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Evaluation of the *In-Vivo* Anti-Fibrotic Properties of the Leaf Extract of Indian Jujube (*Ziziphus Mauritiana*) on Chemically Induced Hepatic Fibrosis

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Abstract

Worldwide, liver injury is a major public health issue. Thus, novel, effective, and preventive therapy are needed. Ziziphus Mauritiana (*Z. mauritiana*) is believed to treat numerous organ toxicities. This is due to its antioxidants. Chronic liver inflammation, such as non-alcoholic steatohepatitis, promotes liver fibrosis and mortality. However, no authorized liver fibrosis treatment exists. This study examined the antioxidant and liver-protective activities of hydroethanolic extracts from *Z. mauritiana* leaves (ZMLE). The study focused on the liver damage caused by 2-nitropropane (2-NP) in rats, namely fibrosis. We investigated the impact of ZMLE on preclinical models. The rats received intraperitoneal injections of 2-NP twice a week for a duration of 12 weeks. Following this, they were simultaneously treated with ZMLE at doses of 200, 400, and 800 mg/kg body weight daily for another 12 weeks. The anti-fibrotic effect was assessed using serological and tissue homogenate tests, including measurements of AST, ALT, ALP, reduced GSH, MDA, SOD, GPx, CAT, proinflammatory cytokines, tissue hydroxyproline content, CYP-450 content, UDPGT, Na+-K+-ATPase and Ca+-K+-ATPase activity. Additionally, histochemical analysis using H&E staining was performed. The oral administration of ZMLE, particularly at high doses, effectively reduced the occurrence and severity of liver toxicity induced by 2-NP. This was achieved by activating the liver's antioxidant defense mechanisms, regulating liver functions, and reducing the production of lipid peroxidation, pro-inflammatory mediators, and collagen content. Nevertheless, the extent of inhibition differed based on the dosage. This study provides evidence that ZMLE mitigates liver fibrosis through its antioxidant and anti-inflammatory properties.

Keywords: 2-nitropropane (2-NP), Liver fibrosis, *Ziziphus mauritiana* Leaves, Antioxidant, Pro-inflammatory mediators, Anti-inflammatory.

1. INTRODUCTION

The aberrant accumulation of fibrous tissue in the liver, which occurs as a result of continuous inflammation and injury to liver cells, is the defining characteristic of the clinical condition known as hepatic fibrosis (1). It is a crucial organ that performs various activities, including detoxification, metabolism, and the creation of proteins that are necessary for blood coagulation. The liver is an organ that is extremely important (2). A wound-healing reaction is triggered when there is chronic damage to the liver. This reaction involves the deposition of collagen and other proteins, which ultimately leads to the creation of scar tissue. Liver fibrosis often emerges as a result of chronic liver diseases, including chronic viral hepatitis (hepatitis B or C), excessive alcohol consumption, non-alcoholic fatty liver disease (NAFLD), autoimmune hepatitis, and particular genetic disorders (3). As the fibrous tissue accumulates, it has the potential to disrupt the normal organization of the liver, prevent it from functioning properly, and ultimately lead to more serious conditions, such as cirrhosis or liver failure. In the early stages of liver illnesses, the process of liver fibrosis can be dynamic and reversible to some degree. This is especially true in the early stages of the disease. The course of fibrosis can be slowed down or even reversed if the underlying cause of liver injury is recognized and treated in an appropriate manner. This is possible if patients receive the

appropriate treatment (4). Fibrosis may proceed to more severe stages if, on the other hand, the underlying reason continues to exist or if the condition continues to worsen. This would result in the liver becoming less resilient and more potentially prone to difficulties. Both the implementation of effective therapies and the prevention of the progression to severe liver problems are based on the early diagnosis of liver fibrosis and the regular monitoring of the condition, both of which are vital (5). Using a variety of diagnostic techniques, such as blood tests, imaging examinations, and liver biopsies, it is possible to determine the proper course of treatment and evaluate the severity of fibrosis. This can be performed by determining the suitable course of treatment. To develop successful treatment regimens and provide the highest possible level of care, medical professionals need to have a thorough understanding of the causes, risk factors, and progression of liver fibrosis. Researchers are looking into potential new treatments for liver fibrosis as well as molecular mechanisms. The primary objective of this research is to develop individualized pharmaceuticals. 2-NP is a chemical molecule that can cause liver injury and potentially lead to disorders like liver fibrosis. Exposure to certain chemicals, particularly those with hepatotoxic qualities, can contribute to this damage (6) . It is important to emphasise that the liver plays a vital role in metabolising a range of substances, and when exposed to specific chemicals, poisons, or medications, it can get overwhelmed, leading to liver damage. Nevertheless, the precise influence of 2-NP on the liver can vary based on factors such as the extent of exposure, duration, and individual sensitivity (7). *Z. mauritiana*, also known as Indian Jujube or Ber, has not been the subject of any clinical investigations or scientific research that has been conducted for the purpose of treating liver fibrosis. It is also essential to keep in mind that this subject is persistently developing, and as a result, additional research might have been conducted since that time (8). Taking this into consideration, the species of *Z. mauritiana* might be beneficial for the treatment of chronic liver diseases such liver fibrosis. Because of its antioxidant characteristics, the leaf extract of *Z. mauritiana* has been shown to be effective in treating liver fibrosis in animal tests. In the proposed research, the hepatoprotective activity of ZMLE is evaluated in relation to chemical-induced liver fibrosis.

Chemicals and reagents

Olive oil was obtained from the Indian company MP Biomedicals. Serum biochemical analysis kits were acquired from Span Diagnostic LTD, Sachin, Gujarat, India. The quantification of proinflammatory cytokines was conducted in accordance with the manufacturer's guidelines using commercially available reagents (R&D systems, Minneapolis, MN).

Plant source and preparation of extract

Z. mauritiana (Lamk.) leaves were collected from the wild regions of the Meerut district in Uttar Pradesh, India, and verified by Dr. Vijai Malik, Professor, Department of Botany, CCS University, at Meerut, Uttar Pradesh, India. A voucher specimen Bot/730/13-5-2022 containing a plant sample has been preserved at CCS University in Meerut, Uttar Pradesh, India.

Preparation of ZMLE

The leaves had been washed and dried in the air in a shaded environment at room temperature until they reached a consistent weight. The dried materials were later pulverized by using a laboratory size mill and blender. ZMLE dry and powdered leaves (250g) underwent six successive percolations using a mixture of ethanol and water (8:2) at ambient temperature. The extracts underwent filtration using Whatmann paper, followed by centrifugation (10000×g, 4°C, 10 min), and were subsequently concentrated under reduced pressure using a rotary evaporator at 40±5°C. In the end, the extract was vacuum-dried in a desiccator and refrigerated at 4°C until use. The entire process yielded an extract (ZMLE) at a rate of 09-13% (w/w) concerning the dried starting material.

Preliminary Phytochemical Screening

On preliminary phytochemical evaluation of ZMLE using the reported method, the ZMLE showed positive tests for Cardiac glycosides, Coumarins, Emodins, Phenol/Polyphenols, Quinones, Saponins, Tannins and Terpenoids, while negative results for Alkaloids, Phlobatannins, Steroids, flavonoids.

Determination of the Total Phenolic Content

The determination of the total phenolic content (TPC) in the extracts was carried out employing Folin reagent (9). To summarize, 0.3 mL of the extract was combined with 1.5 mL of diluted Folin–Ciocalteu reagent (1:10) and 1.2 mL of 7.5% (w/v) Na_2CO_3. Following a 1-hour incubation at room temperature in darkness, the absorbance of the mixture was measured at 765 nm over a blank. The Total Phenolic Contents (TPCs) were assessed utilizing a standard curve generated with gallic acid and were reported as gallic acid equivalents (GAE) in mg per gram of the extract.

Determination of the Total Flavonoid Content

The estimation of total flavonoid content (TFC) in the extracts was evaluated employing the aluminum chloride colorimetric technique(10). To summarize, a mixture 0.1 mL of 1 M potassium acetate, 1.5 mL of methanol, 2.8 mL of distilled water, and 0.1 mL of aluminum chloride (10%) were mixed into a volume of 0.5 mL of extracted solution. After an incubation period of 30 minutes at room temperature, the absorbance of the reaction solution was obtained at 415 nm. Quercetin served as the reference standard, and the outcomes were presented as quercetin equivalents (QE) in milligrams per gram of the extract.

Antioxidant Activity

DPPH Radical Scavenging Capacity

To summarize, experimental procedure involved adding 0.1 mL of ZMLE at various concentration to 2.9 mL of a 0.002% methanolic DPPH solution. The reaction mixtures were allowed to incubate for 30 minutes at room temperature, and the absorbance at 517 nm was subsequently obtained against a blank (11). Ascorbic acid served as the standard. The capacity to neutralize the $DPPH^{\cdot}$ radical was computed as follows:

$$\% \text{ of antioxidant activity} = [(Ac - As) \div Ac] \times 100$$

where: 'Ac' represents the absorbance of the negative control at the time of solution preparation.; and 'As' signifies the absorbance of the sample after 30 minutes.

