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Supplementation of *Madhuca longifolia* Seed oil augments diclofenac-induced organ toxicities: A biochemical and histopathological approach

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Diclofenac medication has been extensively used for anti-inflammatory, anti-pyretic, and analgesic actions. Its abiding usage and overdose have induced toxicity and harmful effects on the liver, kidney, and gastrointestinal tract. The research aims to scrutinize the protective effect of *Madhuca longifolia* seed oil against diclofenac-induced toxicity in female Wistar albino rats. A period of 10 days of study was aimed at 7 groups; Group 1 was assigned as normal control. Group 2 has been administered diclofenac (50 mg/kg b.w. /day, *i.p.*) only on the last two days of each study period. Group 3 and Group 4 have been pre-treated with 1 mL, and 2 mL of *Madhuca longifolia* seed oil, respectively, and diclofenac was induced as per Group 2. Group 5 was treated with the standard drug silymarin and diclofenac. Group 6 and Group 7 were given 1 mL and 2 mL of *Madhuca longifolia* seed oil alone. After the study period, parameters like liver enzyme markers, renal enzyme markers, and antioxidants were measured, and tissue samples were analyzed for histopathological changes. The results proved that pre-treatment of 1 mL of*Madhuca longifolia* seed oil has efficacy against diclofenac-induced toxicity.

Keywords: Antioxidant, Diclofenac, Gastrointestinal toxicity, Hepatotoxicity, *Madhuca longifolia seed* oil, Renal toxicity

Belonging to the modern era, pharmaceutical drugs are the prompt and impending savior for existing and emerging contemporary diseases. Therein, the most frequently used drugs are antibiotics and non-steroidal anti-inflammatory drugs (NSAIDs)[1]. These medications are known to cause hepatotoxicity, renal toxicity, gastrointestinal tract toxicity, and injuries to other organs upon their abiding usage. Among assorted NSAIDs, diclofenac alias 2-[2-[(2, 6-Dichlorophenyl) amino] phenyl] acetic acid is one of the broadly used medications for their analgesic, anti-inflammatory, antipyretic, and anti-phlogistic actions[2]. It functions by obstructing Cyclooxygenase-1 (COX-1) and Cyclooxygenase-2 (COX-2) enzymes responsible for producing prostaglandins. COX-1 generates prostaglandins for gastric cytoprotection and homeostatic functions, while COX-2 plays a significant role in the synthesis of prostaglandins under pathophysiological conditions such as pain and inflammation[3]. Despite the therapeutic value of diclofenac, it causes adverse health effects.

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Suppl. Data available on respective page of NOPR

The liver, kidney, and gastrointestinal tract are the life-sustaining organs in drug metabolism. The liver is a cardinal organ that is basal for the body's metabolic activity, detoxification, immune system, bile secretion, and elimination of wastes[4].It is the primary organ for drug and xenobiotics metabolism, making it easily susceptible to toxic effects. More than a thousand pharmaceutical drugs have been reported to cause liver injury. Where drug-induced liver damage can account for about 50% of acute liver failures, 10% of acute hepatitis, and 5% of hospital admissions. Diclofenac has been reported to cause serious hepatotoxicity. Its strange mechanism causing adverse hepatic effects may arise due to the concurrence of the drug-hepatoprotection adduct and the formation of toxic metabolites in the liver[5].

The kidney is the central organ for maintaining homeostasis, clearance of harmful metabolites from the body, and detoxification[6]. Toxicants and continued drug exposure to the kidney produce major diseases in them as their normal function like toxin elimination, filtration, and excretion gets altered and induce kidney injury. About 15-20% of acute kidney injuries emanate from therapeutic drug exposure[7]. The most prevalent type of drug class that chiefly source kidney toxicity is NSAIDs. Renal dysfunction heaves insight as renal prostaglandin levels are diminished by the suppression activity of

diclofenac, which induces damage to kidney metabolism, decreases glomerular filtration rate, and forms Reactive Oxygen Species (ROS) creating oxidative stress further leading to acute renal failure.

The gastrointestinal tract is another leading destination for adverse drug events such as gastrointestinal bleeding caused by impaired vascular integrity and histopathological changes[8]. Drugs that undergo enterohepatic cycling are the foremost ones to provoke gastrointestinal injury contrasted to the drugs that don't meet with enterohepatic cycling. The gastrointestinal tract is protected by ulcer healing agents as well as mucoprotective agents[9,10]. These agents protect from acidity by counterbalancing with bicarbonate ions secretion maintaining the gastrointestinal environment[11]. Prostaglandins are the ones that engender a protective layer to the intestine when the diclofenac hinders it causes gastrointestinal tract ulceration bleeding, inflammation, and ultimately gastrointestinal tract toxicity.

Silymarin is a standard ancient reputable medicine that has been used for centuries to treat liver, kidney, and gallbladder-related diseases[12]. It is a polyphenolic flavonoid extracted from the seeds of *Silybum marianum* milk thistle that elicits significant antioxidant, anti-bacterial, anti-fungal, antiviral, anti-carcinogenic, and hepatoprotective properties protecting tissues and organs from chemical toxicities and injuries. Though silymarin is accepted, it has low bioavailability in humans as it is extensively excreted as sulfate-glucuronide conjugates in humans' bile and possesses numerous side effects anaphylaxis, bloating, and nausea. Further, its mechanism of action, clinical efficacy, drug interaction, and food interactions are less known[13].

