ORIGINAL ARTICLE

Hepatoprotective and Antioxidant Properties of Stem Bark Extract of *Mimosa pigra* in Rats

Sanusi Hassan Wara[1]*, Kareemah Umar Dandare[1], Muazu Abubakar Gusau[1], Aliyu Abdullahi Ebbo[2]

[1] Department of Biochemistry and Molecular Biology, Faculty of Chemical and Life Sciences, Usmanu Danfodiyo University, Sokoto, P.M.B. 2346, Sokoto, Nigeria
[2] Department of Veterinary Pharmacology and Toxicology, Faculty of Veterinary Medicine, Usmanu Danfodiyo University, Sokoto, P.M.B. 2346, Sokoto, Nigeria

ABSTRACT

Introduction: Liver diseases are responsible for 3.5% of mortality in Africa. Despite incredible advances in modern medicine, drugs for liver diseases are not effective. This work evaluates the hepatoprotective and antioxidant effects of stem bark fractions of *Mimosa pigra* in Carbon tetrachloride-induced liver toxicity in albino rats. **Materials and Methods:** Lethal Dose (LD_{50}) was determined according to Organization for Economic Co-operation and Development. Liver function tests were employed to evaluate hepatoprotective activity, and antioxidant assays were used to elucidate the mechanisms of action. **Results:** The LD_{50} was greater than 5000 mg/kg. Group treated CCl_4 only (group 2) showed significant increase ($p< 0.05$) in liver enzymes and non-enzyme serum activities, while serum albumin and total protein levels decreased ($p < 0.05$) compared to control group (group 1). Vitamins (C and E), glutathione and antioxidant enzymes were decreased in group 2. Dosages of CCl_4 and stem bark methanol extract of *Mimosa pigra* and its fraction suppressed the raised serum liver enzymes and Malondialdehyde (groups 4 to 6). Raised total protein, albumin and antioxidant enzymes occurred in the treatment groups (3 to 6). Butanol fraction was the most active, exhibiting same effect as silymarin (conventional drug). Reduced activities of microsomal aniline hydroxylase and malondialdehyde were observed in group administered butanol fraction compared to group 2 ($p<0.05$). **Conclusion:** Butanol fraction of *M. pigra* exerts hepatoprotective activity comparable to silymarin as natural antioxidant and detoxifying enzymes.
Malaysian Journal of Medicine and Health Sciences (2026) 22:3: 138-145. doi:10.47836/mjmhs.20.s12.21

Keywords: Hepatoprotective, Antioxidants, LD_{50}, Liver diseases, *Mimosa pigra*

Corresponding Author:
Sanusi Hassan Wara, PhD
Email: hassan.sanusi@udusok.edu.ng
hassanwara@gmail.com
Tel: +2348036355866

INTRODUCTION

Mimosa pigra is a giant tree, also known in Hausa language as Farin gumbi in Northern Nigeria. It is of the genus Mimosa and family of fabaceae. It has thorny impassable spinney, majorly in wetlands. The plant is utilized in sub-Saharan Africa as a stimulant, relieving toothaches and swelling, as antidiarrheal and for management of liver diseases [1, 2].

Liver disease is a global health problem where nearly 2 million deaths occur annually globally due to complications including cirrhosis, viral hepatitis and hepatocellular carcinoma [3, 4]. Liver injury occurs due to frequent exposure to various agents, such as chemicals, alcoholic beverages, viruses and immune-mediated disorders [5]. Reports indicate over 2 billion people consume alcohol worldwide with about 75% diagnosed with alcohol related disorders including cirrhosis classified as the twelfth leading cause of death worldwide. The range of alcoholic liver toxicity could be fatty liver to acute hepatitis, fibrosis, cirrhosis and chronic liver failure. Liver cancer is listed as the third deadliest cancer in the world [6, 7]. Hepatocellular carcinoma is spreading worldwide and constitutes 75% of all liver cancers [8].

Plants and their compounds have been used several years for liver diseases [9]. Medicinal plants and their formulations, singly and in combination, were found to have hepato-protective effects and accelerate healing of damaged liver cells [10, 11]. ''Many natural medications exist whose main goal is to protect the liver against attacks'' [5 p. 2]. ''Milk thistle extracts were used as early as the 4th century B.C., became a favored medicine for hepatobiliary diseases in the 16th century and experienced a revival in central Europe in the late 1960s'' [12 p. 1101]. Conventional drugs

such as amiodarone, sulfasalazine etc. employed to cure liver diseases possess several limitations including adverse side effects, complications and impairment of other functions of the liver [5, 13]. Therefore, search for new drugs with minimum side effects from different sources must continue to be pursued. Stem bark of *M. pigra* has been used for treatment of liver diseases in North Western Nigeria for decades. It is important to scientifically validate its hepatoprotective activity and postulates its biochemical mechanism of actions as a basis for future drug development. Therefore, this research was aimed at evaluating hepato-protective activities and antioxidant profiles of stem bark extract of *M. pigra*.