ABTS Radical Scavenging Capacity

The assessment of ABTS radical scavenging involved measuring the discoloration of the free radical $ABTS^+$ (12), with certain modifications. $ABTS^+$ was generated by combining a 7 mM ABTS stock solution in water with an equal amount of a 2.45 mM potassium persulfate solution. The mixture was left to stand for 12–16 hours at room temperature in the dark until the reaction reached completion and the absorbance became stable. The $ABTS^+$ solution underwent dilution to attain an initial absorbance of 0.70 ± 0.02 at 734 nm within a phosphate-buffered saline solutionwith a pH of 7.4. Subsequently, 1 mL of the ABTS+ solution was introduced to the test samples, and the absorbance was measured 6 minutes after mixing. The percentage of inhibition was calculated as follows:

$$\%\text{inhibition} = [(A_{\text{control}} - A_{\text{sample}})/A_{\text{control}}] \times 100$$

A_{control} = indicates the negative control's absorbance during solution formation;

A_{sample} = denotes the absorbance of the sample after a 6-minute duration.

Animals

The evaluation of hepatoprotective activity involved albino rats of either sex, with a weight range of 110-145 g. These rats were obtained from the central animal house facility of TIPER, Meerut. The rats were housed under a 12-hour light/dark cycle at a controlled temperature of 25±2°C. The rats were provided with unrestricted access to a standard pellet diet (Amrut Laboratory Rat Feed, Pune, India) and water *ad libitum*. The research received approval from the ethics committee CPCSEA, with reference letter no IAEC/PH-22/TIPER/170, and adherence to ethical norms was rigorously maintained throughout all experimental procedures.

Acute toxicity studies

ZMLE was orally administered to the female rats after an overnight fasting period using an oral gavage, with a dosage of 10 ml/kg body weight. The animals were divided into three groups, each consisting of three rats, as mentioned earlier (13). The initial dose of ZMLE at 50 mg/kg was administered to group 1. All rats were closely

monitored for behavioral changes, toxicity, and mortality during the first 4 hours and subsequently up to 48 hours. Group 2 received the next higher dose of ZMLE, 2000 mg/kg body weight, at 48-hour intervals when no signs of toxicity or mortality were observed in group 1. Meanwhile, group 3 served as the negative control group, receiving vehicle treatment in accordance with the OECD guideline. Continuous observations were made every 30 minutes up to 4 hours, followed by monitoring up to 24 hours after administration, and then once daily for a total duration of 14 days. The observation aimed to evaluate the onset of toxic symptoms, encompassing changes in skin and fur, eyes, mucous membranes, and behavioral alterations, which were meticulously documented. Additionally, the animals were monitored for signs of convulsions, tremors, diarrhea, salivation, lethargy, sleep, coma, and mortality. Daily records were maintained for food consumption and water intake. The body weights of the animals were documented on a weekly basis, and the percentage of body weight change was calculated using the provided equation.

$$\%\ Body\ weight\ change = \frac{Body\ weight\ at\ the\ end\ of\ each\ week - \ Initial\ body\ weight}{Initial\ body\ weight} \times 100$$

Drugs and dosing schedule

The animals were categorized into six distinct groups: group I (Control treatment), group II (Disease control treatment), 2NP, group III (Standard treatment; Silymarin), group IV (ZMLE-200), V (ZMLE-400) and VI (ZMLE-800). Animals in group I received distilled water at a volume of 10 mL/kg body weight. Animals in groups II, III, IV, V, and VI were subjected to 2-NP administration at a dose of 100 mg/kg body weight twice weekly via the intraperitoneal (*i.p.*) route for a duration of 12 weeks, dissolved in olive oil and administered subcutaneously. Concurrently, but at different times of the day, animals in group III were orally administered with silymarin suspension (50 mg/kg body weight) once daily for the entire 12-week period. Animals in groups IV, V, and VI were orally administered with ethanolic extract at doses of 200 mg/kg, 400 mg/kg, and 800 mg/kg body weight, respectively.

Collection of Blood Samples

Blood samples were obtained through the retro-orbital venous plexus puncture method without anticoagulant administration under mild anesthesia. The blood sample collected at the end of study period was for the estimation of all biochemical parameters. All animals from various groups were euthanized 24 hours after the last dose through cervical dislocation. Liver tissue was extracted, weighed, cleaned with ice-cold saline, and dried by blotting with filter paper. The largest liver lobe was preserved in a 10% formaldehyde solution for histopathological examination. The remaining liver tissue was stored at -80°C and later used for evaluating tissue biochemical parameters.

Estimation of Biochemical Parameters

Liver function test

The evaluation of hepatic parameters, including AST, ALT, ALP, and total bilirubin, was conducted using kits obtained from Span Diagnostic Ltd, Sachin, Gujarat, India.

Preparation of Cytosolic and Microsomal Fraction

The frozen liver tissue samples stored at -80°C from different experimental rat groups were thawed, quickly blotted, and weighed. Liver tissues were individually homogenized with ice-cold 0.1 N KCl (pH-7.4) in a pre-cooled Teflon-coated glass homogenizer for several minutes, resulting in a 10% w/v tissue homogenate. The liver homogenate underwent centrifugation at 8000×g for 15 minutes (at 4°C), and the resulting supernatant was subjected to ultracentrifugation (WX-Ultra 90, T-890 rotor, Thermoscientific, USA) at 78,000×g for 90 minutes. The obtained supernatant served as the cytosolic fraction, and a portion of it underwent additional ultracentrifugation at 1,05,000×g for 1 hour to isolate microsomes (precipitate). The cytosolic fraction was utilized to determine SOD, CAT, GPx, and reduced GSH, while the microsomal fraction was used to assay UDP-glucuronosyltransferases (UDPGT) activity, CYP-450 content, and lipid peroxidation. (14)

The estimation of membrane-bound enzymes, including CYP-450, was carried out using the procedure detailed by Yuan Xie et al. UDPGT activity towards p-nitrophenol was determined through the established method described by Black in 1994. GST activity was assessed according to the protocol introduced by Hossain et al, with certain modifications aligned with the GST assay by Habig et al.(15). The determination of Na^+-K^+-ATPase activity was conducted using two reaction mixtures, following the method reported by Hossain et al. in 2013 and as outlined by Pradip K. Sarkar(16). The estimation of Ca^{2+}-ATPase in the plasma membranes was conducted using the method reported by Lotersztajn. SOD and CAT levels were measured in liver tissue homogenate following the methodology described by Marklund et al.(17) and Sinha (18) respectively. GPx by Rotruck et al.(19), and total GSH by Moron et al.(20). In liver tissue homogenates, the calculation of lipid peroxidation (LPO) was performed using thiobarbituric acid reactive substances (TBARS), following the method outlined by Ohkawa et al.(21).

Estimation of Proinflammatory Cytokines

The proinflammatory cytokines including interleukin-1β(IL-1β), tumor necrosis factor-α (TNF-α), IL-6 and IL-10, and profibrotic cytokines (TGF-β) were measured by commercially available kits (R&D systems, Minneapolis, MN) as per the manufacturer's instruction with enzyme-linked immunosorbent assay and their amount represented as pg/mg protein.

Determination of Hydroxyproline in Liver Tissue

The liver tissue was removed, weight of 30–100 mg and homogenized in 5% trichloroacetic acid solution (x10 volume) using a cell homogenizer at 8,000×g (4°C) for 2 min in an ice bath. Cells were centrifuged (2,500 × g, 4°C) for 20 min and the supernatant washed twice with distilled water. Then, 6N HCl was added at 110°C completely at the beginning and reacted for 16 h. Following completion of the reaction, toluene (3 ml) was added, and the mixture was agitated for 20 min. Following centrifugation (3,000×g, 20°C) for 10 min, the organic layer was collected and p-dimethylaminobenzaldehyde added. Hydroxyproline in the sample was detected by spectrophotometrically at 565 nm using a colorimetric analyser (22).