Phytomedicines would be the ulterior solution for drug-induced toxicities of their protective and reviving quality[14,15]. *Madhuca longifolia* commonly called the mahua tree appertaining to the family Sapotaceae extensively grown in India and Ceylon. The discrete parts of the mahua tree, such as leaves, bark, flowers, seeds,and other parts are known to have ethnopharmacological properties for healing different maladies. Amongst, *Madhuca longifolia Seed* oil has a composition of 37% oleate, 25% palmitate, 23% stearate, and 12.5% of linoleate. This *Madhuca longifolia Seed* oil exhibits edibility and medicinal traits that possess hepatoprotective, anti-ulcer, anti-microbial, anti-pyretic, anti-inflammatory, anti-tumor, analgesic, and wound healing properties. *Madhuca longifolia* seed oil's higher anti-radical capacity and phytochemicals indicate that it is a powerful source of antioxidants that will exhibit its oxidative stability and nutritional benefits.

In previous research, the protective effects of *Madhuca longifolia* seed oil on diclofenac-induced toxicity have not been studied. The current study was therefore initiated to scrutinize the impact of pre-treatment of *Madhuca longifolia* seed oil on diclofenac-induced hepato-, renal- and gastrointestinal toxicity in female Wistar albino rats.

Materials and Methods

Chemicals and reagents

The NSAID diclofenac was procured from Unique Pharmaceutical Laboratories Private Limited, Mumbai, Maharashtra, India. The Standard drug silymarin was obtained from Micro Lab Private Limited, Solan, Himachal Pradesh, India. Antioxidant assays were evaluated under the standard protocol, whereas, serum biomarkers of liver and kidney function were appraised using diagnostic kits acquired from Span Diagnostics Limited, Surat, Gujarat, India. Commercially accessible *Madhuca longifolia* seed oil was purchased and authenticated the purity of oil from Tamil Traders Private Limited, Coimbatore, Tamil Nadu, India.Following the volume selection guidelines of the Organization for Economic Cooperation and Development (OECD), oil dosages of 1 mL and 2 mL were fixed concerning rat body weights[16].

Animals

For this experiment, the female Wistar albino rats, each weighing 175±15 grams, were used. These rats were acquired from VIT Animal house, Vellore, Tamil Nadu, India. Underthe Indian Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines, the Institutional Animal Ethics Committee (IAEC) of VIT has authorized the experimental operating method (Reg. no: VIT/IAEC/13/Feb13/21). Formerly, in the experiment, the rats were accustomed to the new environmental conditions for one week. The rats were kept in well-ventilated polypropylene rat cages with stainless steel cover lids to feed rats with clean water, and commercial rat pellet feed was purchased from Hindustan Lever Limited (HLL), Mumbai, Maharashtra, India.

Gas chromatography-mass spectrometry (GC-MS) analysis

GC-MS provides a good integrative approach for the characterization of organic compounds found in plant extracts as it integrates a fast separation technique (GC) with an identification system (MS). Accordingly, we identified the bioactive compounds present in the extract and quantified them based on the peak size. The greater the scale of the peaks, the higher the concentration of a compound in a sample. GC-MS analysis of *Madhuca longifolia* seed oil reported the presence of compounds such as n-hexadecanoic acid, oleic acid, andbeta-amyrone. Based on the peak size, compounds were quantified as oleic acid (75.826%), n-hexadecanoic acid (3.517%), and beta-anyone (2.541%).Oleic acid was reported to be the major component of *Madhuca longifolia* seed oil based on the analysis. The GC-MS chromatogram of *Madhuca longifolia* seed oil identified a variety of pinnacles revealing the source of dynamic mixes that revealed the plant's medicinal quality (Supplement file).

Experimental groups and treatment

The animals were grouped into seven experimental groups containing six rats each and were treated for 10 days (Table 1).

At the end of each experimental period, rats were euthanized onthe 11th day. The blood samples were obtained after ether anesthesia. The liver, kidney, stomach, and intestine were sieved out from the rats and stored for further analysis.

Appraisal of biochemical activities

Blood samples collected in tubes were centrifuged at 2000 rpm for 10 min to separate serum for biochemical analysis. To inspect hepatoprotective activity, biochemical markers such as albumin, total protein, Alkaline Phosphatase (ALP), Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), total and direct bilirubin, triglycerides, high-density lipoprotein (HDL) and total cholesterol levels were assessed. For renal protective activity, functional markers like urea, uric acid, and creatinine levels were estimated. This serum assay was performed following the manufacturer's standard protocol employing relevant diagnostic kits.

Appraisal of antioxidant activities

Antioxidant activities like superoxide dismutase (SOD), catalase (CAT), and glutathione S-transferase (GST) were assayed by the ideal method[17–19]. Sections of the liver, kidney and intestine were homogenized using Potter–Elvehjem homogenizer and then stored in 0.1 M ice-cold Phosphate Buffer Saline (PBS) with pH 7.4.

Scrutinizing histopathological changes

For microscopic interpretation, procured liver, kidney, and intestine were cleansed in cold PBS and subsequently restrained in 10% formalin. The tissues were sectioned at the 5µm thickness using a microtome. Eventually, the tissue sections were stained with hematoxylin and eosin. These stained sections were examined under the light microscope and photographed the histopathological changes in the treated rat groups, as well as the control group, wherefore compared and documented.