MATERIALS AND METHODS

Materials, chemicals and reagents

The solvents for the extraction included methanol, n-hexane, ethylacetate and n-butanol saturated with water (BDH Chemicals, Poole, England). The solvents employed were of analytical grade.

Experimental animals

A total of thirty [30] male and female albino rats weighing 150-200g were utilized for this research. The rats were acquired from the animal house of Biological Sciences Department, Faculty of Chemical and Life Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria. The animals were accommodated in hygienic polymer cages and maintained on rat chow (2500kcal/kg) and tap water *ad libitum* to acclimatize for at least seven [7] days before the experiment. Animals care was done according to WHO guidelines [14]. Ethical clearance for the study was obtained from the Usmanu Danfodiyo University, Sokoto, Nigeria, Health and Biomedical Research Ethics Committee (HBREC/23/051).

Plant collection and identification

The stem bark of *M. pigra* was gathered from Kalgo town, Kebbi State, Nigeria. The plant was authenticated by a taxonomist at Botany Unit, Department of Biological Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria. A voucher specimen number (UDUH/ANS/0275) was obtained.

Preparation of plant extracts

The stem bark of *M. pigra* was cleaned, air dried inside laboratory and cut into tiny pieces and pulverized. A 500g portion was extracted with 1L of methanol (95%) for 2,880 minutes (twice) and filtered using Whatman filter paper 1. The filtrate was evaporated in a rotary evaporator at 45^{o}C and residue obtained was screened for hepato-protective activity in CCl_4-intoxicated rats. Further fractionations with solvents of decreasing polarity were conducted.

Fractionation of the methanol extract

A 100g portion of the methanol residue was subjected to fractionation in water and serial partition with solvents such as hexane (3x400 mL), ethyl acetate (3x400 mL) and butanol (saturated with water 3x400 mL). Fractions obtained were evaporated to dryness and screened for hepatoprotective activity.

Lethal Dose (LD_{50})

The acute oral toxicity study was conducted using the procedure of Organization for Economic Co-operation and Development for testing of chemicals [15]. A total of five (5) rats were selected randomly and used for the experiment. The extract was administered to each animal orally using limit test dose (5000 mg/kg body weight). Feed was withheld after dosing for 3-4hrs. The rats were checked at first 30 minutes after dosing, then periodically at 8, 14, 24 and 48 hours intervals. Symptoms observed included hair loss, refusal to eat food, drowsiness, hyper-salivation, tremors and convulsion. The rats were examined for up to 14 days for possible signs of late toxicity. The LD_{50} was less than 5000mg/kg if three (3) or more animals died within 48 hours. However, the LD_{50} was greater than 5000mg/kg if one, two or none died within 48 hours.

Animal grouping and sample collection

Hepatotoxicity induction was conducted by the method of Guntupalli et al. [16] with few modifications. Albino rats (wister strains) were divided in to Six (6) groups of six (6) animals each. Group one (1) received 1ml of liquid paraffin orally (per os), once daily for fourteen days (control). Group two (2) were injected 1ml/kg body weight of Carbon tetrachloride (CCl_4) intraperitoneally. Group three (3) received Silymarin per os (100mg/kg body weight) once daily for 14 days and simultaneously with CCl_4. Group four (4), five (5) and six (6) were administered 80, 160 and 240 mg/kg body weight of the respective *M. pigra* (*per os*), extracts, once daily for fourteen (14) days and simultaneously with CCl_4. The dosage of CCl_4 (30%) was administered in liquid paraffin to induce toxicity for every seventy (72) hours in groups 2,3,4,5 and 6. The rats were fasted overnight and killed 48 hours after the last administration of CCl_4. Blood samples were allowed to clot and centrifuged, sera obtained were utilized for the estimations of Aspartate Transaminase (AST), Alanine Transaminase (ALT), Alkaline Phosphatase (ALP), Total Protein (TP), Total Cholesterol (TC), γ-Glutamyl Transferase (γ-GT), Albumin (ALB), Direct Bilirubin (DB) and Total Bilirubin (TB). A portion of rat liver was perfused using cold 1.15% KCl, homogenized and centrifuged at 9000g for 30mins to get post mitochondrial supernatant for the analyses of enzymatic and non-enzymatic antioxidants. Induction of hepatotoxicity and treatment were also done for the hexane, ethylacetate and butanol fractions using same procedure [16].

