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Evaluation of Protective Potentials of Methanolic Extract of *Jatropha tanjorensis* against Liver Damage Induced by Paracetamol Overdose

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Authors' contributions

This work was carried out in collaboration between all authors. Author AA designed the study, authors AMO and TDO performed the experiment, statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

This study investigated the potential of methanolic leaf extract of *Jatropha tanjorensis* (MEJT) in alleviating paracetamol-induced hepatotoxicity in male Wistar rats. Rats were pretreated with MEJT at doses of 125 mg/kg and 250 mg/kg body weight before exposure to paracetamol. The effects of MEJT were assessed through various parameters, including body weight changes, liver enzyme levels, lipid profiles, oxidative stress markers, and histopathological evaluations. The results demonstrated that paracetamol overdose significantly increased body weight gain percentage, liver-to-body weight ratio, and the activities of serum AST, ALT and ALP as well as concentration of

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serum bilirubin, indicating liver damage. MEJT pretreatment effectively normalized these parameters, reducing liver enzyme levels and improving lipid profiles. Histological examination revealed that MEJT preserved hepatocyte morphology and mitigated hepatic steatosis and fat infiltration. Additionally, MEJT significantly decreased the percentage of fragmented DNA and pro-inflammatory cytokines (IL-6 and TNF-α), while restoring normal levels of SOD and GSH and inhibiting *Bax* expression, suggesting a reduction in oxidative stress and apoptosis. These findings underscore MEJT's antioxidant and anti-inflammatory properties, attributed to its reported flavonoid and tannin contents. The extract's ability to suppress oxidative stress and inflammation highlights its potential as a therapeutic agent for liver diseases. Further research should explore the detailed mechanisms of MEJT's protective effects and its long-term efficacy and safety for clinical use.

Keywords: Jatropha tanjorensis; hepatoprotective; paracetamol overdose; oxidative stress; inflammation; liver function.

1. INTRODUCTION

The liver plays a crucial role in the digestion, vitamin storage, detoxification and metabolism of both endogenous and exogenous substances in humans and mammals [1]. However, its functionality can be compromised by viruses, hepatotoxins, and xenobiotics. According to the World Health Organization (WHO), approximately 46% of global morbidity and 59% of mortality are attributed to chronic liver diseases [2]. Specifically, data published by WHO in 2018 indicated that fatalities due to liver disease in Nigeria have reached 60,044, making Nigeria the second-highest in the world with an age-adjusted death rate of 64.44 per 100,000 population [2]. Furthermore, a study conducted in the southwest region of Nigeria by Olusegun et al*.* [3] reported that drug-induced liver injury accounted for 0.7% of liver disease cases.

One of the most abused drugs is paracetamol, which, despite being safe at therapeutic doses, can cause severe liver injury, hepatic necrosis, nephrotoxicity, extra-hepatic lesions, and even death when taken in overdose [4,5]. According to Mitchell et al. [6], the toxicity begins with a reactive metabolite that binds to proteins. These findings suggest that cytochrome P_{450} enzymes metabolically activate paracetamol to a reactive metabolite that depletes glutathione (GSH) and forms covalent bonds with proteins. James *et al.* [7] also demonstrated that replenishing glutathione (GSH) can halt this toxicity.

Due to the cost, scarcity, and side effects of conventional drugs used in the treatment and management of liver diseases, a significant portion of the population now turns to medicinal plants for managing liver conditions. One such plant is *Jatropha tanjorensis*, which belongs to the family Euphorbiaceae. It is a common weed found in field crops within the higher rainforest zones of West Africa and is known in Nigeria as "hospital too far" or "Catholic vegetable" [8]. The leaf extract of *J. tanjorensis* has been shown to have hepatoprotective [9,10], hematopoietic [11], hypolipidemic [12,13], hypoglycaemic, and antidiabetic [14], anticancer [10], anti-anaemic [15], antimicrobial [16], and anti-malaria [17] properties.

Preliminary phytochemical analysis of the methanolic extract of Jatropha tanjorensis revealed the presence of saponins, glycosides, flavonoids, alkaloids, anthraquinones, phenols, and tannins [18]. Given its phenolic, flavonoid, and tannin contents, this study aimed to investigate the hepatoprotective effects of methanolic leaf extract of Jatropha tanjorensis on paracetamol-induced liver damage.