The formula for content calculation was as follows:

Hydroxyproline content (μg/mg wet weight) = (measured OD value − blank OD value)/ (standard OD value-blank OD value) × standard concentration (5 μg/mL) × total hydrolysate volume (10 mL/tissue wet weight (mg)

Histopathological studies

For histological examination, liver tissue was fixed in 10% buffered formalin. Standard laboratory technique is used for liver histology. After fixing the liver tissue in formalin for 48h, the tissues were washed with tap water for about 4h. The tissues were dehydrated by soaking for 1h each in 70%, 80% and 100% alcohol followed by soaking in xylene for about 30 minutes. The tissue was finally soaked overnight in hot liquid paraffin and was proceeded to form blocks. The block was cooled at -20ºC for 1h, sectioned to 5 μm using a microtome (Leica, Bensheim, Germany), and dried in a hot air oven. The sections were deparaffinized with excess xylene and rehydrated with 100%, 95%, 80%, and deionized water. Before haematoxylin staining, the slides are dried. Slides were kept in haematoxylin solution for 3 minutes, then rinsed for 5 minutes. The slides were then soaked in acid ethanol and washed with water. Excess water was blotted from the slide. The slides were stained with eosin for 30-45 seconds, dehydrated with 95% and 100% ethanol, and then treated with xylene for 15 minutes. After staining, the sections were mounted by DPX, covered using a cover slip and observed under light microscope using Zeiss microscope (Axioplan 2 Imaging, Axiovision software). The degree of hepatic damage was assessed by examining hepatocyte structure, portal vein, hepatic and bile ducts, and inflammatory cell infiltration.

Statistical analysis

The results were expressed as mean ±SEM; n=6 animals in each group; $^{*}P<0.05$: Statistically significant, $^{**}P<0.01$: Statistically very significant and *** $P<0.001$: Statistically highly significant from normal control. $^{\#}$ $P<0.05$: Statistically significant, $^{\#\#}$ $P<0.01$: Statistically very significant and $^{\#\#\#}$ $P<0.001$ Statistically highly significant from disease control. Statistical analysis was carried out using Graph Pad Prism software (version 8.1). One way

ANOVA or two-way ANOVA was used, followed by Bonferroni multiple comparison tests. Normal control groups and disease control groups were compared with other.

2. RESULTS AND DISCUSSION

Phytochemical analysis enables us to comprehend the chemical composition of plants and harness their advantageous properties in the fields of health, nutrition, agriculture, and environmental science. It promotes progress in numerous scientific disciplines and enhances human well-being.

The *Z.Mauritiana* leaves were extracted using a mixture of ethanol and water (8:2) at room temperature. The extraction process yielded 09-13% (w/w) of the extract (ZMLE) based on the weight of the dried starting material. The ZMLE were subjected to qualitative preliminary screening for phytoconstituents, which revealed the presence of several phytoconstituents. The ZMLE was subjected to preliminary phytochemical screening using the established method. The ZMLE showed positive results for cardiac glycosides, Coumarins, Emodins, Phenol/Polyphenols, Quinones, Saponins, Tannins, and Terpenoids. The results were negative for alkaloids, phenols, steroids, and flavonoids (Table 1).

Table 1. Phytochemical analysis of ZMLE Lam. Leaves

Metabolites	Observation	Results
Alkaloids	No Brownish-red precipitate formed	Negative
Phlobatannins	No red precipitate Formed	Negative
Cardiac glycosides	Brown ring formed at interphase	Positive
Coumarins	Yellow coloration formed	Positive
Emodins	Yellow coloured precipitate formed	Positive
Phenol/Polyphenols	Blue green to dark blue coloration formed	Positive
Quinones	Formation of red coloration observed	Positive
Saponins	Formation of continues effervescence observed	Positive
Steroids	No dark green coloration observed	Negative
Tannins	Dark blue or greenish grey coloration observed	Positive
Terpenoids	Reddish brown colour formed at interface	Positive

The measurement of the overall amount of phenolic and flavonoid compounds is a highly effective analytical method that has diverse uses in assessing the antioxidant capacity, health benefits, and purity of compounds produced from plants. This method has a significant impact on various scientific areas and industries (23).

The extraction methods and solvents are accountable for dissolving the naturally occurring components of the plants. Furthermore, plant components can exhibit either polar or non-polar characteristics. Phenolic compounds exhibit enhanced solubility in polar organic solvents because of the hydroxyl group present. Consequently, hydroethanolic was chosen as the solvent for extraction. The hydroxyl groups present in plant extracts play a crucial role in helping the process of scavenging free radicals. Phenolic content in each extract was tested using the Folin–Ciocalteu reagent as a reference. The data were obtained from a calibration curve ($y = 0.0379x - 0.0349$, $R^2 = 0.9975$) of gallic acid (0–30 µg/mL) (Figure. 1A) and reported as gallic acid equivalents (GAE) per gramme of dry extract weight (Table 2). The ZMLE have a phenolic component concentration of 307.14± 9.8 mg GAE/g.

Flavonoids are a type of secondary metabolite that possesses antioxidant properties. The effectiveness of their antioxidant activity is determined by the quantity and arrangement of unbound hydroxyl (OH) groups. The flavonoid concentrations in selected plant extracts were quantitatively measured using the colorimetric technique with aluminium chloride. The results were obtained from the calibration curve ($y = 0.0411x - 0.0332$, $R^2 = 0.9978$)

of quercetin (0–30 µg/mL) (Figure. 1B) and were represented in quercetin equivalents (QE) per gramme of dry extract weight (Table 2). The ZMLE have a flavonoid concentration of 132.62 ± 6.00 mg QE/g.

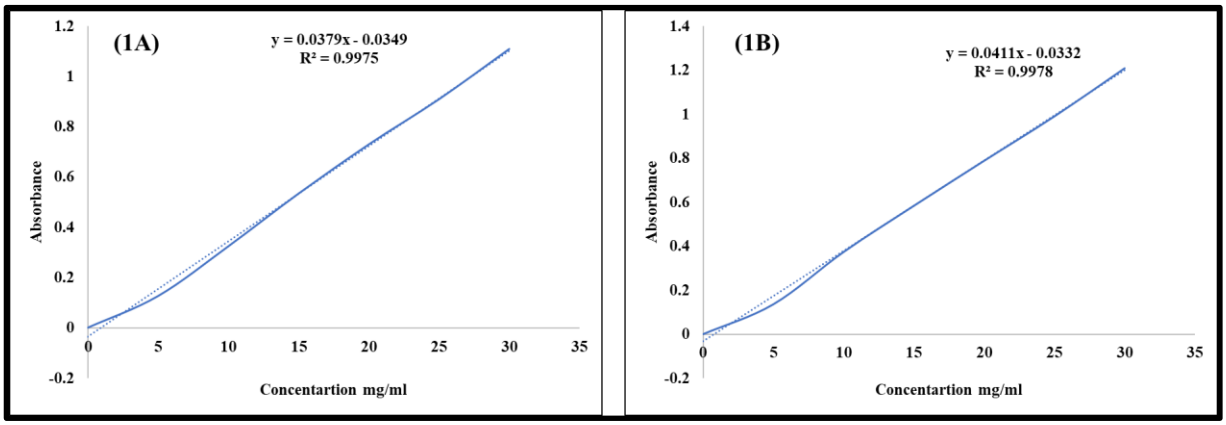

Figure 1. A. Calibration curve of gallic acid. B. Calibration curve of Catechin. Each point represents the mean of three experiments.

Table. 2. Total phenolic and flavonoids content in ZMLE Lam. leaves.

Total phenolic mg GAE/g extract	Total flavonoids mg Catechin/g extract
307.14± 9.8	132.62 ± 6.00

Figure 2A shows, ZMLE DPPH radical scavenging. As the concentration of ZMLE increased, their radical-scavenging activity increased. The most effective DPPH radical scavenging was seen at 1000 µg/ml concentration. An electron or free radical entity reduces the absorption spectrum range of DPPH, an organic radical, from 515–528 nm. A simple, widely used method for testing plant extracts' radical-scavenging capacity is the DPPH test. Plant antioxidants neutralise the stable purple DPPH radical, turning it yellow (23).

Figure 2A compares BTH and ABTS+'s radical-scavenging capabilities. The stable ABTS radical cation was produced using potassium persulfate. After constant absorbance, antioxidant plant extract was added to the reaction medium, and decolorization was used to assess antioxidant potency. At a dosage of 1000 µg/ml, the ABTS radical scavenging observed was comparable to the DPPH assay. The results matched literature. The aqueous ethanolic extract (80%) of ZMLE leaves showed antiradical and antioxidant activity (ABTS and DPPH) linked with polyphenolic components (24).

