



Original article

Protective effect of the ethanolic and methanolic leaf extracts of *Madhuca longifolia* against diclofenac-induced toxicity in female Wistar albino rats



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ABSTRACT

Background: Diclofenac is commonly prescribed Non-Steroidal Anti-Inflammatory Drug (NSAIDs) as it has anti-inflammatory, analgesic and anti-pyretic properties. Long term usage and over-dosage of diclofenac is associated with adverse effects like drug-induced liver injury, gastrointestinal and renal toxicity. The therapeutic uses of medicinal plants have gained a prominent role in recent years. *Madhuca longifolia* is a tree found throughout India, which is known to have several pharmacological activities. The aim of our study is to investigate the potential effect of the ethanolic and methanolic leaf extracts of *M. longifolia* against diclofenac-induced toxicity.

Methods: The rats used for the experiment were divided into seven groups. Group-1 was the normal control. Group-2 was administered with diclofenac (50 mg/kg b.w./day/ip) on the 4th and the 5th day. Group-3 was treated with diclofenac and ELEML (500 mg/kg b.w./day/po) on all 5 days. Group-4 was treated with diclofenac and MLEML (500 mg/kg b.w./day/po) on all 5 days. Group-5 was treated with diclofenac and silymarin (25 mg/kg b.w./day/po) was given to the rats of group-5 along with diclofenac. Group-6 and group-7 were treated with ethanolic leaf extract and methanolic leaf extract of *M. longifolia* respectively. After the study period, the rats were evaluated for parameters like liver and renal markers, antioxidants and histopathological changes.

Result: This study has proved the beneficial effect of ethanolic and methanolic leaf extract of *M. longifolia* against diclofenac-induced toxicity wherein ethanolic leaf extract showed a better result than methanolic leaf extract.

Conclusion: Our study has concluded the beneficial effect of ethanolic and methanolic leaf extract of *Madhuca longifolia* against DFC-induced toxicity. This study proves that it has potential effect on hepato, renal and gastro toxicity in female Wistar albino rats. It can further be studied to understand its mechanism in treating toxicity.

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Introduction

The liver, stomach, intestine and kidney are the chief organs of humans. The liver is a vital organ that plays a unique role in the synthesis of biochemical compounds, metabolize protein, lipids and carbohydrates, and detoxifies drugs and other xenobiotics [1]. Hepatic injury is a severe metabolic disorder which occurs due to elevated oxidative stress and the use of various drugs and xenobiotic which can even cause death by

eradicating the liver's common function [2]. Other than drug-induced hepatotoxicity the prevalent causes for liver disease may also result in fatty liver disease associated with metabolic syndrome, alcoholic hepatitis, hepatitis A, B and C virus infection, hereditary disease, and biliary obstruction [3]. It is reported that diclofenac (DFC) give rise to severe liver damage in close to 3.6 per 100,000 patients using DFC. About 8% of this damages has resulted in death [4].

The stomach and intestine are important parts of the gastrointestinal tract with the primary role of digestion, absorption of nutrients, biotransformation and excretion [5]. Gastric and intestinal mucosa is often damaged by the intake of unhealthy food which contains combination of various substances like xenobiotic

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[6]. The prolonged use of Non-Steroidal Anti-Inflammatory Drugs (NSAIDs), excessive intake of alcohol and smoking, stress, and *Helicobacter pylori* agents also cause of gastric ulcer which is common among 10% of the world population [7].

The kidney is another vital organ which plays an important role in fluid osmolality, controlling body fluids and the electrolyte concentration, acid-base balance and elimination of toxins from our body. Damage or injury caused by the endogenous and exogenous toxicants could lead to functional default in excretion and detoxification of kidney which results in nephrotoxicity [8]. In the global population approximately 20% of people are with acute renal failure in which 66% are caused due to drug-induced nephrotoxicity [9].

Due to its anti-inflammatory, analgesic and anti-pyretic properties DFC is a widely prescribed NSAID [10]. Prostaglandin is a mediator of inflammation and fever. DFC has the potential to inhibit the action of the enzyme cyclooxygenase (COX) to suppress the prostaglandin biosynthesis [11]. COX occurs as two isoforms like COX-1 which produces prostaglandin that is responsible for protecting the gastric mucosa integrity and COX-2 which is a cytokine that induces prostaglandin which is known to cause pain and inflammation [12,13]. DFC is therapeutically beneficial however its long term usage and overdose can lead to idiosyncratic toxicity including gastric, renal and hepatotoxicity [14].

Madhuca longifolia belongs to the sapotaceae family commonly known as butternut tree, which is a fast-growing tree found widely in Nepal, India and Srilanka. The common Indian name for *M. longifolia* is Mahua, Mahwa or Illupai. It is cultivated in the warm and humid regions. It is a deciduous tree which can grow up to 20 m of height [15]. The ethnomedicinal uses of *M. longifolia* contain various phytochemical compounds such as flavonoids, vitamin A and C, histidine, glutamic acid, tannins, volatile oil, beta carotene and xanthophylls [16,17]. The aqueous and alcoholic extract of *M. longifolia* has been reported to show analgesic activity [18]. The ethanolic extract of *M. longifolia* has a significant role in nephro and hepatoprotective activity against acetaminophen-induced necrotic damage of hepatic and renal tissue [19]. Ether benzene-95% crude ethanolic extract of leaves and bark of *M. longifolia* shows a remarkable reduction in the time taken to heal a wound [20]. The methanolic extraction proved to have potential benefits of anti-inflammatory, anti-pyretic and analgesic properties because of the presence of flavonoids in the plants [21]. The methanolic extract of bark is known to have anti-diabetic and anti-hyperglycemic activity [22,23]. The aqueous extract of leaves has been proven to have an effective anti-ulcer property [24].

Silymarin (SLY) is a polyphenolic flavonoid derived from the fruit and seeds of milk thistle [25]. It is scientifically named as *Silybum marianum* which is known to have anti-inflammatory, antioxidant and anti-carcinogenic properties [26]. It is commonly used for treating hepatic and gall bladder disorders [27]. The mechanism of action is that it alters or inhibits the cell transporters, β -glycoprotein, the estrogenic and nuclear receptor to protect the damage caused by drug-induced toxicity [28]. It has been proven to be a neuroprotective, gastroprotective, and nephroprotective agent [29–31]. So, in the current study, we have used SLY as the standard drug.

The protective effect of ethanolic leaf extract of *M. longifolia* (ELEML) and methanolic leaf extract of *M. longifolia* (MLEML) against DFC-induced toxicity has not been reported till date. Therefore, the following study was undertaken to check the potential effect of ELEML and MLEML against dose-dependent DFC-induced toxicity in rats.