Measurement of hepatic function markers

Serum AST and ALT were estimated by Reitman and Frankel method [17]. Serum ALP, ALB, TP, TB and

γ-Glutamyl Transferase were determined by standard methods [18, 19, 20, 21, 22] respectively. Estimation of oxidative stress biomarkers such as catalase [23], reduced glutathione [24], superoxide dismutase [25], ascorbic acid [26, 27] and vitamin E [27, 28] were done according to standard methods.

Qualitative phytochemical screening

The tests for the existence of alkaloids, anthraquinones, anthraquinone glycosides, cardiac glycosides, flavonoids, glycosides, phenols, saponins, saponin glycosides, steroids, tannins, terpenoids and volatile oils were conducted employing normal methods [29, 30, 31, 32]

Antioxidant properties of the most potent fraction (butanol)

Induction of hepatotoxicity was done using same method [16]. The livers of the rats were removed and rinsed in ice-cold 1.15% potassium chloride, dried, homogenized in phosphate buffer PH 7.4 and centrifuged at 9000g for 30mins to get post mitochondrial supernatant fraction. The supernatant was frozen to ice. The microsomes were re-suspended in 0.2M Sucrose. The microsomes were used for estimations of glucose-6-phosphatase [33,34], proteins [35, 36], aniline hydroxylase [37], malondialdehyde[38] and cholesterol [39].

Statistical analysis

All data of this work were analyzed by SPSS version 17, one way Analysis of Variance (ANOVA) and the data presented as mean ± standard deviation. P values of ≤0.05 were regarded as significant.

RESULTS

Phytochemical screening of the methanol stem bark extract and its fractions showed presence of tannins, saponins, flavonoids, saponin glycosides and alkaloids.

Acute oral toxicity of methanol stem bark extract of *Mimosa pigra*

Oral dosing of 5000 mg/kg body weight of stem bark extract (methanol) of *M. pigra* in the rats did not produce behavioral signs and symptoms of severe pain or death in the treated animals. Behavioural changes such as diarrhea, fatigue, salivation, refusal to eat and drink and respiratory distress were not observed over a period of fourteen days.

Liver function indices of methanol stem bark extract

Rats administered CCl_4 and methanol stem bark extract of *M. pigra* have shown significant increases ($p<0.05$) in the levels ALP, ALT, AST, TB and DB, while the level of ALB was reduced in the negative control (group 2, administered only CCl_4) as compared to control group (group 1). Rats treated methanol stem bark extract of *M. pigra* significantly ($p<0.05$) reduced the activities of ALP, AST, ALT and the concentration of TB compared to group 2. The silymarin cured group also indicated reduced elevated levels of TB, ALP, AST, ALT but increased ALB was observed in group 3 (Table I).

Antioxidants of methanol stem bark extract

Antioxidant Vitamins and Enzymes of Rats administered methanol stem bark extract of *M. pigra* and CCl_4 have indicated Group 1 to have the highest concentration of the antioxidant vitamins followed by group 3 and 6. Catalase (CAT) levels remained almost the same in all the groups but increases in activity of Superoxide Dismutase (SOD) were observed in groups 1, 3 and 6. The Glutathione concentration showed greater variations between the groups with Group 1 showing highest and group 2 the lowest concentrations (Table II).

Liver function indices of hexane stem bark fraction

ALP and AST showed significant increases in activity ($p<0.05$) in group 2 and 4 but decrease drastically in groups 2, 3 and 6 with lower activity of ALT when

Table I: Liver function indices of Rats administered Carbon tetrachloride and Methanol Stem bark extract of *Mimosa pigra*

Groups	ALP (U/L)	AST (U/L)	ALT (U/L)	ALB (g/dL)	TB (mg/dL)	DB (mg/dL)
1	52.53±2.79	51.58±1.93	14.67±1.09	2.51±0.02	15.83±0.46	5.98±0.83
2	235.25±14.45^{a}	250.59±9.17^{a}	78.72±2.49^{a}	0.11±0.00^{a}	50.38±2.36^{a}	12.62±0.48^{a}
3	74.52±5.93^{b}	70.99±1.38^{b}	27.07±0.93^{b}	2.47±0.18^{b}	17.89±0.31^{b}	6.28±0.17^{b}
4	169.09±6.48^{abc}	179.83±2.83^{abc}	67.00±1.74^{abc}	1.52±0.12^{abc}	37.96±2.29^{abc}	10.76±0.44^{abc}
5	119.99±6.43^{bc}	129.06±4.77^{bc}	39.92±2.06^{bc}	1.99±0.02^{bc}	27.79±1.05^{bc}	8.15±0.26^{bc}
6	86.85±4.30^{ab}	89.49±1.39^{ab}	31.30±1.68^{b}	2.18±0.03^{b}	19.75±0.12^{b}	6.58±0.23^{b}

Values are expressed as mean ± standard error of five (5) replicates. a= significantly different vs group 1: $p<0.05$; b= significantly different vs group 2; c= significantly different vs group 3: $p<0.05$; using analysis of variance (ANOVA), Duncan Bonferroni multiple comparison, Instat Graph Pad Software (San Diego, USA).. ALB: Albumin, ALP:Alkaline phosphatase, AST: Aspartate amino transferase, ALT: Alanine amino transferase, TB: Total bilirubin, DB:Direct bilirubin.

