, and ALP with high levels of malondialdehyde (MDA) in liver homogenate, along with necrosis and inflammatory cell infiltration in the liver and kidney. Hesperidin was found to be hepatoprotective against meloxicam-induced hepatotoxicity in another study conducted by Mahmoud (2017) who mentioned that the liver histology and serum liver enzymes (ALT, AST, and ALP) of rats given meloxicam (7.5 mg/kg/day) for 28 days showed notable alterations. Hesperidin co-administration reduced

meloxicam-induced hepatic damage.

In addition to its antioxidant and anti-inflammatory properties, grape seed extract may have hepatoprotective and neuroprotective effects, which may explain its protective effect against meloxicam hepatorenal toxicity. In addition to neutralizing the adverse effects of free radicals, grape seed extract (GSE) reduces the risk of organ damage, improves the balance between oxidants and antioxidants, and reduces inflammatory mediator release by regulating cellular oxidative damage (Hassan and Al-Rawi, 2013; Sochorova *et al*., 2021).

Abdel-Hafez *et al*. (2017) reported that in male albino rats, grape seed extract was studied to determine how it protected against renal cortical damage caused by paracetamol. Paracetamol significantly increased serum urea and creatinine levels as well as significantly decreased renal superoxide dismutase levels in the study. The proximal and distal convoluted tubules also showed marked degeneration, dense nuclear staining, cytoplasmic vacuolization, and a partial loss of brush borders. If compared to the control and GSE groups, PCT and DCT showed less PAS reaction and more COX-2 and caspase expression. Most tubules were dilated, irregular, and filled with hyaline casts. In combination with paracetamol, grape seed extract significantly improved these biochemical and histological changes. When compared to control and GSE, distal and proximal convoluted tubules showed less PAS reaction and more COX2 and caspase expression. In addition, these biochemical and histological changes were significantly ameliorated by concomitantly administering GSE with paracetamol.

Sengottuvelan (2013) investigated the hepatoprotective effects of GSE against alcohol-induced liver damage in rats. Rats were given ethanol (6 g/kg/day) for 4 weeks, which caused significant increases in serum liver enzymes (AST, ALT, ALP) and oxidative stress markers. Co-administration of GSE (100 mg/kg/day) attenuated the alcohol-induced liver injury and oxidative stress.

The hepatotoxic (liver-damaging) effects of meloxicam have been studied in various animal models, and the proposed mechanisms involve several interrelated pathways. Include oxidative stress, Inflammation and apoptosis, mitochondrial dysfunction, Metabolite-mediated, toxicity and Hemodynamic changes (Boelsterli et al., 2002).

The hepatoprotective (liver-protective) effects of grape seed extract (GSE) have been attributed to several mechanisms, including antioxidant and free radical scavenging, Anti-inflammatory effects by modulation the expression of pro-inflammatory cytokines, such as TNF-α, IL-6, and IL-1β, thereby reducing inflammation in the liver Kohut *et al*. (2024). In addition, Modulation of apoptosis and cell signaling such as the NF-κB and MAPK pathways, which are involved in the regulation of apoptosis (programmed cell death) and cell proliferation. This can help protect the liver from injury and promote regeneration Yang *et al*. (2014). Mitochondrial function and energy metabolism, GSE can improve mitochondrial function and energy metabolism in the liver, thereby enhancing the liver's ability to cope with various stressors and toxins (Bagchi et al., 1998).

The nephroprotective (kidney-protective) effects of grape seed extract (GSE) have been attributed to several mechanisms, including modulation of kidney function and hemodynamics, GSE has been shown to improve kidney function, increase glomerular filtration rate, and enhance renal blood flow, thereby improving the overall kidney health and reducing the risk of kidney injury, Inhibition of fibrosis and proteinuria, GSE can inhibit the development of renal fibrosis and reduce the excretion of proteins in the urine (proteinuria), which are important indicators of kidney disease progression (Yousef, 2010).