Materials and methods

Chemicals and reagent

DFC was purchased from Unique Pharmaceutical Labs, Pvt, Ltd, Mumbai, Maharashtra, India. SLY tablet was brought from Micro lab, Pvt, Ltd, Solan, Himachal Pradesh, India. The liver and renal enzyme markers diagnostic kits were obtained from Span Diagnostics Ltd, Surat, Gujarat, India. As per the standard protocol the antioxidant parameters were performed on liver, kidney, stomach, and intestine.

Plant extraction

M. longifolia leaves were collected fresh on December 2016 which were recognized and authenticated by Prof. Jayaraman, Director of Institute of Herbal Botany, Plant Anatomy Research Centre, Chennai, Tamil Nadu, India. The enrolment number of authentication certificate is PARC/2016/3322. The leaves were immersed in water to eliminate the dust, microbes and insects present on the surface of the leaves and it was allowed to dry at room temperature ($27 \pm 2^\circ\text{C}$) for 4 weeks. Then the leaves were ground into fine powder. To prepare the methanolic leaf extract 5 g of leaf powder was allowed to mix in 50 ml of methanol and the same protocol was followed for the ethanolic leaf extract. They were incubated in conical flasks for 24 h at 25°C with constant shaking. Later this was filtered using Whatman filter paper no-1 and the extract was concentrated using rotatory vaporizer at 40°C under low pressure. The remnants were kept in a hot air oven till

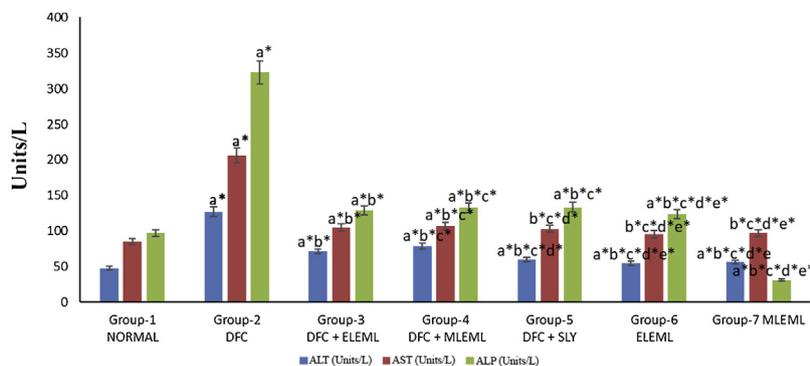


Fig. 1. Hepatoprotective effect of ELEML and MLEML on ALP, AST and ALT of DFC-induced rats.

Each value shows the mean \pm SD of six rats. Comparison were made as follows: 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. The symbols represent statistical significance at $*p < 0.05$. Statistical analysis was calculated by one-way ANOVA followed by the Student Newman-Keul's test.

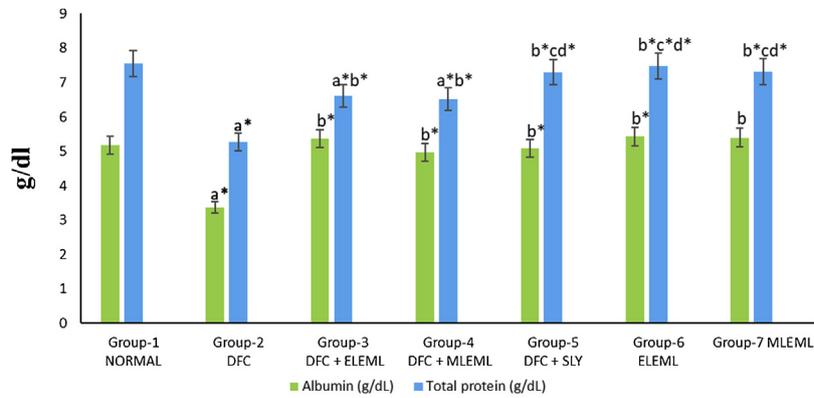


Fig. 2. Hepatoprotective effect of ELEML and MLEML on albumin and total protein of DFC-induced rats.

Each value shows the mean ± SD of six rats. Comparison were made as follows: 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. The symbols represent statistical significance at *p < 0.05. Statistical analysis was calculated by one-way ANOVA followed by the Student Newman-Keul's test.

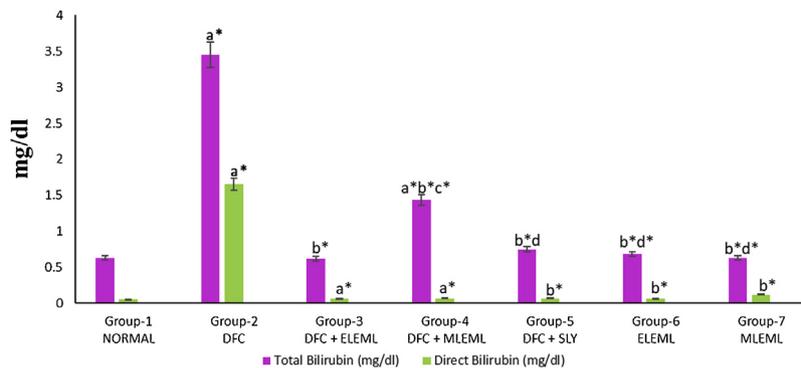


Fig. 3. Hepatoprotective effect of ELEML and MLEML on total and direct bilirubin of DFC-induced rats.

Each value shows the mean ± SD of six rats. Comparison were made as follows: 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. The symbols represent statistical significance at *p < 0.05. Statistical analysis was calculated by one-way ANOVA followed by the Student Newman-Keul's test.

Table 1
Effect of ELEML and MLEML on total cholesterol, HDL and triglyceride of DFC-induced rats.

Parameters	Group - 1 Normal control	Group 2: DFC (50 mg/kg b.w./day, ip)	Group 3: DFC + ELEML (500 mg/kg b.w./day, po)	Group 4: DFC + MLEML (500 mg/kg b.w./day, po)	Group 5: DFC + SLY (25 mg/kg b.w./day, po)	Group 6: ELEML (500 mg/kg b.w./day, po)	Group 7: MLEML (500 mg/kg b.w./day, po)
Total cholesterol (mg/dL)	102.11 ± 1.59	37.93 ± 1.782a*	85.70 ± 1.42a*b*	84.94 ± 1.79a*b*	93.82 ± 1.66a*b*c*d*	94.02 ± 1.70a*b*c*d*	92.88 ± 1.51a*b*c*d*
HDL (mg/dL)	61.16 ± 2.25	27.37 ± 1.56a*	56.06 ± 1.63a*b*	54.57 ± 1.57a*b*	58.32 ± 2.02a*b*	56.84 ± 2.05a*b*	56.66 ± 1.04a*b*
Triglyceride (mg/dL)	116.95 ± 1.73	267.20 ± 1.76a*	162.05 ± 1.83a*b*	158.78 ± 1.70a*b*	159.48 ± 1.96a*b*	142.58 ± 1.15a*b*c*d*e*	155.61 ± 1.62a*b*c*e*f*

Each value shows the mean ± SD of six rats. Comparison were made as follows: 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. The symbols represent statistical significance at *p < 0.05. Statistical analysis was calculated by one-way ANOVA followed by the Student Newman-Keul's test.

the left over ethanol and methanol evaporated and stored at 4°C for further use.