The objectives of the study were to:

i. determine the effect of the *Jatropha tanjorensis* extracts on the body and liver weight and serum liver function markers in paracetamol-induced liver damage in experimental rats
ii. measure the effect of the *Jatropha tanjorensis* extract on serum lipid profile and oxidative stress markers in paracetamol-induced liver damage in experimental rats
iii. assess the effect of the *Jatropha tanjorensis* extract on DNA fragmentation of paracetamol-induced liver damage in experimental rats.
iv. assess the effect of the *Jatropha tanjorensis* extract on pro-inflammatory markers including interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-alpha) in paracetamol-induced liver damage in experimental rats.

v. examine the effect of the *Jatropha tanjorensis* extract on histoachitecture of liver and liver immunohistochemical changes of experimental rat exposed to toxic dose of paracetamol-induced liver damage

2. METHODOLOGY

2.1 Collection of Plant and Preparation of Extracts

Fresh leaves of *Jatropha tanjorensis* were collected from Ogbomoso South Local Government Area in June 2023 and authenticated by a botanist at the Department of Pure and Applied Biology, Ladoke Akintola University of Technology, Ogbomoso, Nigeria (voucher specimen number LHO 816). The leaves were thoroughly rinsed under running water to remove debris and air-dried at room temperature to constant weight. The dried leaves were reduced to coarse powder using an electric blender. Two hundred and fifty (250) grams of the powdered leaves were exhaustively extracted in 2 liters of 98% methanol through cold maceration with intermittent shaking for 72 hours. The resulting mixture was filtered using Whatman No. 1 filter paper, and the filtrate was concentrated *in vacuo* using a rotary evaporator at 40°C under reduced pressure. The concentrated extract was then allowed to dry at room temperature and subsequently stored at 4°C until further use [19].

2.2 Chemicals and Reagents

Paracetamol was manufactured by May and Baker Nigeria Plc, Otta, Nigeria, Silymarin was a branded drug (Silybon-70) from Micro Laboratory Ltd, India, Methanol was a product of Loba Chemie Pvt. Ltd., Mumbai. Alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), triglyceride (TG), total cholesterol (TC), high density lipoprotein (HDL), total and direct bilirubin assay kits were products of Beacon Diagnostics Pvt. Ltd, India. All other chemicals and reagents used were of analytical grade.

2.3 Experimental Design

A total of 25 male Wistar rats, weighing between 120-150g, were purchased from a commercial breeder in Ogbomoso. The animals were moderately fed and housed in clean, well-ventilated cages at the animal holdings of the Department of Biochemistry, Ladoke Akintola University of Technology, Ogbomoso, Oyo State. They were acclimatized under standard laboratory conditions (25°C, 12-hour light/dark cycle) for fourteen days before the commencement of the experiment. The "Principles of Laboratory Animal Care" (NIH publication No. 85-23, revised 1985) were strictly adhered to during this study.

Following acclimatization, the animals were randomized into five groups, as summarized in Table 1. The rats were pretreated with silymarin (70 mg/kg body weight) and methanolic extract of *Jatropha tanjorensis* (MEJT) for seven days, with paracetamol (150 mg/kg body weight) administered only on the seventh day. All administration are via the oral route at about 8:00 am daily. The weights of the animals were recorded on Days 1 and 7 to monitor any changes.

2.4 Collection and Preparation of Samples

Twenty-four hours after the administration of paracetamol, the animals were sacrificed by cervical dislocation. Cardiac blood samples were collected into plain sample bottles and allowed to clot at room temperature for 15 minutes, after which they were centrifuged at 3000 rpm for 10 minutes. The resulting supernatant (serum) was stored at 4°C for subsequent biochemical analysis.

The liver was carefully excised and rinsed in phosphate-buffered saline (PBS) (4°C, pH 7.4, 0.1M). One gram of liver tissue was homogenized in 4 ml of PBS (4°C, pH 7.4, 0.1M). The homogenate was then centrifuged at 3000 rpm for 10 minutes, and the supernatant was collected for biochemical assays.

2.5 Determination of Biochemical Parameters

The activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), as well as total and direct bilirubin, were determined following the protocols provided by the respective kit manufacturers. The concentrations of total cholesterol (TC), triglycerides (TG), and high-density lipoprotein (HDL) were also measured according to the kit manufacturers' instructions. Serum low-density

Table 1. Protocol for treatments

Group	Paracetamol	Treatment
A (Normal control)	Not administered	Distilled water (1ml)
B (Negative control)	Paracetamol (150mg/kg body weight at a single dose on day 7).	Distilled water (1ml)
C (Positive control)	Paracetamol (150mg/kg body weight at a single dose on day 7).	Silymarin (70mg/kg body weight) was for seven days
D	Paracetamol (150mg/kg body weight at a single dose on day 7).	METJ (125mg/kg body weight) was for seven days
E	Paracetamol (150mg/kg body weight at a single dose on day 7).	METJ (250mg/kg body weight)

Administration was done orally. METJ- Methanolic extract of J. tanjorensis

lipoprotein (LDL) and very low-density lipoprotein (VLDL) concentrations were calculated using Friedewald's equation [20]. The activity of the antioxidant enzyme superoxide dismutase (SOD) and the concentrations of reduced glutathione (GSH) and malondialdehyde (MDA) were assessed based on the methodologies described in previous studies [21,22,23].