Statistical analysis

The analyzed results were represented as the mean ± standard deviation. Using the computer software GraphPad InStat (version 3.06), statistical significance between the groups was evaluated by one-way analysis of variance (ANOVA). Following the student's Newman-Keul test, a significance level of (P <0.05) was considered.

Table 1 — Animal grouping and their respective treatment

Groups	Administration	Period
Group 1	Normal healthy control	No treatment
Group 2	Diclofenac (50 mg/kg b.w./day, *i.p.*)	Only on 9th& 10th day
Group 3	1 mL of *Madhuca longifolia seed* oil (Per day, *p.o.*)	All 10 days
	Diclofenac (50 mg/kg b.w./day, *i.p.*)	9th& 10th day
Group 4	2 mL of *Madhuca longifolia seed* oil (Per day, *p.o.*)	All 10 days
	Diclofenac (50 mg/kg b.w./day, *i.p.*)	9th& 10th day
Group 5	Silymarin (25 mg/kg b.w./day, *p.o.*)	All 10 days
	Diclofenac (50 mg/kg b.w./day, *i.p.*)	9th& 10th day
Group 6	1 mL of *Madhuca longifolia seed* oil (Per day, *p.o.*)	All 10 days
Group 7	2 mL of *Madhuca longifolia seed* oil (Per day, *p.o.*)	All 10 days

Results

Reviving capacity of *Madhuca longifolia* seed oil against diclofenac-induced toxicity on liver enzyme markers

Figure 1 shows the hepatoprotective activity of *Madhuca longifolia* seed oil on diclofenac-induced rats during 10 days of experimentation respectively. As the liver plays a prime role in drug metabolism, diclofenac significantly increased the levels (P <0.05) of liver enzyme markers that exhibit toxicity in the rats. *Madhuca longifolia* seed oil had revived the significantly elevated levels (P <0.05) of liver enzyme markers such as ALP, ALT, and AST back to normal. Similar to them, other accompanying liver markers like total bilirubin, direct bilirubin, triglycerides, HDL, and total cholesterol levels also drop down and rebound to approximately normal levels. Decreased serum albumin levels and total protein in the experimental period were also significantly increased (P <0.05) and normalized, as shown in (Fig. 2). However, standard drug silymarin treatment had also subsided the levels (P <0.05) of liver enzyme markers, but the orally administered *Madhuca longifolia* seed oil was highly effective.

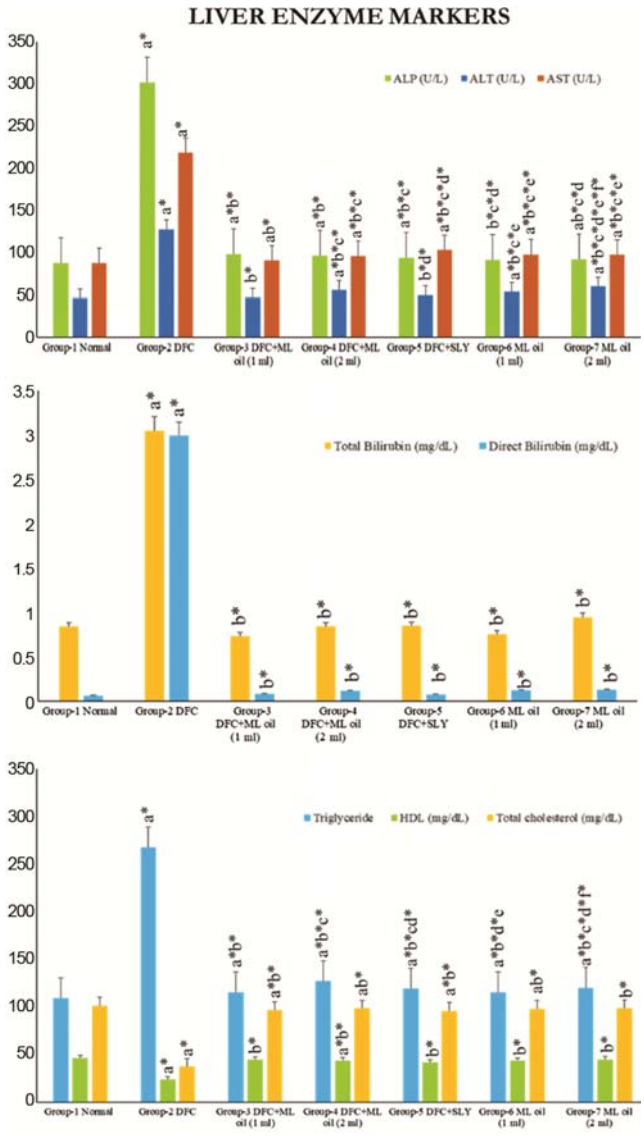

Fig. 1 — Reviving capacity of *Madhuca longifolia* seed oil against diclofenac-induced toxicity on rat liver enzyme markers Each data represents the Mean ± Standard Error of Means (SEM). The symbol asterisk (*) indicates the statistical significance between the groups at P <0.05, which was evaluated by one-way ANOVA following Student Newman–Keul's test. The following comparisons were made in this figure: a- Group 1 *vs*. Group 2, 3, 4, 5, 6 & 7. b- Group 2 *vs*. Group 3, 4, 5, 6 & 7. c- Group 3 *vs*. Group 4, 5, 6 & 7. d- Group 4 *vs*. Group 5, 6 & 7. e- Group 5 *vs*. Group 6 & 7. f- Group 6 *vs*. Group 7. (Abbreviations: DFC- Diclofenac; ML- *Madhuca longifolia*; SLY- Silymarin)