Animals and experimental design

Female Wistar albino rats (160–190 g) were obtained from the Animal house of VIT, Vellore, Tamil Nadu, India. The experimental work which was approved by the ethical committee VIT, Vellore (Reg no: VIT/IAEC/13/Feb13/21), was obtained under the guidelines of the Indian CPCSEA. The animals were maintained under standard laboratory conditions. The animals were kept in

acclimatization for a week before the initiation of experiment. The animals were divided into six per cage and were kept on water and pellet diet. These pellets were brought from the Hindustan lever Ltd, Mumbai, India. The rats were divided into seven groups of six rats each and drugs were administered as follows:

- Group 1: Normal control
- Group 2: DFC (50 mg/kg b.w./day, ip) (on 4th and 5th day)
- Group 3: DFC (50 mg/kg b.w./day, ip) as mentioned in Group 2 + ELEML (500 mg/kg b.w./day, po) for 5 days (On all 5 days)

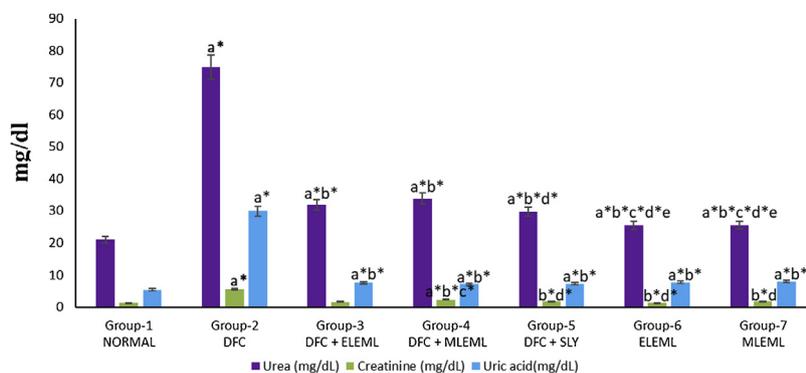


Fig. 4. Nephroprotective effect of ELEML and MLEML on urea, creatinine and uric acid of DFC-induced rats.

Each value shows the mean \pm SD of six rats. Comparison were made as follows: 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. The symbols represent statistical significance at $*p < 0.05$. Statistical analysis was calculated by one-way ANOVA followed by the Student Newman-Keul's test.

Table 2

Protective effect of ELEML and MLEML on Liver antioxidant.

Parameters	Group - 1 Normal control	Group 2: DFC (50 mg/kg b.w./day, ip)	Group 3: DFC + ELEML (500 mg/kg b.w./day, po)	Group 4: DFC + MLEML (500 mg/kg b.w./day, po)	Group 5: DFC + SLY (25 mg/kg b.w./day, po)	Group 6: ELEML (500 mg/kg b.w./day, po)	Group 7: MLEML (500 mg/kg b.w./day, po)
SOD (Units/min/mg protein)	82.51 \pm 1.49	25.35 \pm 1.21a*	74.08 \pm 2.01a*b*	72.75 \pm 1.63a*b*	76.41 \pm 1.32a*b*d	79.28 \pm 1.29b*cd*	76.29 \pm 1.46a*b*d
Catalase (Units/min/mg protein)	75.23 \pm 1.47	42.47 \pm 1.71a*	71.01 \pm 1.89ab*	75.86 \pm 1.97b*c	71.72 \pm 2.13b*d	72.98 \pm 1.92a*b*c*d*e*	75.50 \pm 1.53b*cf*
Reduced glutathione (nmol/mg protein)	48.72 \pm 0.91	31.53 \pm 2.23a*	46.19 \pm 0.81b*	42.59 \pm 0.94a*b*	45.27 \pm 1.67b*	46.00 \pm 1.84b*	44.78 \pm 1.60a*b*

Each value shows the mean \pm SD of six rats. Comparison were made as follows: 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. The symbols represent statistical significance at $*p < 0.05$. Statistical analysis was calculated by one-way ANOVA followed by the Student Newman-Keul's test.

Table 3

Protective effect of ELEML and MLEML on Kidney antioxidant.

Parameters	Group - 1 Normal control	Group 2: DFC (50 mg/kg b.w./day, ip)	Group 3: DFC + ELEML (500 mg/kg b.w./day, po)	Group 4: DFC + MLEML (500 mg/kg b.w./day, po)	Group 5: DFC + SLY (25 mg/kg b.w./day, po)	Group 6: ELEML (500 mg/kg b.w./day, po)	Group 7: MLEML (500 mg/kg b.w./day, po)
SOD (Units/min/mg protein)	94.89 \pm 1.82	36.51 \pm 1.28a*	87.31 \pm 1.25a*b*	85.00 \pm 1.38a*b*	86.750 \pm 1.483a*b*	89.48 \pm 1.85a*b*d*	92.01 \pm 2.00b*c*d*e*
Catalase (Units/min/mg protein)	83.17 \pm 1.95	43.08 \pm 1.64a*	72.30 \pm 1.65a*b*	69.15 \pm 1.24a*b*	72.10 \pm 1.60a*b*	78.43 \pm 1.36a*b*c*d*e*	76.38 \pm 1.64a*b*cd*e*
Reduced glutathione (nmol/mg protein)	47.52 \pm 1.75	21.46 \pm 1.48a*	42.93 \pm 1.95ab*	44.74 \pm 1.62b*	45.02 \pm 2.07b*	46.37 \pm 1.98b*	46.23 \pm 1.98a*b*

Each value shows the mean \pm SD of six rats. Comparison were made as follows: 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. The symbols represent statistical significance at $*p < 0.05$. Statistical analysis was calculated by one-way ANOVA followed by the Student Newman-Keul's test.