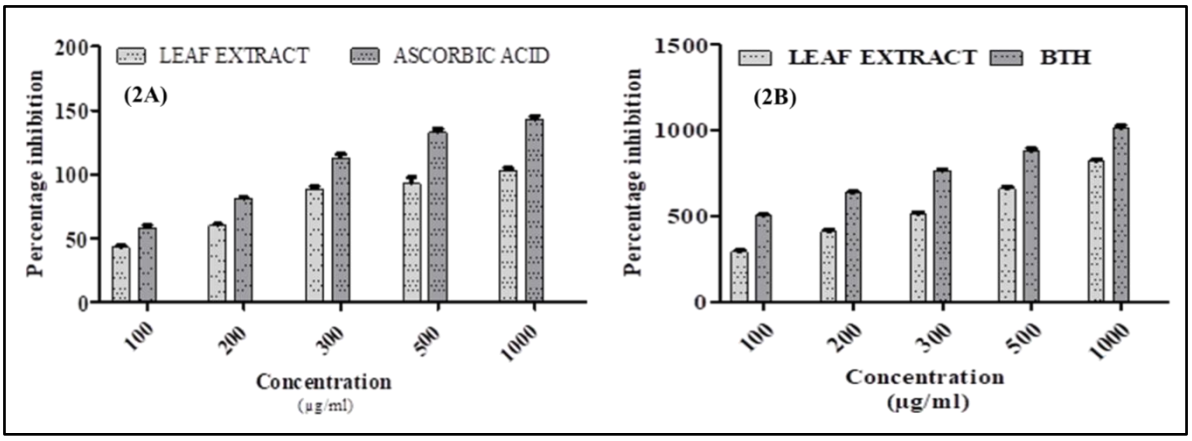

Figure. 2. A. DPPH Assay; B. ABTS assay of ZMLE.

No mortality was observed in rats subjected to the acute toxicity test, following the OECD guideline, with oral administration of ZMLE at a dose range of 50 to 2000 mg/kg. No fatalities were observed for the whole duration

of the short and long-term monitoring period. There were no indications of toxicity in the animals for the entire 14-day trial period. General observations and behavioural analysis can be seen in Table 3. Consequently, the extract is likely to be safe when administered at these quantities, and the lethal dose that causes death in 50% of rats when taken orally is estimated to be more than 2000 mg/kg.

The ZMLE extracts underwent a 14-day acute toxicity study, which revealed no instances of mortality or behavioural and motor-neuronal abnormalities in rats. Owolarafe et al. found that prolonged administration of an aqueous extract of ZMLE leaves can have significant adverse effects on the biological system. This conclusion contradicts the findings presented here, as well as the findings reported by Ramar et al., which indicated that the methanol extract of *Z. mauritana* Lam leaves does not induce any signs of toxicity or mortality in an acute toxicity trial (25,26) .

Table 3. General observation and behavioural analysis

Observations	**Control**		**ZMLE (50 mg/kg)**		**ZMLE (2000 mg/kg)**	
	4h	**24h**	**4h**	**24h**	**4h**	**24h**
Eyes	No Change	No Change	No Change	No Change	No Change	No Change
Skin and fur	No Change	No Change	No Change	No Change	No Change	No Change
Lethargy	Not Observed	Not Observed	Not Observed	Not Observed	Not Observed	Not Observed
Sleep	Normal	Normal	Normal	Normal	Normal	Normal
Diarrhea	Not Observed	Not Observed	Not Observed	Not Observed	Not Observed	Not Observed
Coma	Not Observed	Not Observed	Not Observed	Not Observed	Not Observed	Not Observed
Tremors	Not Observed	Not Observed	Not Observed	Not Observed	Not Observed	Not Observed
Mucous membrane	No Change	No Change	No Change	No Change	No Change	No Change
Behavioural patterns	Normal	Normal	Normal	Normal	Normal	Normal
Salivation	Not Observed	Not Observed	Not Observed	Not Observed	Not Observed	Not Observed

Throughout the study, the rats in the treatment group did not display any notable alterations in their body weight that could be deemed statistically significant in comparison to the rats in the control group, as indicated in Table 4. During the trial, the rats consistently and continuously consumed both feed and water, however the specific results for this experiment are not provided here.

When studying the toxicity and safety of a natural product, it is essential to monitor the body weight and food/water intake of the experimental animals. This information helps understand their physiological and metabolic condition and ensures that any conclusions drawn by the researcher are not influenced by abnormal nutritional status in the rats. The weight growth exhibited by each rat in the present investigation was comparable and adhered to a general pattern. Given that none of the experimental groups exhibited either weight loss or weight gain, it may be inferred that the ZMLE extracts did not have any negative effects on the rats' overall health or metabolic development. Furthermore, the extracts did not induce any noticeable alterations in appetite.

Group	0 Day	Week 1	Week 2	% Change Week 1	% Change Week 2
Control	214.3 ± 3.12	219.0 ± 2.18	233.7 ± 2.68	4.7 ± 1.51	7.56 ± 1.71
ZMLE (50 mg/kg)	223.2 ± 1.32	236.2 ± 2.27	247.4 ± 2.47	4.00 ± 0.95	8.18 ± 1.83
ZMLE (2000 mg/kg)	224.4 ± 3.51	236.0 ± 3.29	246.0 ± 3.43	4.35 ± 1.70	8.26 ± 1.90

Furthermore, it was noted that the rats' consumption of food and water exhibited a steady and predictable pattern during the entire duration of the experiment. The administration of ZMLE did not significantly alter the pattern of body weight and feed consumption, suggesting that the plant extracts had no adverse effects on the rats' growth and development (27).

Table 4. Body weight and percentage change in body weight at 0 day, 1 and 2 week in acute oral toxicity

Data were expressed as mean±SEM (n=3). The P<0.05 was considered as significant (*), p<0.01 considered as very significant (**), p<0.001 considered as highly significant (***), and p>0.05 considered as non-significant (NS).

Table 5 presents the effects of a single oral dose of ZMLE (50 and 200 mg/kg) on the haematological parameters of rats after administration. No statistically significant changes were seen in the analysed parameters as compared to the control groups. Assessing the haematological parameters is essential as it can promptly reveal the systemic effects caused by the administered extract. Based on the results, there was no significant difference (> 0.05) observed in the haematological profiles of the treated rats compared to the control group.

This toxicity study also incorporates some noteworthy biochemical indicators. The kidney and liver are the primary organs susceptible to the deleterious impacts of medicines. Renal function was assessed by measuring serum creatinine, urea, and total protein levels, while liver function was evaluated by measuring AST and ALT levels. The experiment's results indicated that there were no alterations in the kidney or liver functions of the female rats that received treatment. There were no statistically significant changes in the levels of creatinine, urea, total protein, AST, or ALT between the treatment and control groups. Consequently, these findings suggest that ZMLE did not have any detrimental effect on the liver and kidneys of the rats.

Table 5. Haematological and Biochemical parameters of ZMLE Lam in acute oral toxicity

Parameter/Group	Control	ZMLE (50 mg/kg)	ZMLE (2000 mg/kg)
CBC			
HGB	134.7 ± 1.02	144.2 ± 2.087	144.7 ± 1.028
WBC	7.117 ± 0.093	7.290 ± 0.081	7.253 ± 0.118
Differential count			
Neutrophil	0.62 ± 0.03	0.67 ± 0.02	0.66 ± 0.01
Lymphocyte	5.20 ± 0.08	5.16 ± 0.13	5.27 ± 0.13
Monocyte	0.13 ± 0.00	0.1400 ± 0.00	0.1600 ± 0.01
Eosinophil	0.08 ± 0.00	0.27 ± 0.16	0.09 ± 0.00
Basophil	0.02 ± 0.00	0.02 ± 0.00	0.03 ± 0.00
Renal function test			
Urea	3.10 ± 0.10	3.28 ± 0.26	3.70 ± 0.30
Creatinine	30.67 ± 1.8	31.67 ± 1.4	29.68 ± 0.8
Liver function test			

Albumin	32.67 ± 1.45	38.00 ± 0.73	32.6 ± 2.33
Globulin	24.62 ± 0.66	23.00 ± 0.57	23.00 ± 0.32
Total bilirubin	1.067± 0.06	0.96 ± 0.02	1.03 ± 0.03
Conjugate bilirubin	1	1	1
Alkaline phosphatase (ALP)	161.3 ± 0.26	163.3 ± 1.2	162.0 ± 1.15
Alanine Aminotransferase (ALT)	26.00 ± 1.52	25.67 ± 1.20	26.34± 1.45
Aspartate aminotransferase (AST)	92.00 ± 1.1	93.33 ± 1.32	93.67 ± 1.20

Data were expressed as mean±SEM (n=3). The P<0.05 was considered as significant (*), p<0.01 considered as very significant (**), p<0.001 considered as highly significant (***), and p>0.05 considered as non-significant (^{NS}).

Upon microscopic examination, the tissue sections of rats treated with ZMLE did not exhibit any lesions or aberrant histological alterations when compared to their respective control groups (figure 3). The organs exhibited a normal texture and appearance upon physical inspection. The rats treated with ZMLE extracts did not exhibit any significant changes in the relative organ weight index compared to their respective control groups. However, the data is not shown. Histopathological examinations were performed on the brain, heart, kidney, liver, and lungs of all the rats. No evidence of necropsy or aberrant morphological alterations was observed during the gross examination of the organs. The microscopic analysis of the hematoxylin eosin-stained tissue sections revealed minimal alterations in comparison to the control rats tissues.