Group 1; (normal control) liquid paraffin treated group

Group 2; (negative control) 30% CCl_4 in liquid paraffin treated group

Group 3; Silymarin 100mg/kg + CCl_4 treated group

Group 4; 80mg/kg body weight of methanol extract + CCl_4

Group 5; 160mg/kg body weight of methanol extract + CCl_4

Group 6; 240 mg/kg body weight of methanol extract + CCl_4.

compared to ALP and AST. TB has higher concentration in group 1 while ALB and DB showed little increase only in group 2 (Table III).

Liver function indices of ethylacetate stem bark fraction
Increased levels ($p<0.05$) of ALT ALP and AST, T B and DB were observed in group 2 treated with only CCl_4 but the levels were suppressed in groups administered stem bark extract of the plant and CCl_4 in a dose - dependent fashion. The decreased levels of ALB in group 2 were restored in groups 3, 4, 5 and 6 (Table IV).

Liver function indices of butanol fraction of stem bark of *M. pigra*

Increased levels ($p<0.05$) of ALT ALP and AST, TB and DB were seen in group 2 treated with only CCl_4 but the levels were depressed in groups treated simultaneously with stem bark extract of the plant and CCl_4 in a dose dependent mode. The decreased levels of ALB in group 2 were restored in groups 3, 4, 5 and 6. The butanol fraction of the plant has demonstrated to be the most active fraction as indicated from the results (Table V).

Liver function indices of last aqueous fraction of stem bark of *M. pigra*

Significantly ($p<0.05$) increased levels of activities of ALT, ALP and AST, levels of TB and DB were observed in group 2 treated with only CCl_4 but the levels were decreased in groups simultaneously administered stem bark extract of the plant and CCl_4 in a dose-dependent fashion. The decreased levels of ALB in group 2 were reduced in groups 3, 4, 5 and 6 but not as in hexane, ethylacetate and butanol fractions. The last remaining aqueous fraction of the plant demonstrated to be the lowest active fraction as indicated from the results (Table VI).

Antioxidant properties of the most potent fraction (butanol)

Marked significant increases ($p<0.05$) in levels of

Table II: Antioxidant Vitamins and Enzymes of Rats administered Methanol Stem bark Extract of *Mimosa pigra*

Groups	Vit A (IU)	Vit C (mg/dL)	Vit E (mg/dL)	GSH (mg/dL)	SOD (U/mg tissue)	Cat (U/mg tissue)
1	83.32±5.72	81.44±3.62	62.83±1.94	160.62±2.10	27.26±1.99	11.32±0.21
2	$32.51±1.32^{a}$	$25.99±1.28^{a}$	$15.99±2.65^{a}$	$82.02±1.84^{a}$	$3.48±0.29^{a}$	$0.60±0.13^{a}$
3	$71.72±0.82^{b}$	$69.26±0.78^{b}$	$57.48±1.80^{b}$	$144.72±1.69^{b}$	$28.60±1.91^{b}$	$10.64±0.14^{b}$
4	$46.22±136^{abc}$	$44.08±1.49^{abc}$	$27.86±0.53^{abc}$	$106.20±2.38^{abc}$	$5.24±0.35^{abc}$	$4.00±0.25^{abc}$
5	$54.97±1.54^{bc}$	$52.28±0.71^{bc}$	$34.51±1.07^{bc}$	$144.08±13.11^{b}$	$12.38±0.88^{bc}$	$6.56±0.21^{bc}$
6	$66.65±2.08^{ab}$	$67.60±1.03^{b}$	$47.48±1.22^{ab}$	$138.27±0.89^{b}$	$19.50±0.29^{ab}$	$8.58±1.14^{b}$

Values are expressed as mean ± standard error of five (5) replicates. a= significantly different vs group 1: $p<0.05$; b= significantly different vs group 2 ;c = significantly different vs group 3: $p<0.05$; using analysis of variance (ANOVA), Duncan Bonferroni multiple comparison, Instat Graph Pad Software (San Diego, USA). Vit A: Vitamin A, VitC: Vitamin C, VitE: Vitamin E, CAT: Catalase, GSH: Reduced glutathione, SOD: Superoxide dismutase.