Group 4: DFC (50 mg/kg b.w./day, ip) as mentioned in Group 2 + MLEML (500 mg/kg b.w./day, po) for 5 days

Group 5: DFC (50 mg/kg b.w./day, ip) as mentioned in Group 2 + silymarin (25 mg/kg b.w./day, po) for 5 days

Group 6: ELEML alone (500 mg/kg b.w./day, po) for 5 days

Group 7: MLEML alone (500 mg/kg b.w./day, po) for 5 days

After the treatment period the rats were euthanized by using ether anaesthesia and the blood samples were collected by cardiac puncture. Few ml of blood was collected and centrifuged at 2000 rpm for 10 min to separate the serum for further biochemical

analysis. The organs collected from the animals were stored in PBS solution and homogenised for biochemical analysis and also 10% formalin was used to store the organs for the histopathological analysis.

Screening of biochemical activities in rats

The liver enzyme markers such as albumin, Alanine Amino Transferase (ALT), Aspartate Amino Transferase (AST), Alkaline Amino Transferase (ALP), total protein, total cholesterol, High

Table 4
Protective effect of ELEML and MLEML on Stomach antioxidant.

Parameters	Group - 1 Normal control	Group 2: DFC (50 mg/kg b.w./day, ip)	Group 3: DFC + ELEML (500 mg/kg b.w./day, po)	Group 4: DFC + MLEML (500 mg/kg b.w./day, po)	Group 5: DFC + SLY (25 mg/kg b.w./day, po)	Group 6: ELEML (500 mg/kg b.w./day, po)	Group 7: MLEML (500 mg/kg b.w./day, po)
SOD (Units/min/mg protein)	19.43 ± 1.46	9.30 ± 1.99a*	16.02 ± 1.89b*	16.02 ± 1.63b*	16.91 ± 1.96b*	18.51 ± 1.28b*	17.44 ± 1.44b*
Catalase (Units/min/mg protein)	18.39 ± 1.95	8.83 ± 1.54a*	16.59 ± 1.84b*	15.99 ± 1.22b*	16.73 ± 1.56b*	16.86 ± 2.29b*	16.31 ± 1.41b*
Reduced glutathione (nmol/mg protein)	82.03 ± 1.08	51.64 ± 1.30a*	69.48 ± 23.27	77.33 ± 2.49b*	80.29 ± 2.52b*	79.81 ± 2.19b*	77.52 ± 2.49b*

Each value shows the mean ± SD of six rats. Comparison were made as follows: 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. The symbols represent statistical significance at * $p < 0.05$. Statistical analysis was calculated by one-way ANOVA followed by the Student Newman-Keul's test.

Table 5
Protective effect of ELEML and MLEML on Intestine antioxidant.

Parameters	Group - 1 Normal control	Group 2: DFC (50 mg/kg b.w./day, ip)	Group 3: DFC + ELEML (500 mg/kg b.w./day, po)	Group 4: DFC + MLEML (500 mg/kg b.w./day, po)	Group 5: DFC + SLY (25 mg/kg b.w./day, po)	Group 6: ELEML (500 mg/kg b.w./day, po)	Group 7: MLEML (500 mg/kg b.w./day, po)
SOD (Units/min/mg protein)	15.70 ± 1.42	9.63 ± 1.69a*	14.71 ± 1.45b*	14.31 ± 1.27b*	15.42 ± 1.42b*	15.65 ± 1.66b*	14.69 ± 1.44b*
Catalase (Units/min/mg protein)	16.99 ± 1.51	7.53 ± 0.215a*	18.10 ± 1.79b*	15.73 ± 1.42b*	16.42 ± 1.68b*	16.97 ± 1.52b*	15.55 ± 1.67b*
Reduced glutathione (nmol/mg protein)	89.17 ± 1.73	54.69 ± 1.18a*	82.08 ± 1.07a*b*	82.68 ± 1.43a*b*	84.85 ± 1.33a*b*	85.01 ± 1.39a*b*	87.69 ± 1.27a*b*c*d*

Each value shows the mean ± SD of six rats. Comparison were made as follows: 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. The symbols represent statistical significance at * $p < 0.05$. Statistical analysis was calculated by one-way ANOVA followed by the Student Newman-Keul's test.

Density Lipoprotein (HDL), total and direct bilirubin and triglyceride were analysed from serum to evaluate the hepatoprotective activity of ELEML and MLEML. The renal enzyme markers like urea, creatinine and uric acid analysing test were carried out to identify the nephroprotective activity of EMLEML and MLEML. All the serum biochemical activities were examined by using kits purchased from Auto Span Pvt., Ltd.

Screening of antioxidant activities in rats

The organs such as liver, kidney, stomach and intestine tissues obtained from the rats were homogenised in 0.1 M ice-cold phosphate buffered saline (PBS). to evaluate the antioxidant activities such as Superoxide Dismutase (SOD), Catalase (CAT) and Reduced glutathione (GSH).

Screening of histopathological activity

The organs such as liver, kidney, small and large intestine obtained from the rats were rinsed in PBS buffer and preserved in 10% formalin for histopathological analysis. The tissues were stained with haematoxylin and eosin to recognize the structural changes. The samples were analysed to assess the tissue architecture.

Statistical analysis

The statistical analysis of the results were expressed using the one way ANOVA which was followed by student Newman-Keul's test. The results are denoted as mean ± standard deviation and they were considered as statistically significant at the level of $p < 0.05$.

Results

Comparison of ELEML and MLEML on liver enzyme markers of DFC induced rats

Fig. 1 showed the hepatoprotective activity of ELEML and MLEML on ALP, AST and ALT of DFC administered rats. It was observed that there has been a significant increase ($p < 0.05$) in the level of ALP, AST and ALT in DFC-induced rats. In group 3 and 4 treated rats, DFC-induced along with ELEML and MLEML respectively shows a decrease in the level of liver enzyme markers when compared with group 2. SLY administered group showed a similar result on comparing with ELEML and MLEML treated groups, the groups treated with ELEML alone showed a normal level while treated rats with MLEML alone showed a decrease in ALP whereas restoring ALT and AST level when compared to normal group.

Fig. 2 showed the hepatoprotective activity of ELEML and MLEML on albumin and total protein of DFC-induced rats. Albumin and total protein level in DFC-induced group showed a significant decrease ($p < 0.05$). DFC administered group along with ELEML and MLEML restores the level of albumin and total protein. SLY, ELEML and MLEML treated group shows a similar result when compared to normal control group. When compared ELEML showed a better result than MLEML.

Fig. 3 showed a significant rise ($p < 0.05$) on total and direct bilirubin in DFC-induced group. DFC with ELEML showed similar result when compared with the normal control group. DFC along with MLEML showed a decrease in the total bilirubin level when compared with DFC-induced group and direct bilirubin had a normalized level. SLY shows a similar result on comparing it with ELEML and MLEML alone treated groups.