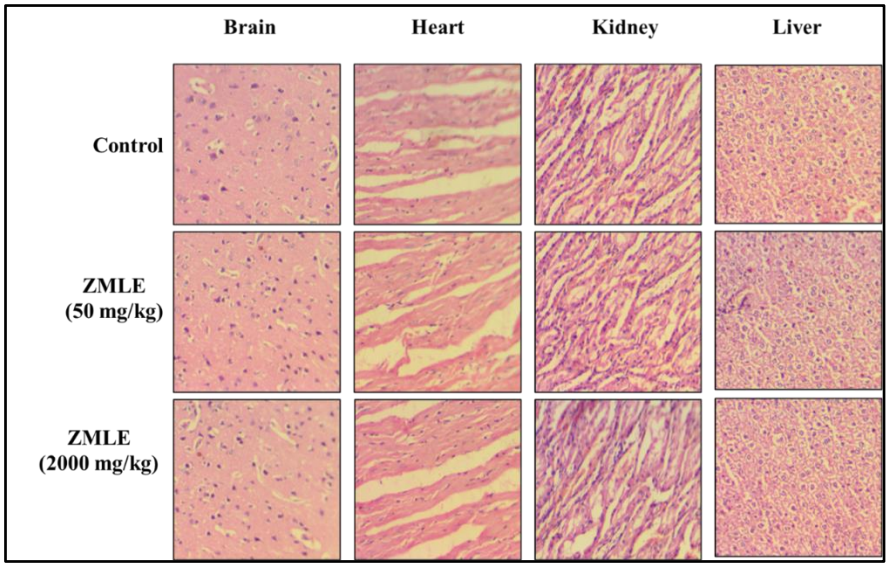

Figure 3. Histopathological examination of various organs of the rat in acute oral toxicity study; Brain, heart, kidney, liver, and lungs of the control group and treated animals; All organs show normal architecture with no sign of toxicity. (Haematoxylin & Eosin, (H&E) ×100).

Liver fibrosis is a severe clinical disorder that results in substantial morbidity and mortality. Hepatic fibrosis is a frequent consequence of long-term liver damage, ultimately resulting in cirrhosis and its associated consequences, such as portal hypertension, liver failure, and hepatocellular cancer (28). 2-NP has been adopted as a suitable model to generate liver toxicity in order to evaluate the efficacy of diphenyl diselenide in mitigating liver damage (29). This is because 2-NP, a chemical known to be harmful to the liver, may cause fibrosis in rats that closely resembles the permanent fibrosis seen in humans.

Administration of 2-NP led to elevated blood AST and ALT activity, as well as increased levels of liver TNF-α, IL-6, and MDA, along with increased production of vascular endothelial growth factor (VEGF) and caspase-3. In contrast, the levels of glutathione (GSH) contents and superoxide dismutase (SOD) activity were decreased (30).

Table 6 demonstrates the impact of 2-NP and ZMLE on both enzymatic and non-enzymatic indicators of liver injury in this research. Rats exposed to 2-NP for 12 weeks exhibited a notable increase ($p<0.05$) in the levels of AST, ALT, ALP, and TB. The administration of ZMLE at doses of 200, 400, or 800mg/kg bw in rats resulted in a significant ($p<0.05$) dose-dependent reduction in levels of AST, ALT, ALP, and TB. Conversely, the administration of 2-NP led to a significant ($p<0.05$) increase in levels of AST, ALT, ALP, and TB, indicating its hepatotoxic potential. Silymarin 50mg/kg as standard produce very significant effect to ameliorate the effect of 2-NP on the AST, ALT, ALP, and TB level.

The repeated treatment of 2-NP resulted in significant hepatocellular damage in rats. The measurement of serum bilirubin levels and the assessment of AST, ALT, and ALP activities are the most reliable and accurate assays used to diagnose liver illnesses. The elevated concentration of ALP is a result of the compromised structural integrity of liver cells. This is since the enzyme alkaline phosphatase is situated in the cytoplasm and is discharged into the bloodstream following cellular injury.

If an injury affects organelles, such as mitochondria, the soluble enzymes, like AST, that are contained within these organelles will also be released, suggesting disruption to the cell membrane. Prior studies have demonstrated that when hepatocytes are exposed to 2-NP , it disrupts the structure and functioning of the cell membrane, leading to an increase in the leakage of AST. Bilirubin, a naturally occurring chemical anion, forms a reversible bond with albumin and is then transferred to the liver. In the liver, it undergoes conjugation with glucuronic acid and is ultimately expelled in the bile. Hepatobiliary illness is diagnosed when the level of bilirubin fraction exceeds the normal range. Elevated levels of bilirubin in the bloodstream indicate abnormalities in the functioning of the liver. Hyperbilirubinemia is a highly sensitive and valuable test for assessing the liver's functional integrity and the severity of necrosis. The measurement quantifies the ability of hepatocytes to bind, conjugate, and excrete substances, which is directly linked to the rate at which erythrocytes are broken down. An elevated total bilirubin level indicates the severity of jaundice, whereas increased aminotransferases and alkaline phosphatase clearly indicate cellular leakage and loss of cellular function. Prior administration of ZMLE to rats, followed by alcohol consumption, led to a notable reduction in levels of AST, ALT, ALP, and TB. This reduction occurred in a dose-dependent manner, in comparison to rats treated with 2-NP. This suggests that the ZMLE may have shielded both the plasma membrane and liver cells from damage caused by 2-NP toxicity. As a result, the release of serum enzyme markers into the bloodstream was reduced. The reduced levels of the enzymes indicate a defence mechanism that protects the liver from the harmful effects of hepatotoxin. Additionally, it is plausible that administering the extract to rats along with 2-NP enhanced the rats antioxidant capacity when given with 2-NP. Literature has revealed that rats co-treated with methanol extract of ZMLE before exposure to CCl4 toxicity showed reduced levels of blood AST, ALT, ALP, and total bilirubin (31).

Table 6. Effect of 2-NP and ZMLE on both enzymatic and non-enzymatic indicators of liver injury.

Groups	ALT (IU/L)	AST(IU/L)	ALP(IU/L)	TB (mg/dl)
Control	58.94 ± 2.4	135.3 ± 3.3	152.8 ± 3.4	0.2 ± 0.01
2-NP	344.5 ± 6.7	328.4 ± 4.4	427.4 ± 6.8	6.8 ± 0.11
Silymarin-50	194.2 ± 4.5	172.1 ± 2.2	244.5 ± 6.4	2.4 ± 0.18
ZMLE-200	279.8 ± 2.5	236.4 ± 8.3	371.2 ± 6.4	4.7 ± 0.15
ZMLE-400	250.8 ± 3.6	189.5 ± 6.7	292.3 ± 4.7	3.9 ± 0.19
ZMLE-800	211.2 ± 4.0	164.6 ± 3.4	268.1 ± 3.3	3.2 ± 0.46

Table 7 displays the results of interventions on enzymes that are bound to membranes, such as CYP-450, UDPGT, Na+/K+-ATPase, and Ca+-ATPase.

At the completion of the 12-week period, there was a substantial decrease in the level of CYP-450 in rats treated with 2-NP compared to the control rats. The application of Silymarin resulted in a considerable ($P<0.01$) improvement in the level of tissue CYP-450, which had been reduced due to the administration of 2-NP. At the end of 12 weeks, the level of UDPGT in rats treated with 2-NP shows a considerable rise compared to normal control rats. Treatment with silymarin considerably ($P<0.01$) enhances the level of tissue UDPGT, which has been reduced due to the administration of 2-NP. Comparable outcomes were observed with the Na+/K+-ATPase and Ca+-ATPase. The treatment of rats with 200, 400, or 800 mg/kg bw of ZMLE led to a significant ($p<0.05$) dose-dependent increase in membrane-bound enzymes.

Hepatotoxicity is caused by the activation or inhibition of CYP-p450 enzymes by medications or substances. Cytochrome P-450 is an example of a supergene oxidase that contains heme. It aids in the elimination of foreign substances and endogenous molecules by liver cells during the process of drug metabolism. Research indicates that hepatotoxicity decreases cyt P-450 levels, hence impacting homeostasis (32). The investigation revealed that 2-NP exhibited a suppressive effect on the activity of the CYP-p450 enzyme. Nevertheless, administration of varying doses (200, 400, or 800mg/kg bw) of ZMLE resulted in a significant ($p<0.05$) enhancement in enzyme activity in rats, with the extent of improvement being dose dependent.

UDPGTs are enzymatic proteins that play a crucial role in the glucuronidation process. Glucuronidation is a phase II metabolic reaction that facilitates the detoxification and elimination of various endogenous and exogenous substances, such as drugs and toxins. The liver is the primary site for glucuronidation, a process in which UDP-glucuronic acid combines with molecules to enhance their water solubility and promote their elimination from the body. Hepatic injury might hinder the operation of UDPGT, resulting in a decrease in their enzymatic activity (32). In this study, it was revealed that the presence of 2-NPresulted in a decrease in the activity of UDPGT. This drop can be attributed to the development of oxidative stress. Nevertheless, upon administering various doses (200, 400, or 800mg/kg bw) of the ZMLE to rats, a significant ($p<0.05$) enhancement in enzyme activity was seen, with the extent of increase being dose dependent.