Group 1; (normal control) liquid paraffin treated group
Group 2; (negative control) 30% CCl_4 in liquid paraffin treated group
Group 3; Silymarin 100mg/kg + CCl_4 treated group
Group 4; 80mg/kg body weight of methanol extract + CCl_4
Group 5;160mg/kg body weight of methanol extract+ CCl_4
Group 6; 240 mg/kg body weight of methanol extract + CCl_4

Table III: Liver function indices of Rats administered Carbon tetrachloride and Hexane fraction of Stem bark of *Mimosa pigra*

Groups	ALP(U/L)	AST(U/L)	ALT(U/L)	ALB (g/dL)	TB (mg/dL)	DB (mg/dL)
1	79.2±1.34	136.3±1.46	25.3±1.86	4.8±0.34	15.8±0.84	2.38±0.15
2	$207.6±1.97^{a}$	$238.1±2.12^{a}$	$104.5±5.15^{a}$	$1.0±0.03^{a}$	$49.7±2.52^{a}$	$13.5±1.13^{a}$
3	$79.1±5.90^{b}$	$148.9±3.11^{b}$	$28.0±2.36^{b}$	$4.1±0.01^{b}$	$18.6±1.33^{b}$	$2.4±0.09^{b}$
4	$202.1±1.28^{a}$	$222.4±2.91^{abc}$	$80.6±5.05^{abc}$	$2.6±0.16^{abc}$	$35.7±1.64^{abc}$	$7.5±0.27^{abc}$
5	$138.7±1.69^{bc}$	$189.9±2.54^{bc}$	$58.2±3.42^{bc}$	$3.5±0.10^{b}$	$30.3±2.73^{bc}$	$5.3±0.35^{bc}$
6	$92.9±5.41^{abc}$	$154.5±1.98^{b}$	$47.7±2.68^{abc}$	$4.0±0.03^{b}$	$23.3±0.87^{ab}$	$3.3±0.20^{ab}$

Values are expressed as mean ± standard error of five (5) replicates. a= significantly different vs group 1: $p<0.05$; b= significantly different vs group 2; c= significantly different vs group 3: $p<0.05$; using analysis of variance (ANOVA), Duncan Bonferroni multiple comparison, Instat Graph Pad Software (San Diego, USA).. ALB: Albumin, ALP:Alkaline phosphatase, AST: Aspartate amino transferase, ALT: Alanine amino transferase, TB: Total bilirubin, DB:Direct bilirubin.

Group 1; (normal control) liquid paraffin treated group,
Group 2; (negative control) 30% CCl_4 in liquid paraffin treated group
Group 3; Silymarin 100mg/kg + CCl_4 treated group
Group 4; 80mg/kg body weight of hexane fraction + CCl_4
Group 5; 160mg/kg body weight of hexane fraction + CCl_4
Group 6; 240 mg/kg body weight of hexane fraction + CCl_4

Table IV: Liver function indices of Rats administered Carbon tetrachloride and Ethyl acetate fraction of Stem bark of *Mimosa pigra*

Groups	ALP(U/L)	AST(U/L)	ALT(U/L)	ALB (g/dL)	TB (mg/dL)	DB (mg/dL)
1	128.1±2.78	108.3±1.71	12.7±0.82	4.0±0.06	15.5±1.51	3.0±0.24
2	290.1±1.48^{a}	254.3±2.17^{a}	82.2±2.42^{a}	1.0±0.01a	59.7±3.58^{a}	16.7±0.81^{a}
3	127.2±1.82^{b}	111.30±2.14^{b}	15.2±1.42^{b}	1.3±0.01^{a}	20.4±0.68^{b}	3.9±0.05^{b}
4	254.8±3.19^{abc}	230.4±2.41^{abc}	70.3±2.02^{abc}	1.9±0.03^{b}	46.7±1.55^{abc}	12.3±0.46^{abc}
5	192.2±1.69^{bc}	156.1±3.07^{bc}	46.4±1.56^{bc}	1.9±0.13^{b}	32.3±1.43^{bc}	6.8±0.35^{bc}
6	152.1±0.85^{ab}	114.3±2.84^{b}	24.5±2.20^{ab}	3.1±0.05^{ab}	20.4±0.77^{b}	3.3±0.17^{b}

Values are expressed as mean ± standard error of five (5) replicates. a= significantly different vs group 1: p<0.05; b= significantly different vs group 2; c= significantly different vs group 3: p<0.05; using analysis of variance (ANOVA), Duncan Bonferroni multiple comparison, Instat Graph Pad Software (San Diego, USA). ALB: Albumin, ALP: Alkaline phosphatase, AST: Aspartate amino transferase, ALT: Alanine amino transferase, TB: Total DB: Direct bilirubin