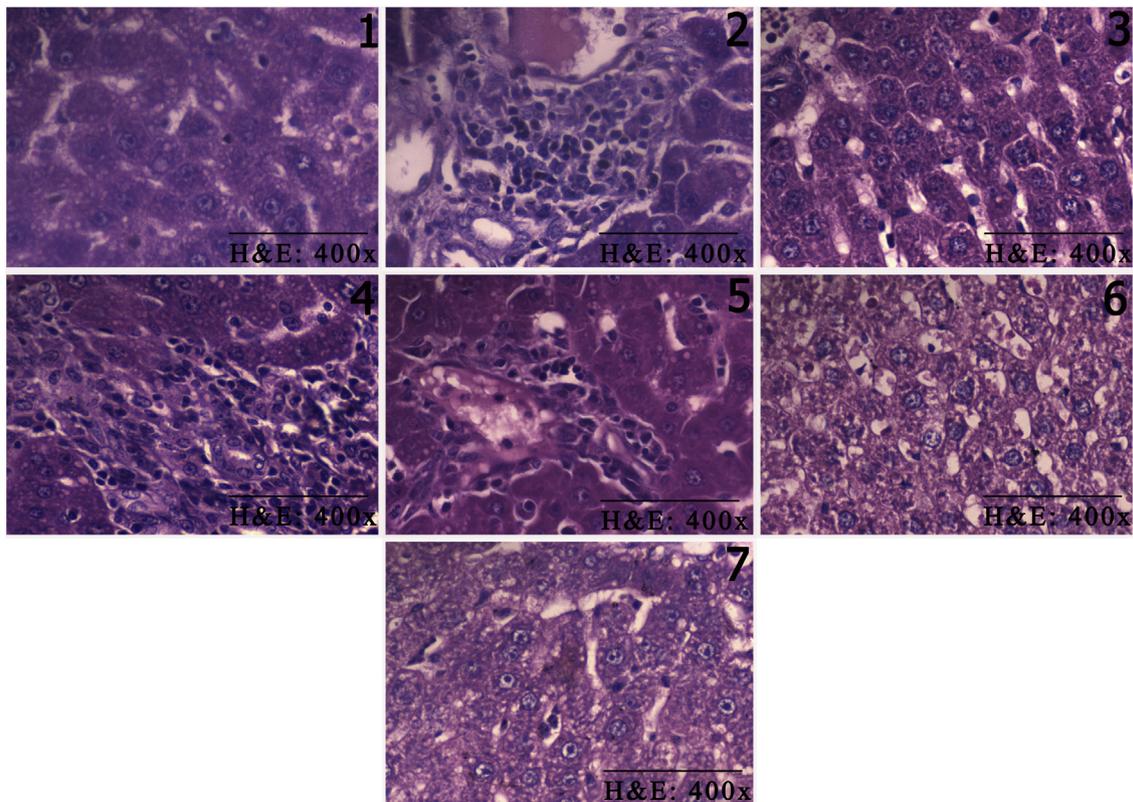


Fig. 5. Effect of ELEML and MLEML on liver histopathology.

Liver histopathology (H&E: 400x) shows the protective effect of ELEML and MLEML of DFC-induced rats: 1) normal control group shows normal liver tissues. 2) DFC-induced toxicity group shows portal inflammation. 3) DFC-induced toxicity treated with ELEML group shows normal liver tissue. 4) DFC-induced toxicity treated with MLEML group shows mild lobular inflammation. 5) DFC-induced toxicity treated with SLY group shows mild pericentral lobular inflammation. 6) ELEML administration group shows feathery degeneration. 7) MLEML administration group shows feathery degeneration changed.

Table 1 shows the DFC-induced rat showed the significant decrease ($p < 0.05$) in the level of HDL and triglyceride is found to be increased in DFC-induced rats when it is compared to the normal group. The level of total cholesterol was found to be decreased in DFC-induced rats. Groups administered with ELEML, MLEML and SLY along with DFC has showed an increase in the level of HDL and total cholesterol however triglyceride level was found to have decreased on comparing with DFC induced group. ELEML and MLEML alone group were found to be nearly equal to group 1 in the level of total cholesterol and HDL values and small rise in the level of triglyceride.

Comparison of ELEML and MLEML on renal enzyme markers of DFC induced rats

Fig. 4 showed the nephroprotective effect of ELEML and MLEML on urea, creatinine and uric acid in DFC-induced rats. The level of renal enzyme markers showed a significant increase ($p < 0.05$) in DFC-induced rats when it is compared with the normal control. ELEML, MLEML and SLY along with DFC treated group shows that the level of urea is reduced to half of DFC alone induced rats group. Also, there is a reduction in other parameters like creatinine and uric acid when compared with Group-2. ELEML and MLEML alone treated group showed a similar result and they were almost able to normalize the renal enzyme markers.

Comparison of ELEML and MLEML on antioxidant of DFC induced rats

Table 2 shows the ELEML and MLEML protective effect on liver antioxidant. It is shown that there is a significant reduction ($p < 0.05$) in the level of SOD, catalase and reduced glutathione

when compared with the control group. These decreased levels of the antioxidant parameters were almost brought back to the normal level by ELEML and MLEML. SLY administered group also shows that it was able to normalize the antioxidant parameters. MLEML alone induced rat showed an equal level of catalase as in the normal group. ELEML alone shows to be effective in normalizing the liver antioxidant parameter than the SLY-induced group. However, the level of reduced GSH was found to be similar in ELEML, MLEML alone and SLY treated group.

Table 3 shows the protective effect of ELEML and MLEML on the kidney antioxidant. DFC-induced rats group shows a significant reduction ($p < 0.05$) in the level of kidney antioxidant parameters. The ELEML and MLEML along with DFC shows that there is an increased level in the antioxidant parameters when compared to the DFC-induced rats group.

The level of SOD and catalase had a better result in Group 3 than in group 4. However, in the reduced glutathione, MLEML along with DFC showed a good result. SLY group showed a result that was almost similar when compared with ELEML and MLEML group. ELEML and MLEML alone treated group found to normalize the kidney antioxidant parameters that was almost similar to that of the normal control group. MLEML alone treated group shows a better result in SOD parameters than the ELEML. Similarly, catalase of ELEML was found to be more effective than in MLEML. However, ELEML and MLEML showed a similar result in the reduced glutathione.