2.6 Determination of Percentage Fragmented DNA

The percentage of fragmented DNA was determined following the method described by Wu et al. (2005). Briefly, liver tissue was homogenized in 10 volumes of Tris-EDTA lysis buffer (5 mM Tris-HCl, 20 mM EDTA, and 2 mL Triton X-100, pH 8.0) and then centrifuged at 27,000 × g for 10 minutes to separate intact DNA (pellet) from fragmented DNA (supernatant). Both the pellet and supernatant (5 mL each) were treated with 3 mL of freshly prepared diphenylamine (DPA) reagent to develop color. The samples were incubated at 37°C for 16-24 hours. The absorbance of the resulting light green or yellowish-green supernatant was measured spectrophotometrically at 620 nm. The percentage of genomic DNA fragmentation was then calculated based on these measurements.
Calculation of % fragmented DNA:

$$\% \text{ fragmented DNA} = \frac{Absorbance of supernatant}{Absorbance of pellet + Absorbance of supernatant} \times 100$$

2.7 Determination of Pro-Inflammatory Markers

The levels of pro-inflammatory cytokines, Interleukin-6 (IL-6) and Tumor Necrosis Factor-alpha (TNF-α), were measured using ELISA kits based on the Sandwich-ELISA principle. For IL-6, 100 µL of either standard or sample was added to each well and incubated at 37°C for 90 minutes. After removing the liquid, 100 µL of Biotinylated Detection Antibody was added and incubated for 1 hour at 37°C, followed by three washes. Then, 100 µL of HRP Conjugate was added, and after a 30-minute incubation at 37°C, the wells were washed five times. Subsequently, 90 µL of Substrate Reagent was added, and the plate was incubated for 15 minutes at 37°C. Finally, 50 µL of Stop Solution was added, and the absorbance was immediately read at 450 nm.

For TNF-α, 100 µL of diluted standard, blank, or sample was added to the appropriate wells and incubated at 37°C for 90 minutes. Following incubation, the liquid was decanted, and 100 µL of Biotinylated Detection Antibody was added to each well, incubated for 1 hour at 37°C, and then washed three times. Next, 100 µL of HRP Conjugate was added and incubated for 30 minutes at 37°C, followed by five washes. Afterward, 90 µL of Substrate Reagent was added, and the plate was incubated for 15 minutes at 37°C, protected from light. Finally, 50 µL of Stop Solution was added, and the optical density of each well was measured at 450 nm using a preheated microplate reader.

2.8 Histological Examination of the Liver

The liver tissues were fixed in 10% formal saline for 24 hours and then processed using an automatic tissue processor. The tissues were dehydrated through a series of ascending alcohol concentrations (70%, 80%, 90%, 95%, and absolute alcohol), cleared in xylene, and embedded in paraffin wax using a semi-automatic tissue embedding center. The resulting tissue blocks were trimmed to expose the tissue surface and sectioned using a rotary microtome at 6 micrometers. After cooling on ice, the sections were further cut to 4 micrometers and floated on a water bath set at 55°C before being

mounted onto clean slides and labeled. The slides were dried on a hotplate set at 60°C for 1 hour. For staining, the sections were dewaxed in xylene for 15 minutes and then dehydrated in absolute alcohol, 95% alcohol, and 70% alcohol. After rinsing in water, the sections were stained with Harris haematoxylin for 5 minutes, briefly differentiated in 1% acid alcohol, and then blued under running tap water for 10 minutes. Counterstaining was done with 1% aqueous eosin for 2 minutes, followed by rinsing in water, dehydration in ascending alcohol grades, clearing in xylene, and mounting in DPX.

2.9 Immunohistochemical Procedure

The tissue sections were first incubated with a blocking buffer, such as 10% fetal bovine serum in PBS or 3% H_2O_2, for 15 minutes at room temperature in a humidified chamber. After removing the blocking buffer and rinsing the sections with wash buffer, a diluted primary antibody was applied and incubated for 1 hour. The slides were subsequently washed twice with wash buffer for 5 minutes each. Next, the sections were treated with Antibody Amplifier and Polymer-HRP Micro-Polymeric-HRP secondary antibody, each applied for 15 minutes, followed by a 5-minute wash with wash buffer. DAB substrate solution was then added to develop the antibody staining, with the color intensity being carefully monitored until the desired level was achieved. Following color development, the sections were washed under running water three times for 2 minutes each, then counterstained with hematoxylin for 10-20 seconds, and rinsed under running tap water for 10 minutes. The tissue slides were dehydrated through four changes of alcohol (95%, 95%, 100%, and 100%) for 5 minutes each, cleared in xylene three times, and coverslipped with mounting solution. Finally, the slides were examined under a microscope.