ATPases are membrane proteins that are dependent on thiol groups and phospholipids in order to maintain their structure and function. The peroxidation of membrane phospholipids not only alters the lipid environment as well as the structural and functional integrity of the cell membrane, but it also has an effect on the functioning of several enzymes that are positioned in the membrane, such as Na^+K^+-ATPase and Ca_2^+ ATPase (33). The Na^+K^+-ATPase pump helps cells maintain osmotic balance and membrane potential. The hepatotoxic effect of paracetamol was a reduction in plasma membrane Na+/K+-ATPase activity (34). This study found that 2-NP decreased Na^+/K^+-ATPase and Ca_2^+ ATPase activity. However, rats fed with 200, 400, or 800mg/kg bw ZMLE showed significant improvements in Na^+/K^+-ATPase and Ca_2^+ ATPase activity. This improvement was dose dependent. This shows that the ZMLE protects the Na^+K^+ and Ca_2^+ ATPase pump, maintaining hepatocyte osmotic balance and membrane potential. Lipid peroxidation affects membrane Ca_2^+-translocase, Ca_2^+-ATPases, and Mg_2^+-ATPases. Due to increased lipid peroxidative damage to cellular membranes, protein-SH depletion may also cause membrane-bound ATPases to function inadequately (35).

Table 7. Effect of 2-NP and ZMLE on membrane bound enzymes.

Groups	CYP-450 (nmol/mg protein)	UDPGT (µmol/mg protein)	Na+/K+-ATPase (mM of Pi liberated/mg protein)	Ca+-ATPase (mM of Pi liberated/mg protein)
Control	1.3 ± 0.11	3.3 ± 0.23	2.5 ± 0.20	1.2 ± 0.15
2-NP	0.4 ± 0.09	1.5 ± 0.09	1.0 ± 0.06	0.7 ± 0.05
Silymarin -50	1.1 ± 0.17	2.9 ± 0.13	2.2 ± 0.13	1.0 ± 0.14

ZMLE-200	0.8 ± 0.11	2.0 ± 0.15	1.5 ± 0.09	0.8 ± 0.03
ZMLE-400	1.0 ± 0.13	2.6 ± 0.19	1.8 ± 0.02	0.9 ± 0.07
ZMLE-800	1.1 ± 0.12	2.9 ± 0.23	2.3 ± 0.07	1.0 ± 0.11

Tissue damage was suggested by decreased SOD, CAT, GPx, and GSH and raised MDA, a biomarker for lipid peroxidation, in 2-NP-treated rats. Silymarin reduces SOD, CAT, GPx, and GSH while increasing MDA. In rats, ZMLE at different doses (200, 400, or 800mg/kg bw) significantly increased SOD, CAT, GPx, and reduced GSH levels, while increasing MDA levels in a dose-dependent manner ($p<0.05$). SOD and CAT enzymes regulate cellular homeostasis and protect against oxidative stress as scavengers. The SOD enzyme transforms superoxide anion ($O2^{-}$) into oxygen (O_2) and hydrogen peroxide (H_2O_2). Additionally, the ubiquitous CAT enzyme neutralises hydrogen peroxide to generate water and oxygen. Like CAT, GPx eliminates H_2O_2.Fibrotic rats have impaired enzymatic antioxidant capacities, indicating unregulated use of ROS created by chemical alteration of 2-NP. LPO (lipid peroxidation) of unsaturated fatty acids is also used to confirm oxidative damage caused by oxygen-lipid interaction. This yields reactive intermediates and partially stable peroxides such malondialdehyde. MDA levels in fibrosis rats indicate increased lipid peroxidation, which damages the liver and impairs oxidative stress protection. Experimental studies on mice with liver injury showed an increase in LPO levels. Oral treatment of ZMLE to rats significantly improved enzymatic antioxidant function. This repair shows the extract has ability to eliminate ROS and prevent 2-NP-induced oxidative stress damage.

The administration of 2-NP resulted in the generation of reactive oxygen species (ROS) and oxidative stress, leading to a decrease in glutathione (GSH) levels and subsequent liver damage. The main function of GSH is to combat oxidative stress and lipid peroxidation. Oral administration of the ZMLE resulted in an elevation of reduced glutathione (GSH) levels in rats, as compared to 2-NP. The test substances effectively elevated the level to a state close to normal, so demonstrating their ability to mitigate oxidative stress generated by 2-NP.

Table 8. Effect of 2-NP and ZMLE on enzymatic and non-enzymatic oxidative stress markers.

Groups	SOD (U/mg protein)	CAT (U/mg protein)	GPx (nmol/min/mg protein)	Reduced GSH (nmol/mg protein)	MDA (nmol/mg protein)
Control	109.1 ± 3.1	34.5 ± 2.3	74.7 ± 2.0	11.2 ± 0.5	7.2 ± 0.3
2-NP	26.4 ± 3.4	12.2 ± 0.6	28.5 ± 1.4	4.8 ± 0.2	15.0 ± 0.4
Silymarin-50	87.1 ± 3.4	26.6 ± 1.7	78.9 ± 2.1	11.1 ± 0.4	7.8 ± 0.3
ZMLE-200	44.8 ± 3.4	18.7 ± 1.2	37.7 ± 4.2	7.5 ± 0.2	12.2 ± 0.5
ZMLE-400	66.6 ± 2.7	21.7 ± 0.7	48.5 ± 2.8	8.5 ± 0.1	10.8 ± 0.3
ZMLE-800	81.4 ± 2.8	25.4 ± 1.6	69.7 ± 2.3	9.4 ± 0.5	8.5 ± 0.6

High oxidative stress and inflammation are linked. TGF-β1, TNF-α, IL-6, IL-1β, and IL-10 were examined as significant plasma inflammatory cytokines in this investigation and shown in figure 4. Inflammatory cytokines in 2-NP group are much higher than in control rats. After 84 days, 2-NP increases liver damage and inflammatory cytokines. Compared to the control group, 2-NP significantly increased ($P<0.01$) inflammatory cytokines like TGF-β1, TNF-α, IL-1β, and IL-6 while lowering IL-10 levels. This suggests 2-NP caused severe liver inflammation. Oral treatment with ZMLE significantly ($P<0.01$) decreased proinflammatory component

synthesis, which was increased by 2-NP administration. Oral ZMLE reduced 2-NP-induced inflammation. The latest study confirms earlier findings.

TNF-β1 and TGF-α levels significantly elevated after 2-NP injection (P<0.01). However, silymarin effectively decreased them. Intervention led to significant IL-6 reductions (P<0.01) and IL-10 improvement (P<0.01). When compared to 2-NP, silymarin reduces inflammatory mediators. ZMLE may reduce 2-NP-induced liver fibrosis. Anti-inflammatory properties may explain this change.

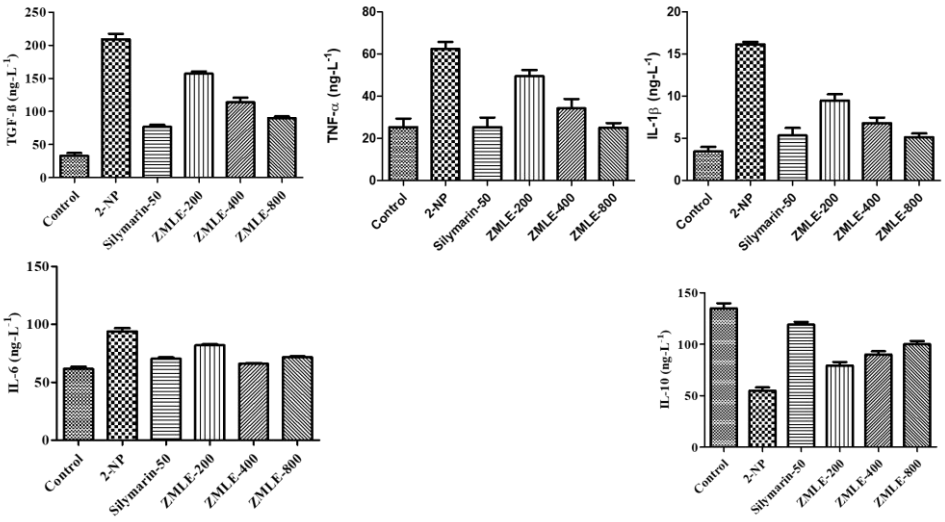

Figure 4. Effect of 2-NP and ZMLE on inflammatory markers.