This study has some limitations. First, the use of different control vehicles (saline for GSE and polysorbate 80 for Meloxicam) may introduce a potential confounding effect, though both vehicles are generally considered inert. Second, the sample collection was conducted 24 hours after the final treatment, which may not fully capture the long-term effects or recovery from toxicity; future studies should include multiple time points. Third, while oxidative stress markers (SOD, CAT, MDA) were used, additional markers like GPx or TAC could provide a more comprehensive assessment. Finally, although the study was randomized and blinded, a prospective power analysis would have strengthened the study design, and future research should address this. Despite these limitations, the rigorous experimental design, including randomization, blinding, and appropriate control groups, ensures the validity and reliability of our findings. The significant differences observed between treatment groups and consistent results across multiple parameters (biochemical, histological, and immunohistochemical) provide strong evidence for the hepato-renal protective effects of GSE against Meloxicam-induced toxicity. Future studies can build upon these findings by addressing these limitations, potentially providing even more comprehensive insights into the long-term protective effects of GSE.

CONCLUSIONS AND RECOMMENDATIONS

This study highlights the significant protective effects of grape seed extract (GSE) against Meloxicam-induced hepato-renal toxicity in rats, demonstrated through

improvements in biochemical, histopathological, and immunohistochemical markers. GSE administration reduced oxidative stress, improved antioxidant enzyme activities (SOD, CAT), and alleviated liver and kidney damage. It modulated key apoptotic and inflammatory markers, including Caspase-3, Bax, and TNF-α, reducing cell death and inflammation. These findings suggest that GSE may serve as a promising adjunct therapy with NSAIDs, enhancing the safety of these drugs. Further research is needed to validate these protective effects in human populations, identify optimal dosing regimens, and explore GSE's long-term efficacy and safety. This study provides a strong foundation for advancing GSE as a potential therapeutic agent to mitigate NSAID-induced toxicity in veterinary and human medicine.

ACKNOWLEDGEMENTS

Sincere and great thanks for prof. dr. Hossam fouad attia, Professor of histology and cytology, faculty of veterinary medicine, Benha university for his great help and support in histopathology and immunohistochemistry.

NOVELTY STATEMENT

This study addresses an important gap in understanding natural compounds that may counteract NSAID toxicity, with potential clinical implications for safer long-term NSAID use."

AUTHOR'S CONTRIBUTION

All authors contributed equally to this article, funding acquisition and project administration and assisted in writing and editing the manuscript.

Funding information

No specific grant was provided for this research by governmental, private, or nonprofit funding organizations.

Ethical statement

The animal study was reviewed and approved by Faculty of Veterinary Medicine, Benha University, Egypt

Conflict of interest

The authors have declared no conflict of interest.

REFERENCES

Abdel-Hafez SMN, Rifaai RA, Abd Elzaher WY (2017). Mechanism of grape seeds extract protection against paracetamol renal cortical damage in male Albino rats. Bratisl. Lek. Listy., 118(4): 233-242. https://doi.org/10.4149/BLL_2017_046

Ahmed MY, Abdul-Fattah JH, Aziz FM (2015). The protective role of vitamin C against the hepatotoxic and nephrotoxic effect of meloxicam in male mice. J. Pharm. Biol. Sci., 10(5): 69-73.

Ávila-Vázquez R, Cruzblanca H, Manzo-Flores P, Vega-López MA, Velázquez-Olvera M (2011). Effect of meloxicam on lipopolysaccharide-induced acute phase response in the rat. Inflammation, 34(3): 167-173.

Bagchi D, Garg A, Krohn RL, Bagchi M, Bagchi DJ, Balmoori J, Stohs SJ (1998). Protective effects of grape seed proanthocyanidins and selected antioxidants against TPA-induced hepatic and brain lipid peroxidation and DNA fragmentation, and peritoneal macrophage activation in mice. Gen. Pharmacol. Vasc. Syst., 30(5): 771-776. https://doi.org/10.1016/S0306-3623(97)00332-7

Bancroft JD, Layton C, Suvarna SK (2013). Bancroft's theory and practice of histological techniques (Amsterdam, the Netherlands: Elsevier).