2.10 Statistical Analysis

The results are presented as mean ± SEM (Standard Error of the Mean). Statistical analyses were performed using one-way analysis of variance (ANOVA) with GraphPad Prism 5.0 software, and significance was determined at $P < 0.05$.

3. RESULTS AND DISCUSSION

3.1 Effects of Methanolic Leaf Extract of *Jatropha tanjorensis* on Weight Gain and Liver-to-Body Weight Ratio in Paracetamol-Induced Liver Damage in Male Wistar Rats

As shown in Table 2, a significant increase ($P < 0.05$) in both percentage body weight gain and the liver-to-body weight ratio were observed in experimental animals following the administration of paracetamol (PCM) overdose. However, no significant difference was found in these parameters between animals pretreated with either MEJT (125 mg/kg and 250 mg/kg body weight) or silymarin (70mg/kg body weight) before exposure to paracetamol overdose and the control group.

The increase in relative liver weight as observed in the animals administered overdose of paracetamol is clinically referred to hepatomegaly and usually result from inflammation of liver cells due to accumulation of NAPQI, a toxic metabolite of paracetamol [24] Thus, prevention of hepatomegaly, and the consequent preservation of body weight, in animals pre-treated with MEJT may be attributed to the anti-inflammatory properties of *J. tanjorensis* [25]. This property could be attributed to the presence of natural antioxidants such as flavonoids and tannins in the leaf extract [18].

Table 2. Effects of methanolic leaf extract of *Jatropha tanjorensis* on percentage weight gain and the relative liver-to-body weight ratio of paracetamol-induced liver damage in male Wistar rats

Groups	% Body weight gain	Liver:Body Weight
A. Normal Control	9.16 ± 1.04^a	3.99 ± 0.05^a
B. PCM only	12.89 ± 0.19^b	5.01± 0.09^b
C. PCM + silymarin	9.49 ± 0.33^a	4.04 ± 0.18^a
D. PCM + MEJT(125mg/kg)	10.05 ± 0.69^a	4.15± 0.14^a
E. PCM + MEJT (250mg/kg)	9.86 ± 0.53^a	4.05 ± 0.19^a

Values were expressed as mean ±SEM (n=5). Different alphabet superscripts along the same column denote significant difference at P<.05. PCM=Paracetamol, MEJT=Methanolic leaf extract of Jatropha tanjorensis

Table 3. Effects of methanolic leaf extract of *Jatropha tanjorensis* (MEJT) on the hepatic parameters of paracetamol-induced liver damage in male Wistar rats

Groups	AST(U/L)	ALT(U/L)	ALP(U/L)	Total Bilirubin µmol/L)	Direct Bilirubin (µmol/L)
A. Normal Control	1.31±0.44[a]	0.87±0.36[a]	1.38±0.56[a]	0.51±0.11[a]	0.23±0.00[a]
B. PCM only	3.06±0.62[b]	2.62±0.35[b]	3.67±0.71[b]	1.31±0.19[b]	0.66±0.07[b]
C. PCM + silymarin	1.31±0.25[a]	1.53±0.42[a]	2.07±0.40[a]	0.60±0.13[a]	0.26±0.08[a]
D. PCM + MEJT(125mg/kg)	1.53±0.22[a]	1.09±0.55[a]	1.73±0.35[a]	1.01±0.13[b]	0.21±0.08[a]
E. PCM + MEJT (250mg/kg)	1.09±0.42[a]	1.31±0.25[a]	1.35±0.35[a]	0.61±0.10[a]	0.25±0.01[a]

Values were expressed as mean ±SEM (n=5). Different alphabet superscripts along the same column denote significant difference at P<.05. PCM=Paracetamol, MEJT=Methanolic leaf extract of Jatropha tanjorensis

Table 4. Effects of methanolic leaf extract of *Jatropha tanjorensis* (MEJT) on the serum lipid profile of paracetamol-induced liver damage in male Wistar rats

Treatments	TC (mmol/L)	TG (mmol/L)	HDL (mmol/L)	LDL (mmol/L)	VLDL (mmol/L)
A. Normal control	1.90±0.11[a]	1.10±0.04[a]	0.20±0.01[a]	1.48±0.12[a]	0.22±0.00[a]
B. (PCM only (negative control)	2.53±0.25[b]	1.93±0.07[b]	0.12±0.00[c]	2.04±0.24[b]	0.38±0.01[b]
C.(PCM + Silymarin (70mg/kg body weight)	2.02±0.03[a]	1.13±0.05[a]	0.22±0.02[a]	1.58±0.05[a]	0.22±0.01[a]
D. (PCM + MEJT(125mg/kg body weight)	2.00±0.04[a]	0.67±0.05[c]	0.20±0.00[a]	1.63±0.05[a]	0.13±0.01[c]
E. (PCM + MEJT(250mg/kg body weight)	1.96±0.01[a]	1.11±0.04[a]	0.23±0.02[a]	1.56±0.02[a]	0.22±0.01[a]