Table 4 denotes the protective effect of ELEML and MLEML on stomach antioxidant. In DFC-induced group there is a significant reduction ($p < 0.05$) in the level of SOD, catalase and reduced glutathione. ELEML and MLEML with DFC-induced rats group showed a similar result in the level of SOD and catalase. MLEML

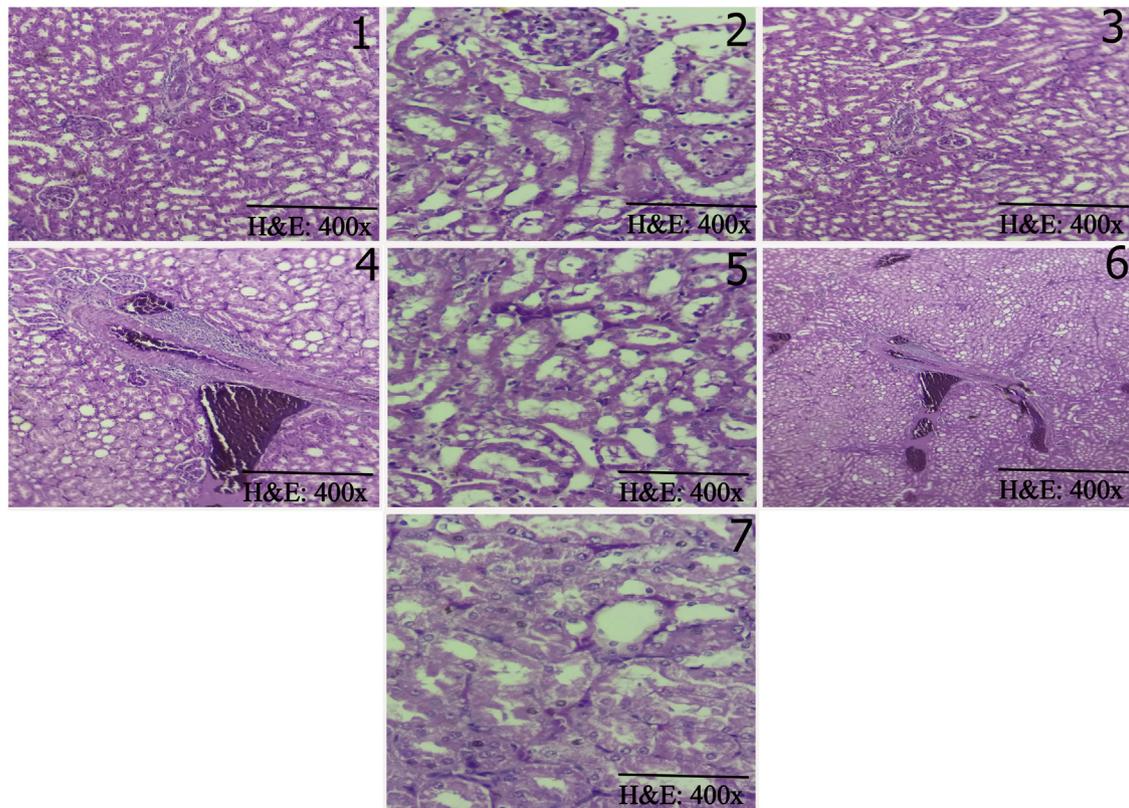


Fig. 6. Effect of ELEML and MLEML on kidney histopathology.

Kidney histopathology (H&E: 400x) shows the protective effect of ELEML and MLEML of DFC-induced rats: 1) normal control group shows normal kidney tissues. 2) DFC-induced toxicity group shows focal avascular tumor necrosis. 3) DFC-induced toxicity treated with ELEML group shows normal kidney tissue. 4) DFC-induced toxicity treated with MLEML group shows mild interstitial nephritis. 5) DFC-induced toxicity treated with SLY group shows focal avascular tumor necrosis. 6) ELEML administration group shows normal kidney tissues. 7) MLEML administration group shows avascular tumor necrosis features changed.

showed a better result in GST when compared with ELEML. SLY treated group also showed a similar result in group 3 and 4. However SLY group shows better result in reduced glutathione when compared to other groups. ELEML and MLEML alone treated group was found to normalize the stomach antioxidant. ELEML alone treated groups was found to be effective when compared with MLEML alone treated group.

Table 5 shows the protective effect of ELEML and MLEML on intestine antioxidant. The level of SOD, catalase and reduced glutathione GST has been found to be significantly reduced ($p < 0.05$) in DFC-induced rats group. ELEML, MLEML and SLY along with DFC was able to normalize the intestine antioxidant parameters. SOD and catalase of ELEML alone treated group showed similar result on comparison with normal control group. MLEML alone treated group showed their ability to maintain the liver antioxidant parameters to a normal level.

Comparison of ELEML and MLEML on histopathological changes of DFC-induced rats

The liver histopathology image (Fig. 5) group 1 shows the normal liver tissues. The DFC-induced rats group shows the portal inflammation as the effect of DFC toxicity. The ELEML and MLEML treated along DFC group shows the normalized liver tissue and mild lobular inflammation respectively. Group 5 SLY treated with DFC shows mild peri central lobular inflammation. ELEML and MLEML alone treated group shows their protective ability as feathery degeneration changes.

Fig. 6 shows kidney histopathology where group 1 shows normal control of kidney tissues. The focal avascular tumor

necrosis was seen in DFC-induced rats group. The ELEML and MLEML along with DFC treated group shows normal kidney tissue and mild interstitial nephritis respectively. SLY group also shows avascular tumor necrosis. ELEML and MLEML alone treated group shows normal kidney tissues and avascular tumor necrosis feature changes.

Fig. 7 shows the effect of ELEML and MLEML on small intestine histopathology. Group 1 is the control group which shows the normal intestine mucosa. DFC-induced group shows lamina propria necrosis and focal inflammation as a result of toxicity. The ELEML and MLEML along with DFC treated group shows small intestine mucosa with decreased intraepithelial lymphocyte. SLY administered group shows lamina propria necrosis and focal inflammation. ELEML and MLEML alone treated group shows normal small intestine mucosa as the protective effect.

Fig. 8 shows large intestine histopathology as group-1 is the normal control of intestine mucosa. Group 2 shows DFC-induced toxicity lead to chronic changes and chronic inflammation. DFC along with ELEML and MLEML treated group shows normal intestinal mucosa which is the result of the protective effect of *M. longifolia* extract from DFC-induced toxicity. SLY along with DFC treated group shows intestinal mucosa and lymphoid aggregated, mucosal and sub-mucosal involvement and destruction of the glands MALTOMA. ELEML and MLEML alone administered shows the normal intestinal mucosa.