Bartels H, Böhmer M, Heierli C (1972). Serum creatinine determination ithout protein precipitation. Clin. Chim. Acta, 37: 193–197. https://doi.org/10.1016/0009-8981(72)90432-9

Boelsterli UA (2002). Mechanisms of NSAID-induced hepatotoxicity: Focus on nimesulide. Drug Safety, 25: 633-648. https://doi.org/10.2165/00002018-200225090-00003

Burukoglu D, Baycu C, Taplamacioglu F, Sahin E, Bektur E (2016). Effects of nonsteroidal anti-inflammatory meloxicam on stomach, kidney, and liver of rats. Toxicol. Ind. Health, 32(6): 980-986. https://doi.org/10.1177/0748233714538484

Coulombe JJ, Favreau LA (1963). new simple semimicro method for colorimetric determination of urea. Clin. Chem., 9: 102–108. https://doi.org/10.1093/clinchem/9.1.102

Crofford LJ, Lipsky PE, Brooks P, Abramson SB, Simon LS, van de Putte LB (2000). Basic biology and clinical application of specific cyclooxygenase-2 inhibitors. Arthrit. Rheumat. Off. J. Am. Coll. Rheumatol., 43(1): 4-13. https://doi.org/10.1002/1529-0131(200001)43:1<4::AID-ANR2>3.0.CO;2-V

da Silva, J. C. G., Dallegrave, E., Rodrigues, G. Z. P., Bigolin, C., Neumann, T. M. S. O., Schuster, A. C., Kayser, J. M., Staudt, L. B. M., Moraes, M. F., Farias, D. G., Schiling, G., Raasch, J. R., Perassolo, M. S., da Silva, L. B., Gehlen, G., & Betti, A. H. (2022). Repeated dose of meloxicam induces genotoxicity and histopathological changes in cardiac tissue of mice. Drug and chemical toxicology, 45(2), 822–833.

Ferrer MD, Busquets-Cortés C, Capó X, Tejada S, Tur JA, Pons A, Sureda A (2009). Cyclooxygenase-2 inhibitors as a therapeutic target in inflammatory diseases. Curr. Med. Chem., 26(18): 3225-3241. https://doi.org/10.2174/0929867325666180514112124

Giribabu, N., Karim, K., Kilari, E. K., Kassim, N. M., & Salleh, N. (2018). Anti-Inflammatory, Antiapoptotic and Proproliferative Effects of Vitis vinifera Seed Ethanolic Extract in the Liver of Streptozotocin-Nicotinamide-Induced Type 2 Diabetes in Male Rats. Canadian journal of diabetes, 42(2), 138–149.

Gupta SK, Mohanty I, Talwar KK, Dinda A, Joshi S, Bansal P (2007). Anti-inflammatory effect of meloxicam and nephroprotective effect in gentamicin-induced acute renal failure in rat. Mol. Cell. Biochem., 306(1-2): 165-173.

Hasona NA, Alrashidi AA, Aldugieman TZ, Alshdokhi AM, Ahmed MQ (2017). *Vitis vinifera* extract ameliorate hepatic and renal dysfunction induced by dexamethasone in Albino