Values were expressed as mean ±SEM (n=5). Different alphabet superscripts along the same column denote significant difference P<.05. PCM=Paracetamol, MEJT =Methanolic leaf extract of Jatropha tanjorensis

Table 5. Effects of methanolic leaf extract of *Jatropha tanjorensis* (MEJT) on oxidative stress markers of paracetamol-induced liver damage in male Wistar rats

Groups	SOD (U/mg protein)	GSH (mg/mg protein)	MDA (mmol/L)
A. Normal Control	1.51 ± 0.34[a]	3.36 ± 0.59[a]	3.64 ± 0.25[a]
B. PCM only	1.35 ± 0.05[c]	2.65 ± 0.53[c]	6.25 ± 0.50[b]
C. PCM + silymarin	1.45 ± 0.05[a]	3.04 ± 0.18[a]	4.10 ± 0.43[a]
D. PCM + MEJT(125mg/kg)	1.40 ± 0.08[c]	2.81 ± 0.38[a]	4.75 ± 0.90[c]
E. PCM + MEJT (250mg/kg)	1.50 ± 0.11[a]	2.93 ± 0.15[a]	4.21 ± 0.66[a]

Values were expressed as mean ±SEM (n=5). Different alphabet superscripts along the same column denote significant difference P<.05.PCM (Paracetamol), MEJT (Methanolic leaf extract of Jatropha tanjorensis)

3.2 Effects of MEJT on Selected Liver Function Parameters in Paracetamol-Induced Liver Damage in Male Wistar Rats

Selected hepatic parameters for experimental animals pretreated with METJ in paracetamol-induced hepatotoxicity model are presented in Table 3. Paracetamol overdose (150 mg/kg body weight) led to a significant increase ($P < 0.05$) in serum activities of AST, ALT and ALP, as well as total and direct bilirubin levels when compared to the control group. Pre-treatment with METJ at doses of 250 mg/kg and 125 mg/kg body weight significantly prevented this increase ($P < 0.05$) in liver enzyme biomarkers and bilirubin concentrations compared to the negative control group.

Elevated serum AST and ALT activities indicate liver necrosis and inflammation of the liver cells brought about by accumulation of the reactive NAPQI radicals. Hyperbilirubinemia and high level of ALP are biomarkers of hepatic obstruction [26]. Prevention of abnormally high levels of these biomarkers in the serum of animals administered both doses of METJ prior exposure to paracetamol overdose and the

protection could either be attributed to the presence of free radical-scavenging or antiinflammatory phytoconstituents of *J. tanjorensis* such as flavonoids [27].

3.3 Effects of Methanolic Leaf Extract of *Jatropha tanjorensis* (MEJT) on Serum Lipid Profile of Paracetamol-Induced Liver Damage in Male Wistar Rats

As shown in Table 4, there is a significant (P < 0.05) increase in serum concentrations of TC, TG, LDL, and VLDL, and a decrease in HDL concentration in the untreated groups compared to the normal control. Treatment with *Jatropha tanjorensis* extract at 250 mg/kg body weight significantly (P < 0.05) reduced TC, TG, LDL, and VLDL levels and significantly (P < 0.05) increased HDL levels in the treated rats compared to the negative control group.

Dyslipidemia is a commom occurrence in liver damage since the liver is the major organ for lipd metabolism. Increase in serum TC, TG, LDL, and VLDL observed in the untreated animals could result from suboptimal absorption from the circulation [26]. The improvement in lipid profiles observed in the extract-treated rats may be attributed to the phytochemical constituents of *J. tanjorensis*. Previous studies have also demonstrated the beneficial effects of *J. tanjorensis* on hyperlipidemia [27,12].

3.4 Effects of Methanolic Leaf Extract of *Jatropha tanjorensis* (MEJT) on Oxidative Stress Markers of Paracetamol-induced Liver Damage in Male Wistar Rats

Paracetamol overdose significantly (P < .05) lowered hepatic SOD activity and GSH concentration, with a concomitant increase in MDA concentration of untreated rats compared to the normal control group. In contrast, no significant differences (P > 0.05) were observed in SOD activity, GSH concentration, or MDA levels in rats treated with 250 mg/kg body weight of the extract compared to the normal control group (Table 5).

Depletion of reduced glutathione in the liver, during overdose of paracetamol, usually result from overproduction of NAPQI radicals which overwhelms glutathione storage. This situation establishes oxidative stress and the unquenched radicals induces lipid peroxidation in the membrane and accumulation of MDA. These series of event accounted for membrane damage to the membrane of the hepatocytes leading to other abnormalities presented in sections 3.2 and 3.4. The observed reduction in MDA levels and the increase in SOD activity and GSH concentration in the extract-treated animals may be attributed to the antioxidant properties of *Jatropha tanjorensis.* The presence of flavonoids in *J. tanjorensis* has been implicated as a key factor in its hepatoprotective effects due to its strong antioxidant capacity [27]. These findings are consistent with previous research highlighting the hepatoprotective potential of *J. tanjorensis* extracts in liver diseases [28].