Reviving capacity of *Madhuca longifolia* seed oil against diclofenac-induced toxicity on renal enzyme markers

Renal enzyme markers such as urea, uric acid, and creatinine concentrations were increased in the diclofenac-induced group as an indication of renal damage and dysfunction in them. Figure 3 shows the renalprotective capability of *Madhuca longifolia* seed oil on diclofenac-treated rats during the study period of 10 days. Upon oral administration,*Madhuca longifolia* seed oil significantly reduced (P <0.05) the aberrant levels of renal markers. Equivalently in the 10 days of experimentation, the abundance upsurge of renal biomarkers in the rats treated with diclofenac compared to the control group was also declined by 4 to 5 folds in the groups treated with *Madhuca longifolia* seed oil and protected kidney by sustaining

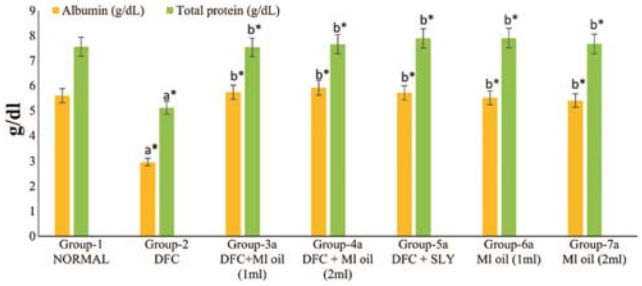

Fig. 2 — Protective activity of *Madhuca longifolia* seed oil on serum albumin and total protein levels in diclofenac-induced rats. Each data represents the Mean ± Standard Error of Means (SEM). The symbol asterisk (*) indicates the statistical significance between the groups at P <0.05, which was evaluated by one-way ANOVA following Student Newman–Keul's test. The following comparisons were made in this figure: a- Group 1 *vs*. Group 2, 3, 4, 5, 6 & 7. b- Group 2 *vs*. Group 3, 4, 5, 6 & 7. c- Group 3 *vs*. Group 4, 5, 6 & 7. d- Group 4 *vs*. Group 5, 6 & 7. e- Group 5 *vs*. Group 6 & 7. f- Group 6 *vs*. Group 7. (Abbreviations: DFC- Diclofenac; ML- *Madhuca longifolia*; SLY- Silymarin)

the normal range and amended the renal function more effectively compared to urea, uric acid, and creatinine restoring competence of silymarin-treated rats.

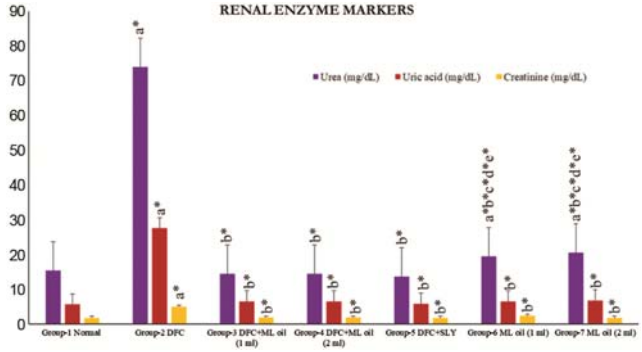

Fig. 3 — Reviving capacity of *Madhuca longifolia* seed oil against diclofenac-induced toxicity on rat renal enzyme markers. Each data represents the Mean ± Standard Error of Means (SEM). The symbol asterisk (*) indicates the statistical significance between the groups at P <0.05, which was evaluated by one-way ANOVA following Student Newman–Keul's test. The following comparisons were made in this figure: a- Group 1 *vs*. Group 2, 3, 4, 5, 6 & 7. b- Group 2 *vs*. Group 3, 4, 5, 6 & 7. c- Group 3 *vs*. Group 4, 5, 6 & 7. d- Group 4 *vs*. Group 5, 6 & 7. e- Group 5 *vs*. Group 6 & 7. f- Group 6 *vs*. Group 7. (Abbreviations: DFC- Diclofenac; ML- *Madhuca longifolia*; SLY- Silymarin)

Reviving capacity of *Madhuca longifolia* seed oil on antioxidant levels of diclofenac-induced rats

Accretion of toxic byproducts aroused as antioxidant enzymes superoxide dismutase (SOD), catalase (CAT), and glutathione S-transferase (GST) levels significantly declined (P <0.05) in the diclofenac-treated rats which provoked cellular damage. Figure 4 demonstrates the antioxidant levels of the liver, stomach, kidney, and intestine after 10 days of pre-treatment with *Madhuca longifolia* seed oil, which has protective and restoring potential as it significantly increased the levels (P <0.05) of SOD, CAT, and GST identical to Group 1. Analogous to the control group, both *Madhuca longifolia* seed oil and silymarin normalized the catastrophic effects induced by diclofenac.