Group 1; (normal control) liquid paraffin treated group
Group 2; (negative control) 30% CCl_4 in liquid paraffin treated group
Group 3; Silymarin100mg/kg + CCl_4 treated group
Group 4; 80mg/kg body weight of ethyl acetate fraction + CCl_4
Group 5; 160mg/kg body weight of ethyl acetate fraction + CCl_4
Group 6; 240 mg/kg body weight of ethyl acetate fraction + CCl_4

Table V: Liver function Indices of Rats administered Carbon tetrachloride and Butanol fraction of Stem bark of *Mimosa pigra*

Groups	ALP(U/L)	AST(U/L)	ALT(U/L)	ALB (g/dL)	TB (mg/dL)	DB (mg/dL)
1	103.8±1.51	64.2±1.72	34.5±1.71	2.6±0.14	25.1±1.94	5.9±0.23
2	322.0±7.50^{a}	239.1±3.45^{a}	83.1±3.54^{a}	0.1±0.001^{a}	71.3±3.87^{a}	17.9±0.51^{a}
3	122.3±2.95^{b}	81.6±2.41^{b}	43.8±1.69^{b}	2.2±0.01^{b}	34.7±1.97^{b}	7.2±0.42^{b}
4	301.5±8.53^{abc}	202.5±5.11^{abc}	71.3±1.91^{abc}	0.5±0.05^{abc}	62.8±2.67^{abc}	14.7±0.93^{abc}
5	266.2±3.80^{bc}	149.7±2.44^{bc}	59.3±2.94^{bc}	1.2±0.09^{bc}	54.0±4.74^{bc}	12.6±0.69^{bc}
6	131.5±1.48^{ab}	92.0±3.96^{ab}	45.9±1.89^{b}	2.0±0.14^{b}	32.3±2.53^{b}	7.9±0.75^{b}

Values are expressed as mean ± standard error of five (5) replicates. a= significantly different vs group 1: p<0.05; b= significantly different vs group 2; c= significantly different vs group 3: p<0.05; using analysis of variance (ANOVA), Duncan Bonferroni multiple comparison, Instat Graph Pad Software (San Diego, USA).. ALB: Albumin, ALP: Alkaline phosphatase, AST: Aspartate amino transferase, ALT: Alanine amino transferase, TB: Total bilirubin, DB: Direct bilirubin.

Group 1; (normal control) liquid paraffin treated group,
Group 2; (negative control) 30% CCl_4 in liquid paraffin treated group
Group 3; Silymarin 100mg/kg + CCl_4 treated group
Group 4; 80mg/kg body weight of butanol fraction + CCl_4
Group 5; 160mg/kg body weight of butanol fraction+ CCl_4
Group 6; 240 mg/kg body weight of butanol fraction + CCl_4

malondialdehyde and cholesterol were observed in group 2 but decreased levels of glucose 6-phosphatase, aniline hydroxylase and TP were observed as compared to control group (group 1). However, they were restored in groups 3, 4, 5 and 6 when compared to groups 1 and 2 (Table VII).

DISCUSSION

Phytocompounds are found in plants and account for their pharmacological activities [40]. In this study, methanol extract and its fractions showed presence of glycosides (0.28%), steroids (0.23%) tannins (0.001%), saponins (1.73%), flavonoids (1.53%) and alkaloids (3.06%). Alkaloids and other phytocompounds, also detected in the extracts of the present work, were reported to have antibacterial, hepato-protective and anti-diabetic properties [41, 42]. Flavonoids also detected in the extract of M. *pigra*, were known to combat free radical-induced oxidative stress [43]. The LD_{50} (5000mg/kg) of methanol extract of *M. pigra* did not produce any signs of toxicity or mortality within 48 hours and 14 days observation periods. Hence, the LD_{50} is ≥ 5000mg/kg body weight. The stem bark extract of the plant may be relatively safe. This finding is similar to the report that no death was recorded in rats administered 5000 mg/kg dose of *Mimosa pudica* (44), a relative of *M. pigra*.

CCl_4 -induced hepatic damage is used as a model for hepato-protective study of drugs, medicinal plants and their phytochemicals [45]. CCl_4 is converted to trichloromethyl radical (CCl_3) by cytochrome P-450 enzyme. CCl_3 reacts with molecular oxygen to form reactive trichloromethyl peroxy radical [46]. The poly unsaturated fatty acids of membranes are vulnerable to oxidative attack causing membrane lesions and loss of cellular homeostasis [47]. Peroxy radicals form covalent bonds with Glutathione causing its depletion and lipid peroxidation. Formation of hydroperoxides is one of the main causes of hepatotoxicity of CCl_4. This is supported by a rise ($p<0.05$) in the levels of ALT, AST, ALP, TB and DB compared to control rats. Rats administered Silymarin, methanol stem bark of *M. pigra* and CCl_4 in a dose - dependent manner had significantly ($p<0.05$) reduced the levels of ALP, AST, ALT and TB.