Due to excessive collagen-based extracellular matrix (ECM), the liver might generate fibrous tissue. The advancement of liver fibrosis depends on collagen buildup. We then measured hepatic hydroxyproline, a marker of collagen synthesis in liver fibrosis rats, to assess collagen expression. Hepatic hydroxyproline levels increased significantly compared to the control group, suggesting excessive liver collagen production. Figure 5 shows liver tissue hydroxyproline concentration.

Collagen contains hydroxyproline in abundance. Hydroxylation of proline creates it. Hydroxyproline in activated hepatic stellate cells (HSCs)' extracellular matrix (ECM) protects liver cells' structure and function. The concentration of this factor in liver tissues is critical for determining liver fibrogenesis rates and progression. Quantifying hydroxyproline in research projects is a diagnostic indicator or anti-fibrotic activity monitor. Hydroxyproline reduction by ZMLE reduces 2-NP-induced hepatic fibrosis.

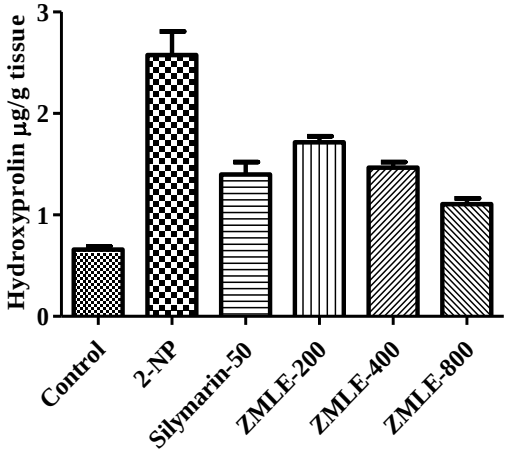

Figure 5: The estimation of hydroxyproline content at the end of the 12 weeks as an indicator of indirect collagen level estimation

Histological analysis of livers from different treatment groups was performed after 12 weeks. Control rats' livers (Fig. 6A) were normal when stained with hematoxylin and eosin under a microscope. Liver connective tissue clearly splits hepatic parenchyma into functional lobules. Hepatocyte configuration in the liver lobule, notably in the middle and central sections, was unclear. Liver cells form plates from the portal to the central vein. There was no liver necrosis, steatosis, inflammation, or fibrosis. From a small central vein, sinusoidal capillaries appear to emerge between liver cell layers. Between the sinusoidal lining cells and hepatocytes are Küpffer, hepatic stellate, and pit cells in Disse. It was harder to find portal areas—small arteries, veins, bile ducts, and lymphatic vessels. Rat livers treated with 2-NP (Fig. 6B) showed substantial liver damage. Tissue damage with cell death in some liver locations, aberrant formations within dead liver cells, swelling and thickening of blood vessels, and severe inflammation and scarring surrounding the liver were signs of these abnormalities. Abnormal liver cell vacuoles, clusters of enlarged immune and scavenger cells, larger fat-storing liver cells, and liver cell size, shape, and appearance were also present. The livers of rats treated with silymarin (Fig. 6C) had less nodular changes and a smoother surface than the control group. The liver changed by decreasing liver cell death and swelling degeneration in central, middle, and periportal areas. Pinkish hyaline was found in dead liver cells. Increased blood flow and portal vein widening, inflammation surrounding the portal areas, tiny and big fluid-filled sacs inside cells, expanded Kupffer cells, and hepatic stellate cells in wider blood vessels were also present. Regrowth of liver cells was also seen. The administration of ZMLE at doses of 200, 400, or 800mg/kg bw resulted in enhanced liver cell structure when compared to rats treated with 2-NP (as shown in Fig. 6D, 6E, and 6F). However, ZMLE was shown to be less potent than silymarin in this regard. The ZMLE, administered at a dosage of 800mg/kg body weight, exhibits similar effects to silymarin, as depicted in Figure 5F. ZMLE exhibits effects that vary in intensity according to the dosage.

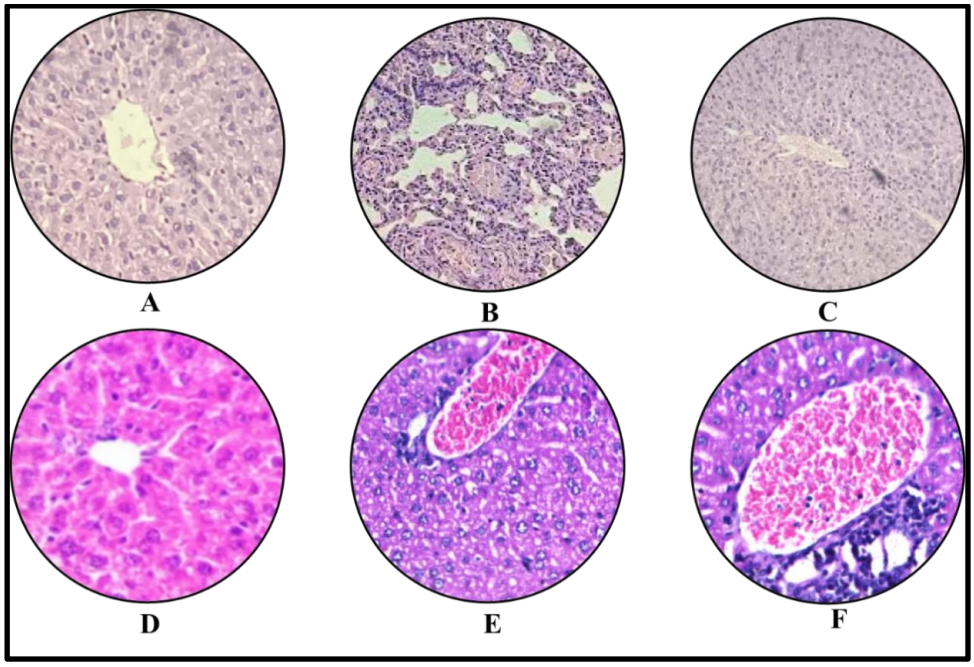

Figure 6: Haematoxylin and eosin-stained liver sections under the microscope (x100); A: Control Rats, B: 2-NP treated rats, C: Silymarin treated rats, D: ZMLE-200 treated rats, E: ZMLE-400 treated rats, F: ZMLE-800 treated rats

3. CONCLUSION

Both silymarin and hydroethanolic *Z. Mauritiana* leaf extract shown efficacy in reducing 2-NP induced liver fibrosis and associated alterations in the liver tissue of adult male albino rats in a dose-dependent manner. Nevertheless, *Z. mauritiana* shown more efficacy when administered to patients as a nutraceutical.

Conflict of interest: None

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Reference

1. Zhang CY, Yuan WG, He P, Lei JH, Wang CX. Liver fibrosis and hepatic stellate cells: Etiology, pathological hallmarks and therapeutic targets. World J Gastroenterol [Internet]. 2016 Dec 12 [cited 2024 Jan 4];22(48):10512. Available from: /pmc/articles/PMC5192262/

2. Robinson MW, Harmon C, O'Farrelly C. Liver immunology and its role in inflammation and homeostasis. Cellular & Molecular Immunology 2016 13:3 [Internet]. 2016 Apr 11 [cited 2024 Jan 4];13(3):267–76. Available from: https://www.nature.com/articles/cmi20163

3. Roeb E. Matrix metalloproteinases and liver fibrosis (translational aspects). Matrix Biology. 2018 Aug 1;68–69:463–73.

4. Ismail M, Pinzani M. Reversal of Liver Fibrosis. Saudi J Gastroenterol [Internet]. 2009 Jan 1 [cited 2024 Jan 4];15(1):72. Available from: /pmc/articles/PMC2702953/

5. Xu X, Poulsen KL, Wu L, Liu S, Miyata T, Song Q, et al. Targeted therapeutics and novel signaling pathways in non-alcohol-associated fatty liver/steatohepatitis (NAFL/NASH). Signal Transduction and Targeted Therapy 2022 7:1 [Internet]. 2022 Aug 13 [cited 2024 Jan 4];7(1):1–39. Available from: https://www.nature.com/articles/s41392-022-01119-3

6. Borges LP, Nogueira CW, Panatieri RB, Rocha JBT, Zeni G. Acute liver damage induced by 2-nitropropane in rats: Effect of diphenyl diselenide on antioxidant defenses. Chem Biol Interact [Internet]. 2006 Mar 25 [cited 2024 Jan 4];160(2):99–107. Available from: https://www.researchgate.net/publication/7327815_Acute_liver_damage_induced_by_2-nitropropane_in_rats_Effect_of_diphenyl_diselenide_on_antioxidant_defenses

7. Singh D, Cho WC, Upadhyay G. Drug-induced liver toxicity and prevention by herbal antioxidants: An Overview. Front Physiol. 2016 Jan 26;6(JAN):167548.