Discussion

Studies on DFC has shown its analgesic activity in animal models that is known to inhibit the COX-2 in the last stage of

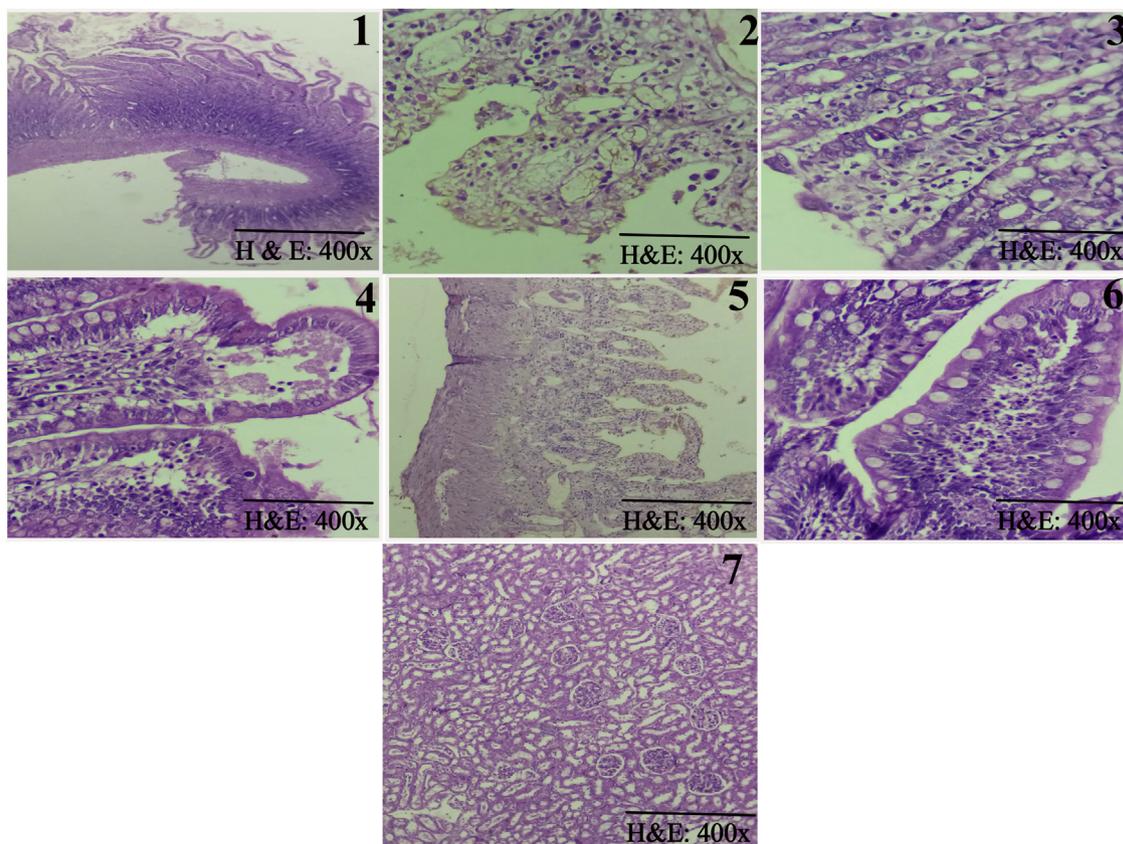


Fig. 7. Effect of ELEML and MLEML on small intestine histopathology.

Small intestine histopathology (H&E: 400x) shows the protective effect of ELEML and MLEML of DFC-induced rats: 1) normal control group shows normal intestine mucosa. 2) DFC-induced toxicity group shows lamina propria necrosis and inflammation (focal). 3) DFC-induced toxicity treated with ELEML group shows small intestine mucosa with decreased intraepithelial lymphocyte. 4) DFC-induced toxicity treated with MLEML group shows small intestine mucosa with decreased intraepithelial lymphocyte. 5) DFC-induced toxicity treated with SLY group shows lamina propria necrosis and inflammation (focal). 6) ELEML administration group shows normal intestine mucosa. 7) MLEML administration group shows normal intestine mucosa.

edema [32]. It is reported to cause the stimulation of pro-inflammatory eicosanoids on its over dosage and long-term usage [33,34]. The prolonged and frequent use of DFC shows an adverse effect that result in hepatotoxicity, as it increases the concentration of aminotransferase in serum, jaundice and liver necrosis. DFC at single oral dose administered of 75 mg/kg showed an acute liver injury and an increase in the liver enzyme markers were observed after 6 h [35]. ALT and AST are cytosolic and mitochondrial enzyme of hepatocyte respectively, the liver damage and excess leakage are the results of increased activity of these ALT and AST in circulatory system. The metabolites transportation across the cell membrane is the main function of ALP enzyme, and elevation of these enzymes indicates liver and bone disease and the accumulation of bile salts leads to a bile duct obstruction and ALP alterations are associated with hyperbilirubinemia [36]. In this study, the level of ALT, AST and ALP were increased significantly ($p < 0.05$) in the DFC-induced rats groups that indicates the liver damage. Similar to our study, other studies in NSAID-induced toxicity showed an increase in the liver enzyme markers level [37]. However the ELEML and MLEML treated along with DFC shows the effectiveness in reducing the liver markers which also had a similar result in the SLY treated group. For the clearance of waste products bilirubin had to break the heme in the red blood cells. In this study the increased level of total and direct bilirubin concentration shows a liver damage [38]. The ELEML and MLEML normalized the bilirubin level as it shows their protective activity. HDL is considered to be a good cholesterol which act as a scavenger and eliminate the LDL

from the arteries. Therefore in the current study the decreased level of HDL shows liver damage. Triglyceride which is known to be bad cholesterol is stored in excess along with fat around the walls of arteries could lead to heart attack [39]. In this study, the increased level of triglyceride shows functional defect in liver as well as tissue damage.

The prominent role of albumin is various exogenous and endogenous substance in transportation and deposition in the blood due to the presence of binding region with various specificity in a limited number [40]. In the current study, it was found that DFC toxicity results in a decreased level of albumin and total protein which indicates the damage to the liver function due to the abnormal levels of liver enzymes. Previous studies have also shown similar results in which albumin and total protein level was found to have decreased due to toxicity [41].

The urea, creatinine and uric acid are renal enzyme markers which are used to examine the functioning of the kidney and renal related diseases. In the current study, elevation of urea, creatinine and uric acid level were caused by DFC-induced toxicity which confirms a kidney disease and other disease such as urinary obstruction, arthritis, muscle disease and hyperthyroidism [42]. It was proven that the elevated renal markers can be reduced and normalized by using the extraction of *M. longifolia*. Similar to this other studies have shown increased concentration of renal enzyme markers that leads to kidney damage [43].