Histopathology changes

Feeding the rats orally with *Madhuca longifolia* seed oil showed a protective effect on the test rats. Morphological changes inthe liver illustrated in (Fig. 5) shows that the healthy control group had normal liver tissue architecture with apparent hepatic cells. Compared to the control group, the liver sections of the diclofenac-treated group were observed with damaged hepatocytes and abnormal

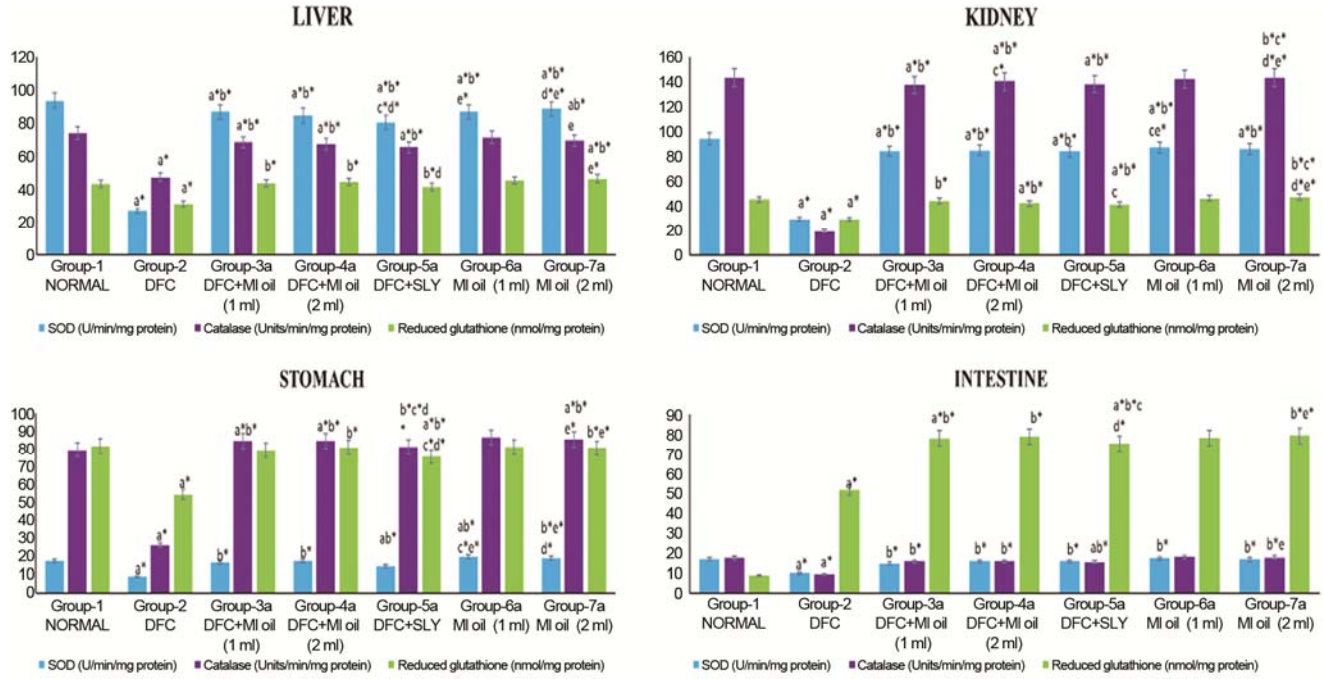

Fig. 4 — Effectual protective activity of *Madhuca longifolia* seed oil on liver, stomach, kidney & intestine's antioxidant level in diclofenac-induced rats. Each data represents the Mean ± Standard Error of Means (SEM). The symbol asterisk (*) indicates the statistical significance between the groups at P <0.05, which was evaluated by one-way ANOVA following Student Newman–Keul's test. The following comparisons were made in this figure: a- Group 1 *vs*. Group 2, 3, 4, 5, 6 & 7. b- Group 2 *vs*. Group 3, 4, 5, 6 & 7. c- Group 3 *vs*. Group 4, 5, 6 & 7. d- Group 4 *vs*. Group 5, 6 & 7. e- Group 5 *vs*. Group 6 & 7. f- Group 6 *vs*. Group 7. (Abbreviations: DFC- Diclofenac; ML- *Madhuca longifolia*; SLY- Silymarin)