3.5 Effects of Methanolic Leaf Extract of *Jatropha tanjorensis* (MEJT) on Percentage Fragmented DNA of Paracetamol-Induced Liver Damage in Male Wistar Rats

Fig. 1 shows that paracetamol overdose significantly (P < 0.05) increased the percentage of fragmented DNA in untreated rats. In contrast, administration of MEJT at both doses significantly (P < 0.05) reduced the percentage of fragmented DNA compared to the untreated groups.

Excessive free radical generation can damage cellular biomolecules, including DNA [29]. The increase in fragmented DNA observed in rats exposed to paracetamol overdose is likely due to free radical-induced damage to genetic material. The significant (P < .05) reduction in DNA fragmentation in rats pretreated with both doses of MEJT may be attributed to the plant's antioxidant properties.

3.6 Effects of Methanolic Leaf Extract of *Jatropha tanjorensis* (MEJT) on the Level of IL-6 and TNF-α of Paracetamol-Induced Liver Damage in Male Wistar Rats

Fig. 2 illustrates that paracetamol overdose significantly (P < 0.05) elevated the levels of IL-6 and TNF-α in untreated rats compared to the normal control group. However, administration of MEJT at both doses (125 mg/kg and 250 mg/kg

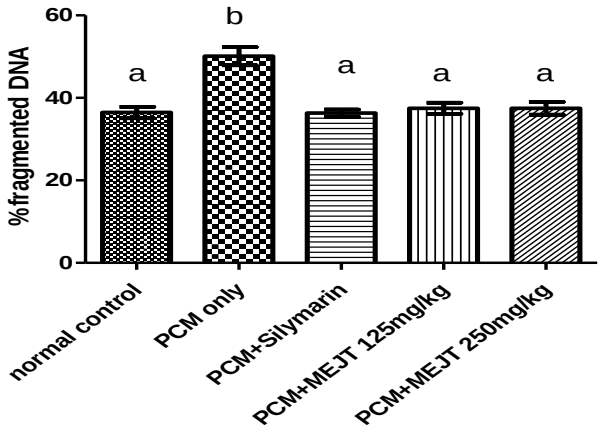

Fig. 1. Effects of methanolic leaf extract of *Jatropha tanjorensis* on percentage Fragmented DNA of paracetamol-induced liver damage in male Wistar rats

Values were expressed as mean ±SEM (n=5). Different alphabet superscripts denote significant difference P<.05. PCM=Paracetamol, MEJT =Methanolic leaf extract of Jatropha tanjorensis

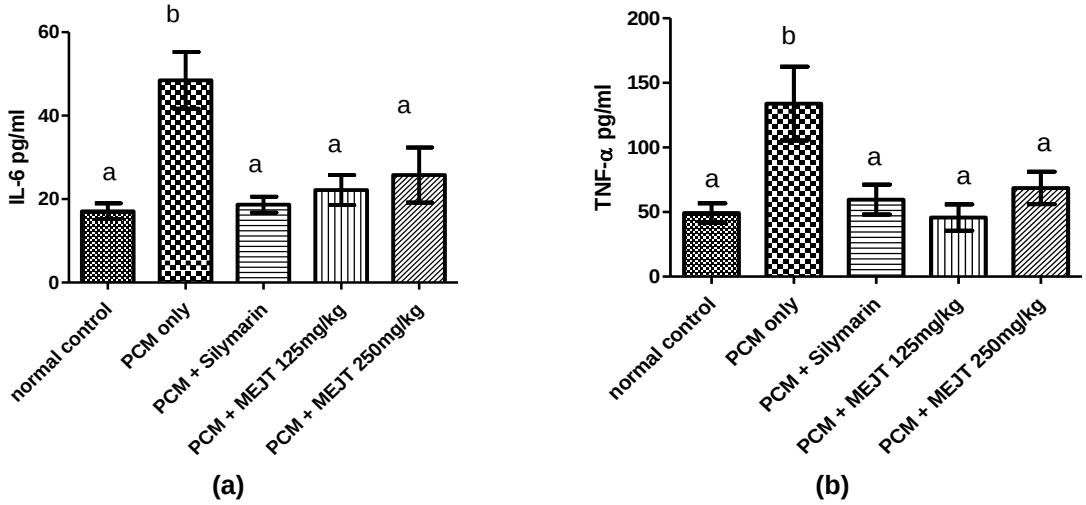

Fig. 2. Effects of methanolic leaf extract of *Jatropha tanjorensis* (MEJT) on level of (a) IL-6 and (b) TNF-α of paracetamol-induced liver damage in male Wistar rats

Values were expressed as mean ±SEM (n=5). Different alphabet superscripts denote significant difference P<.05. PCM=Paracetamol, MEJT=Methanolic leaf extract of Jatropha tanjorensis, IL-6=Interleukin-6, TNF-α =Tumor Necrosis Factor- Alpha

body weight) significantly (P < 0.05) reduced these pro-inflammatory markers compared to the untreated group.