8. Alsayari A, Wahab S. Genus Ziziphus for the treatment of chronic inflammatory diseases. Saudi J Biol Sci [Internet]. 2021 Dec 1 [cited 2024 Jan 4];28(12):6897. Available from: /pmc/articles/PMC8626254/

9. Molole GJ, Gure A, Abdissa N. Determination of total phenolic content and antioxidant activity of Commiphora mollis (Oliv.) Engl. resin. BMC Chem [Internet]. 2022 Dec 1 [cited 2023 Nov 1];16(1):1–11. Available from: https://bmcchem.biomedcentral.com/articles/10.1186/s13065-022-00841-x

10. Mahboubi M, Kazempour N, Nazar ARB. Total Phenolic, Total Flavonoids, Antioxidant and Antimicrobial Activities of Scrophularia Striata Boiss Extracts. Jundishapur J Nat Pharm Prod [Internet]. 2013 [cited 2023 Nov 1];8(1):15. Available from: /pmc/articles/PMC3941883/

11. Baliyan S, Mukherjee R, Priyadarshini A, Vibhuti A, Gupta A, Pandey RP, et al. Determination of Antioxidants by DPPH Radical Scavenging Activity and Quantitative Phytochemical Analysis of Ficus religiosa. Molecules [Internet]. 2022 Feb 1 [cited 2023 Nov 1];27(4). Available from: /pmc/articles/PMC8878429/

12. González-Palma I, Escalona-Buendía HB, Ponce-Alquicira E, Téllez-Téllez M, Gupta VK, Díaz-Godínez G, et al. Evaluation of the antioxidant activity of aqueous and methanol extracts of Pleurotus ostreatus in different growth stages. Front Microbiol. 2016 Jul 12;7(JUL):206086.

13. Al-Afifi NA, Alabsi AM, Bakri MM, Ramanathan A. Acute and sub-acute oral toxicity of Dracaena cinnabari resin methanol extract in rats. BMC Complement Altern Med. 2018;18(1):1–14.

14. Eugenia Letelier Muñoz M, Jara J, Iturra P, Faúndez M. Evaluation of the antioxidant properties and effects on the biotransformation of commercial herbal preparations using rat liver endoplasmic reticulum

Antioxicant capacity of natural products View project Heteroaryl-acrylonitrile derivatives as inhibitors of NADPH Oxidases in an isoform-specific manner. View project [Internet]. 2009. Available from: http://redalyc.uaemex.mx/src/inicio/ArtPdfRed.jsp?iCve=85611769007

15. Habig WH, Pabst MJ, And ~, Jakoby WB. Glutathione S-Transferases THE FIRST ENZYMATIC STEP IN MERCAPTURIC ACID FORMATION*. Journal of Biological Chemistry. 1974;249(22):7130–9.

16. Sarkar PK. A Quick Assay for Na+-K+-ATPase Specific Activity. Zeitschrift fur Naturforschung - Section C Journal of Biosciences. 2002;57(5–6):562–4.

17. MARKLUND S, MARKLUND G. Involvement of the Superoxide Anion Radical in the Autoxidation of Pyrogallol and a Convenient Assay for Superoxide Dismutase. Eur J Biochem [Internet]. 1974 Sep 1 [cited 2023 Dec 25];47(3):469–74. Available from: https://onlinelibrary.wiley.com/doi/full/10.1111/j.1432-1033.1974.tb03714.x

18. Sinha AK. Colorimetric assay of catalase. Anal Biochem. 1972 Jun 1;47(2):389–94.

19. Rotruck JT, Pope AL, Ganther HE, Swanson AB, Hafeman DG, Hoekstra WG. Selenium: Biochemical Role as a Component of Glutathione Peroxidase. Science (1979) [Internet]. 1973 Feb 9 [cited 2023 Dec 25];179(4073):588–90. Available from: https://www.science.org/doi/10.1126/science.179.4073.588

20. Moron MS, Depierre JW, Mannervik B. Levels of glutathione, glutathione reductase and glutathione S-transferase activities in rat lung and liver. Biochimica et Biophysica Acta (BBA) - General Subjects. 1979 Jan 4;582(1):67–78.

21. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem. 1979 Jun 1;95(2):351–8.

22. Qiu B, Wei F, Sun X, Wang X, Duan B, Shi C, et al. Measurement of hydroxyproline in collagen with three different methods. Mol Med Rep [Internet]. 2014 Aug 1 [cited 2023 Dec 26];10(2):1157–63. Available from: http://www.spandidos-publications.com/10.3892/mmr.2014.2267/abstract

23. Aryal S, Baniya MK, Danekhu K, Kunwar P, Gurung R, Koirala N. Total Phenolic Content, Flavonoid Content and Antioxidant Potential of Wild Vegetables from Western Nepal. Plants [Internet]. 2019 Apr 1 [cited 2024 Jan 4];8(4). Available from: /pmc/articles/PMC6524357/

24. Koley TK, Kaur C, Nagal S, Walia S, Jaggi S, Sarika. Antioxidant activity and phenolic content in genotypes of Indian jujube (Zizyphus mauritiana Lamk.). Arabian Journal of Chemistry. 2016 Nov 1;9:S1044–52.

25. Owolarafe T, Ihegboro G, Salawu K, Ononamadu C, Fadilu M, Musa B. Toxicological Investigation of Aqueous Extract of Ziziphus mauritiana Leaves on Wistar Rats. International Journal of Traditional and Complementary Medicine Research IJTCMR. 2022;(2):91–100.

26. Ramar MK, Chidambaram K, Chandrasekaran B, Kandasamy R. Standardization, in-silico and in-vivo safety assessment of methanol extract of Ziziphus mauritiana Lam leaves. Regul Toxicol Pharmacol [Internet]. 2022 Jun 1 [cited 2024 Jan 5];131. Available from: https://pubmed.ncbi.nlm.nih.gov/35218873/

27. Pariyani R, Safinar Ismail I, Azam AA, Abas F, Shaari K, Sulaiman MR. Phytochemical screening and acute oral toxicity study of Java tea leaf extracts. Biomed Res Int. 2015;2015.

28. Rajapaksha I. Liver Fibrosis, Liver Cancer, and Advances in Therapeutic Approaches. Livers 2022, Vol 2, Pages 372-386 [Internet]. 2022 Nov 2 [cited 2024 Jan 5];2(4):372–86. Available from: https://www.mdpi.com/2673-4389/2/4/28/htm

29. Borges LP, Nogueira CW, Panatieri RB, Rocha JBT, Zeni G. Acute liver damage induced by 2-nitropropane in rats: effect of diphenyl diselenide on antioxidant defenses. Chem Biol Interact [Internet]. 2006 Mar 25 [cited 2024 Jan 5];160(2):99–107. Available from: https://pubmed.ncbi.nlm.nih.gov/16445897/

30. Shaheen S, Arafah MM, Alshanwani AR, Fadda LM, Alhusaini AM, Ali HM, et al. Chitosan nanoparticles as a promising candidate for liver injury induced by 2-nitropropane: Implications of P53, iNOS, VEGF, PCNA, and CD68 pathways. Sci Prog [Internet]. 2021 [cited 2024 Jan 5];104(2):1–19. Available from: https://us.sagepub.com/en-us/nam/open-access-at-sage

31. Rajopadhye A, Upadhye AS. Estimation of bioactive compound, maslinic acid by HPTLC, and evaluation of hepatoprotective activity on fruit pulp of Ziziphus jujuba Mill. Cultivars in India. Evidence-based Complementary and Alternative Medicine. 2016;2016.

32. Shin DS, Seo H, Yang JY, Joo J, Im SH, Kim SS, et al. Quantitative Evaluation of Cytochrome P450 3A4 Inhibition and Hepatotoxicity in HepaRG 3-D Spheroids. Int J Toxicol [Internet]. 2018 Sep 1 [cited 2024 Jan 10];37(5):393–403. Available from: https://journals.sagepub.com/doi/10.1177/1091581818780149?icid=int.sj-full-text.similar-articles.1

33. Singh S, Mehrotra S, Pandey R, Sandhir R. Hepatotoxic effects of tert-butyl hydroperoxide (t-BHP) and protection by antioxidants. Indian J Exp Biol. 2005 Aug;43(8):728–31.

34. Corcoran GB, Chung SJ, Salazar DE. Early inhibition of the Na+/K+-ATPase ion pump during acetaminophen-induced hepatotoxicity in rat. Biochem Biophys Res Commun [Internet]. 1987 Nov 30 [cited 2024 Jan 10];149(1):203–7. Available from: https://pubmed.ncbi.nlm.nih.gov/2446617/

35. Yücebilgiç G, Bilgin R, Tamer L, Tükel S. Effects of lead on Na+-K+ ATPase and Ca+2 ATPase activities and lipid peroxidation in blood of workers. Int J Toxicol. 2003 Mar;22(2):95–7.