Antioxidant is a defence mechanism which protects cell activity against free radicals, oxidative stress such as SOD,

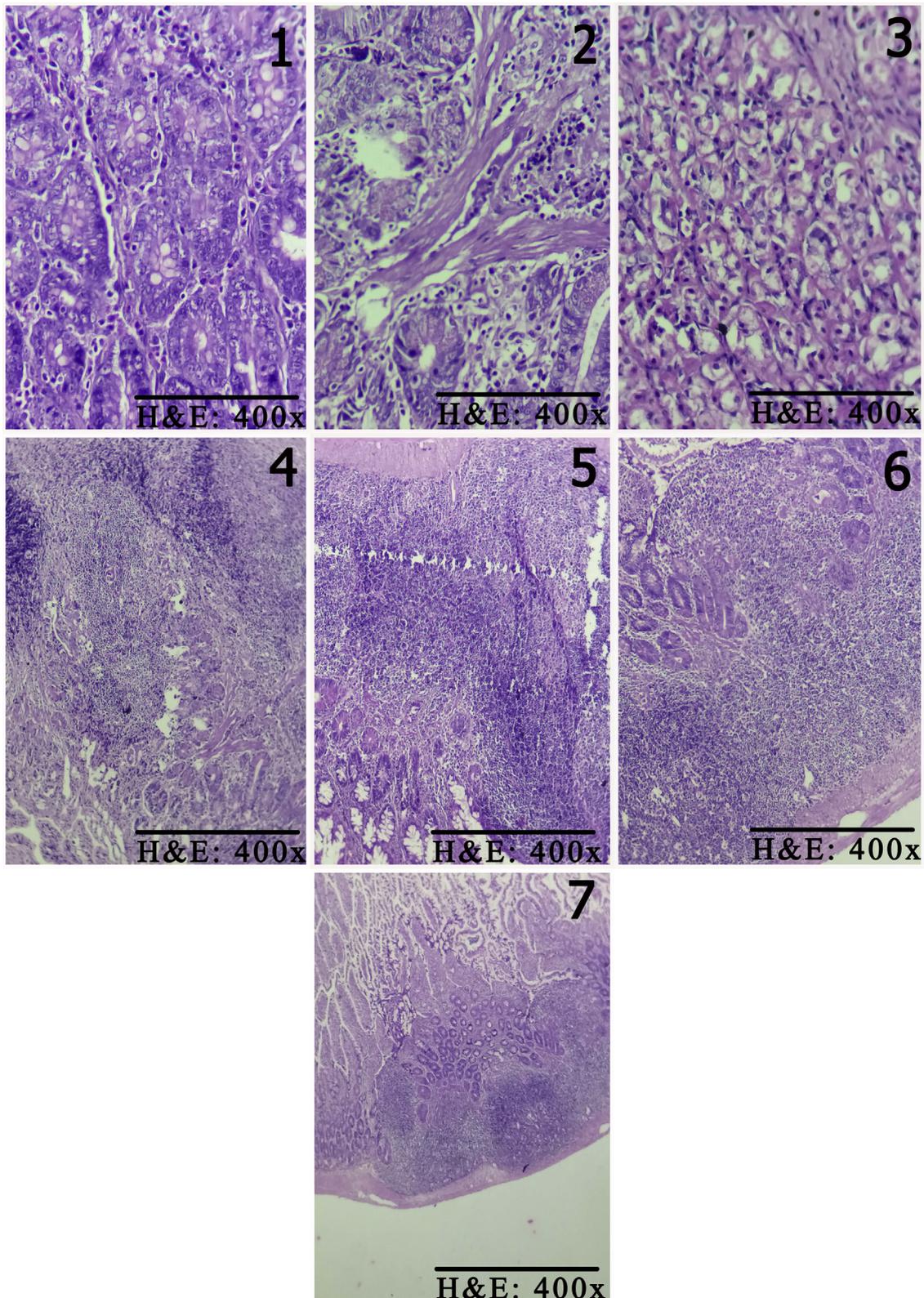


Fig. 8. Effect of ELEML and MLEML on large intestine histopathology.

Large intestine histopathology (H&E: 400x) shows the protective effect of ELEML and MLEML of DFC-induced rats: 1) normal control group shows normal intestine mucosa. 2) DFC-induced toxicity group shows chronicity changes and chronic inflammation. 3) DFC-induced toxicity treated with ELEML group shows normal intestine mucosa. 4) DFC-induced toxicity treated with MLEML group shows normal intestine mucosa. 5) DFC-induced toxicity treated with SLY group shows intestinal mucosa and lymphoid aggregates, mucosal and sub mucosal involvement and destruction of glands MALTOMA. 6) ELEML administration group shows normal intestine mucosa. 7) MLEML administration group shows normal intestine mucosa.

catalase, reduced glutathione. Therefore antioxidants will ciec (ROS) [[44]]. In this study, the liver, kidney, small intestine and large intestine were observed to have decreased in their antioxidant activity when compared to the control group. SOD helps to convert superoxide (O_2^-) to hydrogen peroxide (H_2O_2) and further it is converted to H_2O . Catalase activity reduction indicates the impotence to eliminate hydrogen peroxide by the liver mitochondria and microsomes [45]. Reduced glutathione has an important role in handling enzyme activity synthesized by the hepatic cytochrome P450, decreased level of reduced glutathione and catalase which may indicate that the metabolism of CCl_4 that produces more free radicals leads to tissue damage [46]. SOD converts the O_2^- to H_2O_2 which is further converted into H_2O . Our results showed that ELEML and MLEML treated groups had a normalized antioxidant level which proves the protective properties against free radicals. The similar results have been shown in previous study where DFC reduces the antioxidant level [47].

The histopathology images of the liver, kidney, small intestine and large intestine confirms the beneficial effect of ELEML and MLEML. Their activity shows the protective effect against the DFC-induced toxicity. They show the ability to normalize the portal inflammation in liver tissue, avascular tumor necrosis in kidney tissue, lamina propira necrosis and inflammation in small intestinal mucosa and chronic inflammation in the large intestinal mucosa. The ELEML and MLEML have altered the tissue structure caused by DFC-induced toxicity to the normal tissue architecture. Almost similar results were observed in SLY administered rat groups. Other studies shows an almost similar result in treating inflammation by *Madhuca indica* [24].

Conclusion

Our study has demonstrated the beneficial effect of ELEML and MLEML against DFC-induced toxicity. This study proves that it has potential effect on hepato, renal and gastro toxicity in female Wistar albino rats by decreasing the liver and renal enzyme markers level in the serum, whereas it increased the antioxidant enzyme level in the liver, kidney, stomach and intestine. Also *Madhuca longifolia* proves that it has an ability to normalize the abnormal structural changes in histopathology. Further molecular studies can be done to understand the mechanism of *M. longifolia* against drug-induced toxicity.

Conflict of interest

The authors declare that they do not have any conflict of interest.

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