Table VI: Liver function indices of Rats administered Carbon tetrachloride and Last remaining aqueous fraction of Stem bark of *Mimosa pigra*

Groups	ALP(U/L)	AST(U/L)	ALT(U/L)	ALB (g/dL)	TB (mg/dL)	DB (mg/dL)
1	118.1±1.89	58.9±1.13	61.8±2.82	3.8±0.14	12.1±1.25	3.5±0.34
2	317.3±1.75^{a}	222.3±3.06^{a}	155.3±1.45^{a}	1.1±0.02^{a}	45.4±1.39^{a}	11.3±0.70^{a}
3	121.7±1.96^{b}	74.4±1.53^{b}	79.7±2.88^{b}	3.2±0.08^{b}	11.8±0.73^{b}	4.5±0.23^{b}
4	257.3±2.56^{abc}	189.5±3.04^{abc}	126.9±2.18^{abc}	1.4±0.07^{a}	35.9±1.97^{c}	9.9±0.37^{c}
5	180.9±2.55^{bc}	144.9±1.65^{bc}	98.9±0.65^{bc}	1.7±0.16^{a}	25.9±1.68^{d}	8.3±0.30^{c}
6	125.9±2.28^{b}	100.9±1.44^{ab}	70.3±2.97^{b}	2.8±0.15^{b}	16.0±1.11^{b}	4.8±0.30$_{b}$

Values are expressed as mean ± standard error of five (5) replicates. a= significantly different vs group 1: p<0.05; b= significantly different vs group 2; c= significantly different vs group 3: p<0.05; using analysis of variance (ANOVA), Duncan Bonferroni multiple comparison, Instat Graph Pad Software (San Diego, USA). ALB: Albumin, ALP: Alkaline phosphatase, AST: Aspartate amino transferase, ALT: Alanine amino transferase, TB: Total bilirubin, DB: Direct bilirubin,

Group 1; (normal control) liquid paraffin treated group,
Group 2; (negative control) 30% CCl_4 in liquid paraffin treated group,
Group 3; Silymarin 100mg/kg + CCl_4 treated group,
Group 4; 80mg/kg body weight of the last remaining aqueous fraction + CCl_4
Group 5; 160mg/kg body weight of the last remaining aqueous fraction + CCl_4
Group 6; 240 mg/kg body weight of the last remaining aqueous fraction + CCl_4

Table VII: Effects of Saturated butanol extract of stem bark of *Mimosa pigra* on the activities of microsomal Malondialdehyde, Aniline hydroxylase, Total Protein and Cholesterol in CCl_4 treated rats.

Groups	Malondialdehyde (nmol/mg protein)	Glu-6-Phosphatase (U/mg protein)	Aniline Hydroxylase (U/mg protein)	Cholesterol (µg/mg protein)	Total Protein (mg/g liver)
1	69.51±0.95	9.86±3.65	3.91±0.05	2.99±0.06	4.51±0.45
2	126±5.15^{a}	1.55±0.23^{a}	0.99±0.25^{a}	7.65±0.20^{a}	1.01±0.33^{a}
3	76.98±1.62^{b}	8.36±1.25^{b}	3.53±1.46^{b}	3.11±0.80^{b}	3.91±0.03^{b}
4	100.01±2.81^{abc}	3.92±1.25^{abc}	1.23±0.93^{abc}	5.81±0.32^{ab}	1.90±0.72^{ab}
5	86.28±0.93^{bc}	5.11±1.01^{c}	2.01±1.01^{ab}	4.11±0.41^{abc}	2.72±0.11^{abc}
6	76.59±1.80 ^{bc}	7.98±0.93^{bc}	2.95±0.86^{c}	3.73±0.40^{ab}	3.33±0.52^{ab}

Values are expressed as mean ± standard error of five (5) replicates. a= significantly different vs group 1: p<0.05; b= significantly different vs group 2; c= significantly different vs group 3: p<0.05; using analysis of variance (ANOVA), Duncan Bonferroni multiple comparison, Instat Graph Pad Software (San Diego, USA).

Group 1; (normal control) liquid paraffin treated group
Group 2; (negative control) 30% CCl_4 in liquid paraffin treated group
Group 3; Silymarin 100mg/kg + CCl_4 treated group
Group 4; 80mg/kg body weight of butanol fraction + CCl_4
Group 5; 160mg/kg body weight of butanol fraction + CCl_4
Group 6; 240 mg/kg body weight of butanol fraction + CCl_4

Inhibitions of the generation of free radicals are necessary for protection against CCl_4 hepatotoxicity [48]. The body system has an effective defense mechanism that neutralizes free radical-induced damage. This is accomplished by antioxidant enzymes such as CAT, SOD and Glutathione Peroxidase as a defense [49]. In CCl_4 -induced hepatotoxicity, the balance between reactive oxygen species production and antioxidant defenses may be lost hence leading to oxidative stress. This stress affects cellular functions leading to hepatic necrosis.