Rats. Toxics, 11: 5. https://doi.org/10.3390/toxics5020011
Hassan HA, Al-Rawi MM (2013). Grape seeds proanthocyanidin extract as a hepatic-reno-protective agent against gibberellic acid induced oxidative stress and cellular alterations. Cytotechnology, 65(4): 567-576. https://doi.org/10.1007/s10616-012-9506-6
Honma S, Shinohara M, Takahashi N, Nakamura K, Hamano S, Mitazaki S, Yoshida M (2014). Effect of cyclooxygenase (COX)-2 inhibition on mouse renal interstitial fibrosis. Eur. J. Pharmacol., 740: 578-583. https://doi.org/10.1016/j.ejphar.2014.06.027
Ingrasciotta Y, Sultana J, Giorgianni F, Fontana A, Santangelo A, Tari DU, Trifirò G (2015). Association of individual non-steroidal anti-inflammatory drugs and chronic kidney disease: A population-based case control study. PLoS One, 10(4): e0122899. https://doi.org/10.1371/journal.pone.0122899
Jyothi VGSS, Veerabomma H, Tryphena KP, Khatri DK, Singh SB, Madan J (2023). Acute and sub-acute dermal toxicity of meloxicam emulgel: Analysis of biochemical, hematological, histopathological and immunohistochemical expression. Biochem. Biophys. Res. Commun., 660: 88–95. https://doi.org/10.1016/j.bbrc.2023.04.001
Kohut L, Baldovska S, Mihal M, Belej L, Sirotkin AV, Roychoudhury S, Kolesarova A (2024). The multiple actions of grape and its polyphenols on female reproductive processes with an emphasis on cell signaling. Front. Endocrinol., 14: 1245512. https://doi.org/10.3389/fendo.2023.1245512
Mahmoud FS (2017). Hepatoprotective effect of hesperidin against meloxicam-induced hepatotoxicity in rats. Chem-Biol. Interact., 272: 25-33.
Nishida S, Fujimura H, Miura R, Ozaki S, Hagihara A (2004). Anti-inflammatory and analgesic effects of meloxicam on monosodium urate crystal-induced arthritis in rats. Inflamm. Res., 53(11): 694-699.
Ohkawa H, Ohishi N, Yagi K (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal. Biochem., 95(2): 351-358. https://doi.org/10.1016/0003-2697(79)90738-3
Reitman S, Frankel SA (1957). Colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. Am. J. Clin. Pathol., 28(1): 56–63. https://doi.org/10.1093/ajcp/28.1.56
Rivera-Velez SM, Broughton-Neiswanger LE, Suarez M, Piñeyro P, Navas J, Chen S, Villarino NF (209a). Repeated administration of the NSAID meloxicam alters the plasma and urine lipidome. Sci. Rep., 9(1): 4303. https://doi.org/10.1038/s41598-019-40686-4
Rivera-Velez SM, Broughton-Neiswanger LE, Suarez MA, Slovak JE, Hwang JK, Navas J, Leung AWS, Piñeyro PE, Villarino NF (2019b). Understanding the effect of repeated administration of meloxicam on feline renal cortex and medulla: A lipidomics and metabolomics approach. J. Vet. Pharmacol. Therapeut., 42(4): 476-486. https://doi.org/10.1111/jvp.12788
Sengottuvelan M (2013). Protective effect of grape seed proanthocyanidins on alcohol-induced hepatotoxicity in rats. Chem. Biol. Interact., 206(1): 33-40.
Sochorova L, Baron M, Dadakova K, Kasparovsky T, Sochor J (2021). Health beneficial properties of grapevine seed extract and its influence on selected biochemical markers in the blood, liver and kidneys of Rattus norvegicus. Molecules, 26: 7. https://doi.org/10.3390/molecules26072099
Sriuttha P, Sirichanchuen B, Permsuwan U (2018). Hepatotoxicity of nonsteroidal anti-inflammatory drugs: A systematic review of randomized controlled trials. Int. J. Hepatol., 2018: 5253623. https://doi.org/10.1155/2018/5253623
Tietz NW, Burtis CA, Duncan P, Ervin K, Petitclerc CJ, Rinker AD (1983). A reference method for measurement of alkaline phosphatase activity in human serum. Clin. Chem., 29(5): 751–761. https://doi.org/10.1093/clinchem/29.5.751
Ungprasert P, Cheungpasitporn W, Crowson CS, Matteson EL (2015). Individual non-steroidal anti-inflammatory drugs and risk of acute kidney injury: A systematic review and meta-analysis of observational studies. Eur. J. Intern. Med., 26(4): 285-291. https://doi.org/10.1016/j.ejim.2015.03.008
Uzun B, Atli O, Perk BO, Burukoglu D, Ilgin S (2015). Evaluation of the reproductive toxicity of naproxen sodium and meloxicam in male rats. Hum. Exp. Toxicol., 34(4): 415-429. https://doi.org/10.1177/0960327114542886
Villalba BT, Ianiski FR, Vogt AG, Pinz MP, Reis AS, Vaucher RA, Luchese C (2016). Polymeric nanocapsules as a technological alternative to reduce the toxicity caused by meloxicam in mice. Regul. Toxicol. Pharmacol., 81: 316-321. https://doi.org/10.1016/j.yrtph.2016.09.023
Yang T, Li X, Zhu W, Chen C, Sun Z, Tan Z, Kang J (2014). Alteration of antioxidant enzymes and associated genes induced by grape seed extracts in the primary muscle cells of goats *in vitro*. PLoS One, 9(9): e107670. https://doi.org/10.1371/journal.pone.0107670
Yousef MI (2010). Potential protective effects of grape seed proanthocyanidin extracts against oxidative stress and cytotoxicity of acrylamide in rats. J. Hazard. Mater., 180(1-3): 28-36.
Zhiran J, Menglan L, Junde X, Howell DC, Zhiyun LI, Chen FE (2022). Recent development on COX-2 inhibitors as promising anti-inflammatory agents: The past 10 years. Acta Pharma. Sin. B, 12(6): 2790-2807. https://doi.org/10.1016/j.apsb.2022.01.002