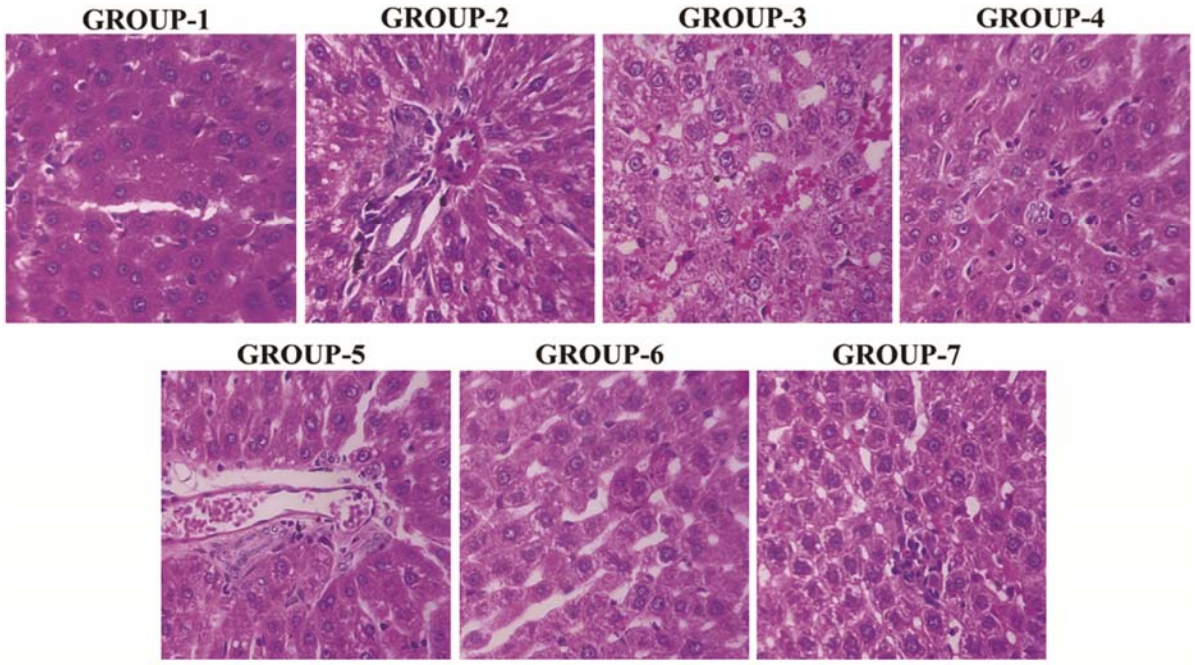

Fig. 5 — Reviving capacity of *Madhuca longifolia seed* oil on liver histopathology. Diclofenac-induced female Wistar albino rats showing protective competency of *Madhuca longifolia* seed oil on liver histopathology (Haematoxylin & Eosin stained). The numbers specified on photomicrographs indicate the Group numbers, respectively. (1) Shows normal liver histology. (2) Shows severe periportal inflammation and possible interface hepatitis. (3 & 4) Shows mild hemorrhage with no evidence of inflammation. (5) Shows focal hepatocyte necrosis and mild to moderate periportal inflammation. Normal liver tissue morphology with no Histopathological alterations was seen in (6 & 7)

hepatic parenchyma, causing periportal inflammation and interface hepatitis. It is evident that pre-treated rats with *Madhuca longifolia* seed oil followed by diclofenac administration had shown a protective effect by minimizing the consequences of diclofenac to milder conditions of periportal inflammation and interface hepatitis with reviving hepatic parenchyma in the liver tissue. Whereas, silymarin-treated groups possessed mild to moderate periportal inflammation and necrosis of hepatocytes with signs of feathery degeneration. Rats treated with *Madhuca longifolia* seed oil alone showed normal liver tissue morphology with no histopathological alterations.

Histopathological changes in kidney sections portrayed in (Fig. 6) show normal glomerulus and renal tubules in the control group. In contrast, diclofenac treatment-induced damage to the renal tubule cells provoked toxic ATN-like features in renal parenchyma. Animals treated with *Madhuca longifolia* seed oil had reduced the toxic effects of diclofenac, which showed signs of protection by diminishing characteristics of ATN. Silymarin-treated animals also possessed a protective effect that showed mild to moderate inflammation. *Madhuca longifolia seed* oil alone treated group shows normal kidney tissue histology that appears similar to that of the control group.

Figure 7 depicts the histomorphological examination of the intestine. Mucosal and sub-mucosal ulceration and inflammation induced by diclofenac's administration recovered the tissues back to the normal intestinal mucosa by the treatment with *Madhuca longifolia* seed oil. In silymarin-treated rats, architectural distortion and lymphoid aggregation were observed, which were not seen in the *Madhuca longifolia* seed oil-treated group. Normal colon mucosa was seen in the *Madhuca longifolia* seed oil alone treated group as well as the control group.

Discussion

Diclofenac is the most recommended NSAID it is safe when consumed at a prescribed concentration, and for a shorter term, principally upon their consistent utilization and higher dosage, it is accounted for hepatotoxicity, renal toxicity, and gastrointestinal toxicity[20]. In the present investigation, the injuries to organs were analyzed by the antioxidant assays, and the leakage of their individual