The study shows that MEJT effectively mitigates the increased expression of TNF-α and IL-6 induced by paracetamol overdose. These findings suggest that MEJT is effective in reducing elevated liver markers and ameliorating inflammation due oxidative stress associated with hepatic injury.

3.7 Effects of Methanolic Leaf Extract of *Jatropha tanjorensis* (MEJT) on the Histopathology of Livers of Rats of Paracetamol-Induced Liver Damage in Male Wistar Rats

Histological examination revealed normal hepatocyte morphology in the normal control group (Fig. 3A), rats pre-treated with silymarin (Fig. 3C) and rats pre-treated with 250 mg/kg

body weight MEJT (Fig. 3E). Severe to chronic hepatic steatosis (blue arrow) and fat infiltration (white arrow) were observed in rats administered paracetamol and untreated rats (Fig. 3B) while there was mild inflammatory cell aggregation (slender arrow) in rats pre-treated with 125 mg/kg body weight MEJT (Fig. 3D).

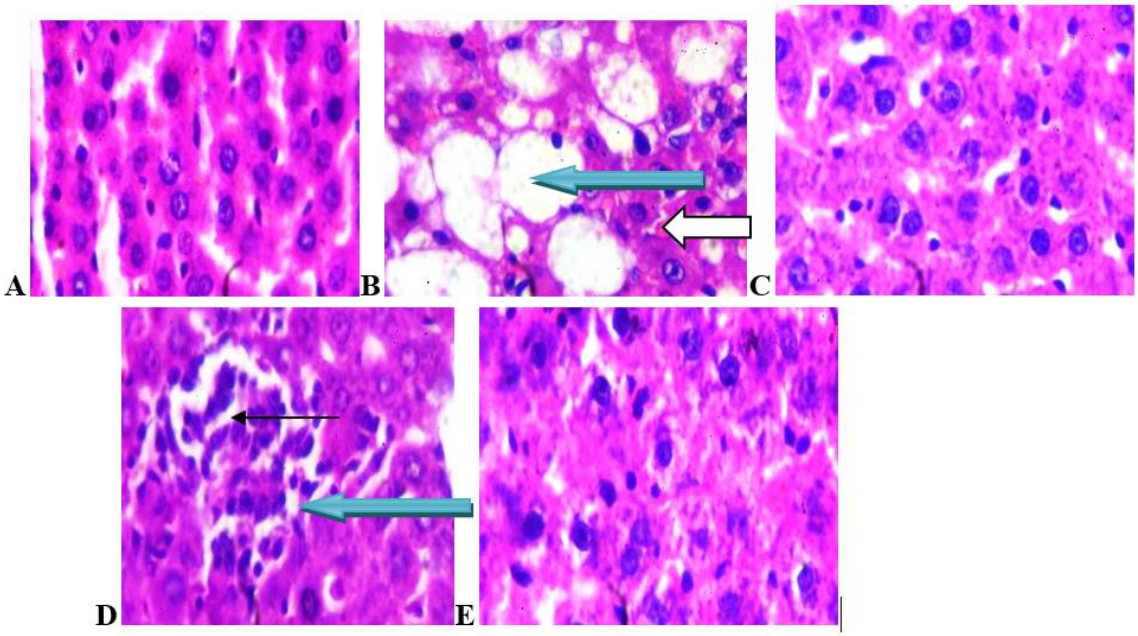

Fig. 3. Photomicrographs of liver section stain by haematoxylin and eosin (400X)
of A (normal control), B (PCM only), C (PCM + Silymarin), D (PCM+ MEJT 125mg/kg body weight), E (PCM+ MEJT 250mg/kg body weight). PCM=Paracetamol, MEJT=Methanolic leaf extract of Jatropha tanjorensis