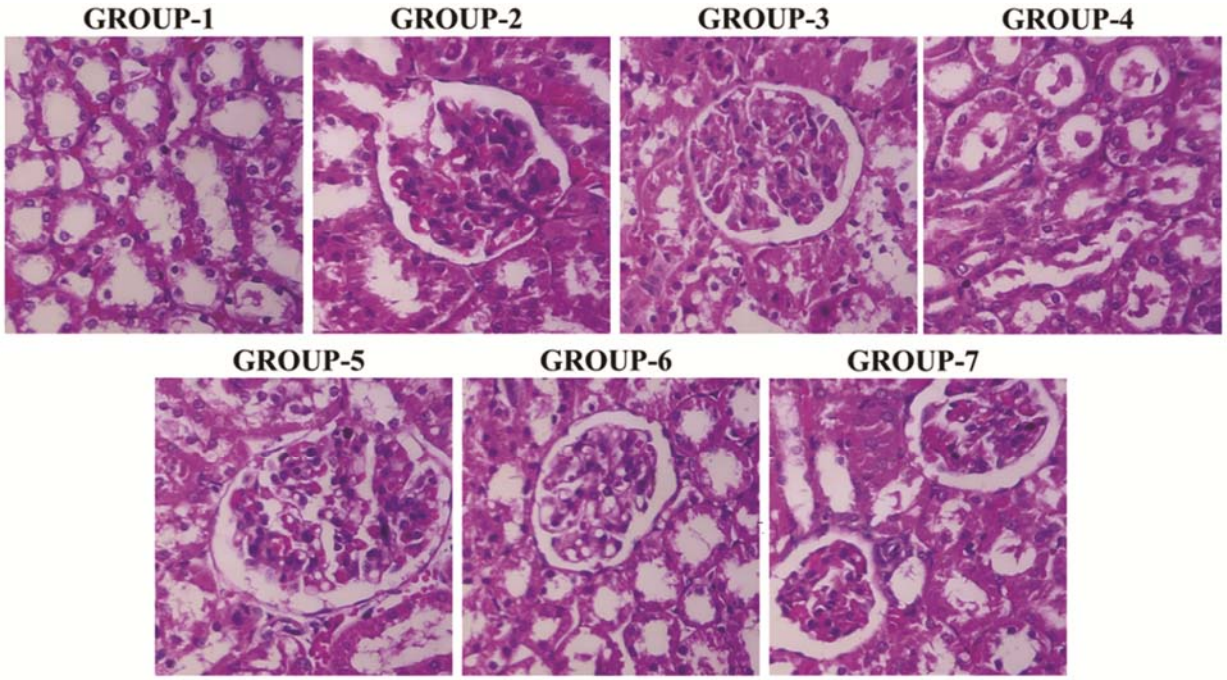

Fig. 6 — Reviving capacity of *Madhuca longifolia seed* oil on kidney histopathology. Diclofenac-induced female Wistar albino rats showing protective competency of *Madhuca longifolia* seed oil on kidney histopathology (Haematoxylin & Eosin stained). The numbers specified on photomicrographs indicate the Group numbers, respectively. (1) Possess normal glomerulus and tubules. (2) Possess renal parenchyma with focal features of toxic Acute Tubular Necrosis (ATN). (3) show focal ATN changes and renal parenchyma with benign features of ATN, respectively. (4) Possess no evidence of tubular necrosis and inflammation. (5) possess mild to moderate glomerular inflammation and vascular congestion. Normal kidney tissue histology was shown in (6 & 7)

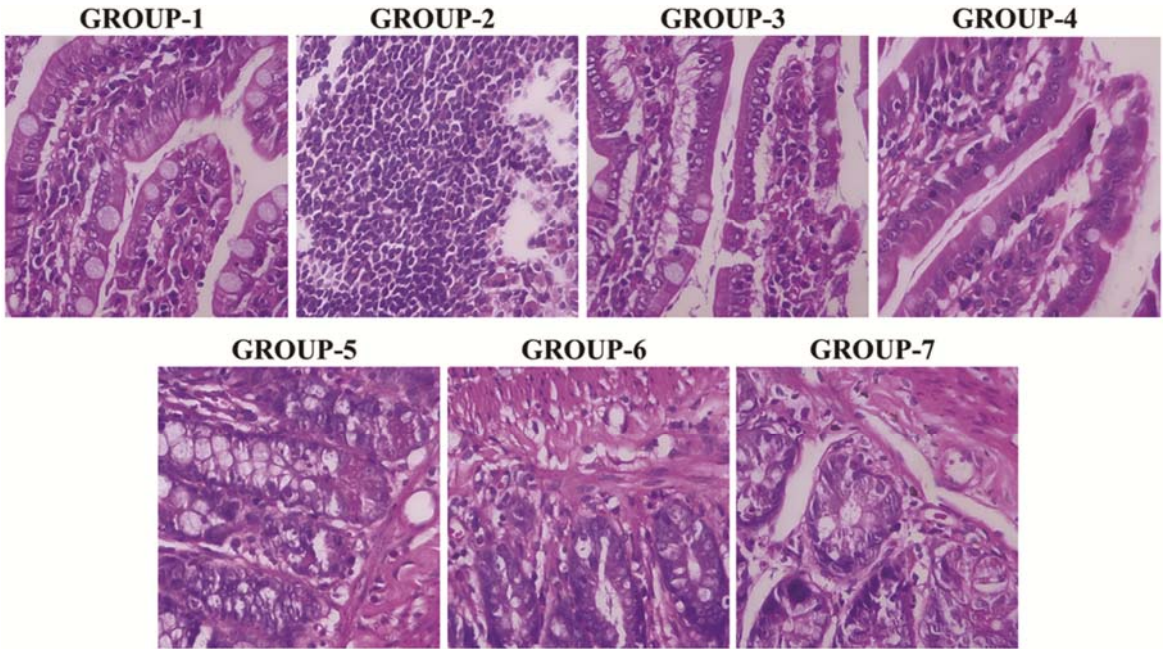

Fig. 7 — Reviving capacity of *Madhuca longifolia seed* oil on intestine histopathology. Diclofenac-induced female Wistar albino rats showing protective competency of *Madhuca longifolia* seed oil on intestine histopathology (Haematoxylin & Eosin stained). The numbers specified on photomicrographs indicate the Group numbers, respectively. (1) Illustrates normal colon mucosa. (2) Illustrates mucosal ulceration with lymphoid aggregation. (3 & 4) possess normal intestinal mucosa. (5) illustrate architectural distortion, increased lymphocytes, and chronicity change. In (6 & 7) Normal intestinal mucosa morphology like that of the control group was seen

biomarkers in blood circulation, which is a direct result of damage. Hepatic damage in the diclofenac-induced rats was confirmed by the increased activity of liver enzyme markers like ALP, ALT, AST, total bilirubin, direct bilirubin and triglycerides, HDL, and total cholesterol discharge in the blood due to the hepatocellular plasma membranedamage[21].Similarly, there was a substantial decline in serum albumin and total protein, subsequently causing hepatic dysfunction.The component of diclofenac-initiated hepatotoxicity seems to be multifactorial such as drug adduct formation, mechanisms of drug idiosyncrasy, the formation of reactive chemical metabolites and drug excretion, and the immunological process of stimulating proinflammatory mediators, inflammatory cytokines and chemokines that advances tissue harm.Our results proved that *Madhuca longifolia seed* oilin pre-treated rats normalized the toxicity produced by diclofenac and caused a significant decrease in liver function parameters and an increase in serum albumin and total protein levels. The capacity of *Madhuca longifolia seed* oil in preserving the serum albumin, total protein, and liver markers have a potential impact on treating diclofenac-induced toxicity, which renormalized liver function.