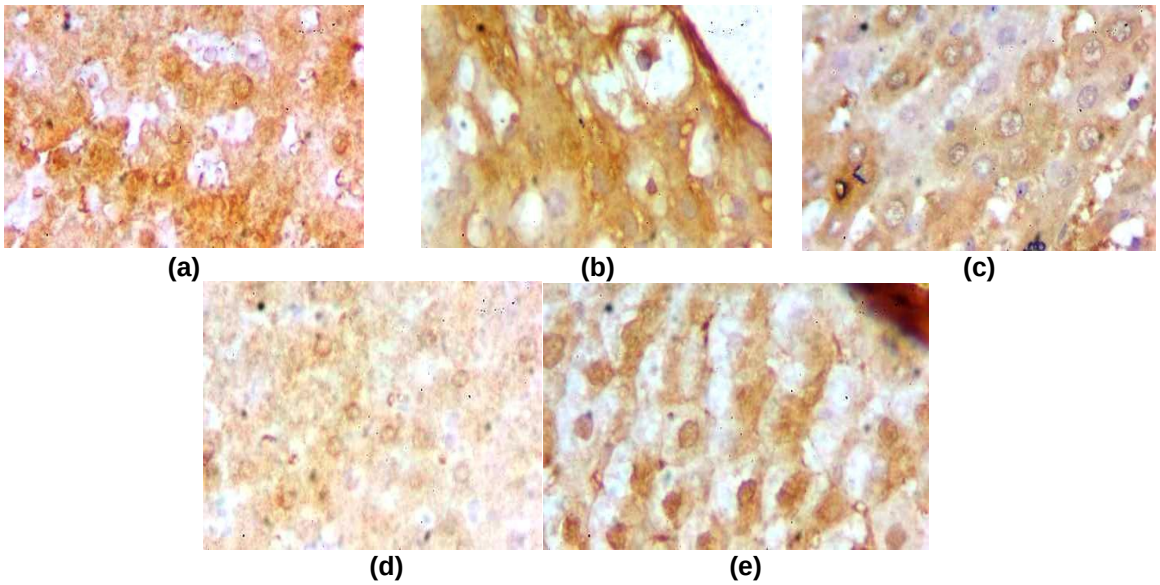

Fig. 4. Photomicrographs of liver section stain with *Bax* protein (400X)
of A (normal control), B (PCM only), C (PCM + Silymarin), D (PCM+ MEJT 125mg/kg body weight), E (PCM+ MEJT 250mg/kg body weight). PCM=Paracetamol), MEJT=Methanolic leaf extract of Jatropha tanjorensis, Bax =Bcl-2-associated X protein

Table 6. Expression of *Bax* protein levels in paracetamol-induced Wistar rats using ImageJ image processing program

Groups	*Bax* protein level (µM)
A. Normal Control	70.55± 0.07[a]
B. PCM only	73.29± 0.11[b]
C. PCM + silymarin (70mg/kg body weight)	68.87± 0.10[a]
D. PCM + MEJT(125mg/kg body weight)	57.29± 0.13[c]
E. PCM + MEJT (250mg/kg body weight)	66.01±0.08[a]

Values were expressed as mean ±SEM (n=5). Different alphabet superscripts denote significant difference P<.05. PCM=Paracetamol), MEJT=Methanolic leaf extract of Jatropha tanjorensis, Bax =Bcl-2-associated X protein

These histological findings support the biochemical findings of impaired lipid metabolism (section 3.3) as well as inflammation of the liver cells (section 3.6). absence of steatosis and inflammation in the livers of animals pre-treated with 250 mg/Kg body weight is a testament to the anti-inflammatory properties of *J. tanjorensis.* This is in tandem with the findings of Ezeonu et al. [30].

3.8 Effects of Methanolic Leaf Extract of *Jatropha tanjorensis* (MEJT) on the Expression of *Bax* in Paracetamol-Induced Liver Damage in Male Wistar Rats

Immunohistochemical analysis revealed moderate to severe expression of Bcl-2-associated X protein (*Bax*) in livers of rats exposed to paracetamol overdose. In contrast, rats pre-treated with silymarin and MEJT (250 mg/kg body weight) exhibited moderate *Bax* expression compared to the normal control group, while MEJT (125 mg/kg body weight) showed weak *Bax* expression (Fig. 4). Quantitative analysis using ImageJ confirmed a significant increase in *Bax* levels in rats exposed to paracetamol. However, no significant difference in *Bax* expression was observed between the silymarin and 250 mg/kg MEJT groups and the control group (Table 6).

Bcl-2-associated X protein (*Bax*) is a pro-apoptotic protein that induces apoptosis via the modification of the mitochondrial membrane depolarization and content leaking, leading to necrotic apoptosis [31]. Prevention of up-regulation of *Bax* in the extract-treated animals suggests that MEJT efficiently mitigates liver damage by mitigating necrotic apoptosis [32-35].

4. CONCLUSION

This study concludes that the methanolic leaf extract of *Jatropha tanjorensis* (MEJT) protects the liver by preventing oxidative stress and inflammation, hepatomegaly, genetic damage, necrosis as well as upregulation of Bax and apoptosis. Ultimately the morphology and biochemistry of the hepatocyte is preserved. These findings projects METJ as a promising agent for mitigating paracetamol-induced liver damage. Its antioxidant properties and inhibition of apoptosis underscore its potential as a hepatoprotective treatment.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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