Diclofenac-induced renal toxicity appeared throughan increase in the serum measurements of urea, uric acid, and creatinine. Accumulation of serum renal biomarkers happens when there is a lack of clearance, which demonstrates that harm to renal tissues has occurred, possibly triggering renal toxicity[22]. Although analgesic and antipyretic properties of NSAID are manifested by inhibiting COX-1 and COX-2 enzymes, they have an assortment of renal effects like the decline in renal blood flow and glomerular filtration rate because the reduction in renal prostaglandins engenders toxicity[24]. The comparative outcome was found in other studies on diclofenac-induced renal damage by hindering renal prostaglandin synthesis. Pre-treatment with *Madhuca longifolia seed* oil hasprotected diclofenac-induced renal impairment. Furthermore, it was able to down-regulate the abnormal levels of serum urea, uric acid, and creatinine to the normal range.

Analogously, gastrointestinal toxicity is the most widely perceived negative impact of diclofenac, which is evident by the mucosal injury in histopathology.A such injury might have happened from COX-1 inhibition by diclofenac, which diminished the production of mucus and bicarbonate that initiate mucosal injury in the intestine and aggravate the condition of gastric damage[25]. Gastrointestinal complications may cause a decline in food consumption in rats leading to weight loss. The relevant result was found in different investigations on the increased risk of gastrointestinal disorders caused by diclofenac[4,26,27]. Our study demonstrated that *Madhuca longifolia seed* oil treatment had repaired the gastrointestinal injuries caused by diclofenac.

Additionally, diclofenac is also a significant inducer of oxidative stress that stimulates cellular damage by ROS production. SOD, CAT, and GST are first-line defense antioxidants that scavenge free radicals and reduce ROS damaging effects by converting the toxic reactive species into non-toxic products[28]. In the current study, diclofenac-treated rats show reduced antioxidant activities culminating in free radical formation compared to the healthy control group, where there is a right balance between free radicals and antioxidants. *Madhuca longifolia seed* oil pre-treated rats showed normal antioxidant levels evidencing its protective role and reinstating the antioxidant status against diclofenac-induced toxicity.

Our study confirmed that pre-treatment with two doses of *Madhuca longifolia seed* oil (1 mL and 2 mL) had significantly decreased serum liver enzyme markers and renal enzyme markers levels of diclofenac-induced rats also, it prevented abnormal range and enhanced their antioxidant activity. This potency might be due to the presence of bioactive components, mainly flavonoids, and alkaloids, that are capable of reducing oxidative stress and acting as hepatoprotective agents and nephroprotective agents. Other findings provide a potential mechanism of terpenoids in *Madhuca longifoliathat* showed anti-ulceration and anti-inflammatory properties.A probable mechanism of*Madhuca longifolia seed* oil against diclofenac toxicity might be owing to its phytoconstituents; for example, oleic acid (76%) has been suggested as a potential agent against oxidative stress. Oleic acid protects rats against steatohepatitis by diminishing cellular and Mito-oxidative stress-mediated lipotoxicity, caspase 8/Poly (ADP-ribose) polymerase (PARP)-mediated apoptosis, and c-Jun N-terminal kinase (JNK)-mediated inflammations exacerbated by saturated palmitic acid[29]. Our findings supported this mechanism since *Madhuca longifolia* seed oil improved antioxidants, lowering oxidative stress, liver enzymes, and lipid markers while

preventing organ damage. Other supportingresearch reported the mechanism of drug-induced oxidative stress and herbal supplementation on renal and gastrointestinal toxicity[30–34]. Also,flavonoids have strengthened the mucus system, protecting intestinal morphology from gastricdamage induced by NSAIDs through decreased mucus secretion. Both doses showed similar results of histopathological changes in repairing the inflammation and necrosis to the improved histopathological condition of the liver, kidney, and intestinal tissue. This aspect of the research proved that a 1 mL concentration of *Madhuca longifolia seed* oil for10 days of study the period was observed to have much more efficacy as a protective agent against diclofenac-induced toxicity.

Conclusion

Our study has proven that pre-treatment with *Madhuca longifolia* seed oil has promising beneficial results on diclofenac-induced toxicities inthe liver, kidney, and intestine.*Madhuca longifolia* seed oil was able to attenuate toxicity by altering liver and renal biomarker levels in diclofenac-induced female Wistar albino rats. Also, *Madhuca longifolia* seed oil could preventhistopathological changes and normalize antioxidant activity. The present investigation concludes by proving the hepatoprotective, renal protective, and gastroprotective activities of *Madhuca longifolia* seed oil against diclofenac-induced toxicity.Future examinations ought to be done on exploring thebioactive components of *Madhuca longifolia* seed oil and should also be executed to understand the mechanism of action of *Madhuca longifolia* seed oil on its protective role.

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Conflict of interest

All authors declare no conflict of interest.

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