The quest to unravel the mechanism of action on the most active fraction (butanol) relies on probing the antioxidant activities. In this study, significant decreases ($p<0.05$) in the levels of vitamins A, C, and E, Reduced Glutathione, Superoxide Dismutase and Catalase in group administered CCl_4 only were observed as compared to control rats. Nevertheless, groups of rats treated stem bark extract (methanol) of *M. pigra* and silymarin with CCl_4 had significantly ($p<0.05$) increased levels of vitamins, Superoxide Dismutase, Catalase and reduced Glutathione. This is similar with the reported severe oxidative stress and massive production of reactive oxygen species, leading to depletion of Glutathione, vitamins C and E [50]. The hepatoprotective activity of *M. pigra* may be due to molecular mechanisms such as inhibitions of free radicals and reactive oxygen species, reduction of inflammation and increased production of growth factors that are important for replacement of damaged liver cells.

Observed increases in levels of antioxidants and vitamins by the butanol fraction, clearly demonstrate that *M. pigra* has antioxidant activities.

CONCLUSION

The results have indicated that administration of butanol fraction of *M. pigra* simultaneously with CCl_4 to be the most potent hepatoprotective fraction of the plant comparable to silymarin followed by ethyl acetate. The results suggest that the action of butanol fraction of *M. pigra* stem bark may be modulated via natural

antioxidant, hepatoprotective and detoxifying enzymes due to the biological activity of the compounds in the plant.

ACKNOWLEDGEMENT

Authors wish to acknowledge the support of Laboratory staff, Department of Biochemistry and Molecular Biology, Usmanu Danfodiyo University, Sokoto, Nigeria.

REFERENCES

1. Majeed I, Rizwan K, Ashar A, Rsheed T, Amarowics R, Kausar H, et al. A comprehensive review of the ethnotraditional uses and biological and pharmacological potential of the genus Mimosa. Int. J Mol. Sci. 2021; 22(14): 7463. doi:10.3390/ijms22147463.
2. Khatun H, Barmon J, Lopa SS, Maniruzzaman MD, Robiul Islam MD, Mamun AL. The methanolic extract of *Mimosa pigra* leaves reduces the severity of diarrhoea as well as inhibits the growth of diarrhea-causing bacteria. Int. J Pharm. Pharm. Sci. 2022; 14(3): 47-52. doi:10.22159/ijpps.2022v14i3.43603.
3. Devarbhavi H, Asrani SK, Ara JP, Nartey YA, Pose E, Kamath PS. Global burden of liver disease. J Hepatol. 2023; 79(2):516-537. doi:10.1016/j.jhep.2023.03.017.
4. Yaschilal MB, Getnet M, Kidan H. Hepatoprotective and antidiarrheal activities of the hydromethanol crude extract and solvent fractions of *Schinus molle* L (Anacardiaceae)leave and fruit in mice. Metabol. Open. 2024; 21:100272. doi: 10.1016/j.metop.
5. Mas'ud H, Estella AT, Bayaga H, Njinkio NB, Charles NF. Evaluation of Hepatoprotective activity and acute toxicity of *Guiera senegalensis* leaf extract on wistar rats. J Adv. Med. Pharm. Sci. 2024; 26(5): 1-17. doi: 10.9734/jamps/2024/v26i5682.
6. Cano L, Foucher F, Musso O. Geographic diversity of human liver cancers mirrors global social inequalities. Front. Oncol. 2025; 15:1565692. doi:10.3389/fonc.2025.1565692.
7. Nagarjuna D, Karthikeyan E. Alcohol-associated liver disease: A review. Gastroenterol. Endosc.2025;3(2):65-85. https://doi.org/10.1016/j.gande.2025.01.003.
8. Jiang Z, Zeng G, Dai H, Bian Y, Wang L, Cao W et al. Global, regional and national burden of liver cancer 1990-2021:a systematic analysis of the global burden of disease study 2021. BMC Public Health. 2025; 25:931. https://doi.org/10.1186/s12889-025-22026-6.
9. Anurag S, Pritt V, Mukesh KS. A comprehensive review on hepatoprotective activity of some medicinal plants. Int. J Pharm. Res. Appl. 2023; 8(2):664-672. doi:10.35629/7781-08026646
10. Arman M, Chowdhury KAA, Bari MdS, Khan MF, Huq MMdA, Haque MdA, et al. Hepatoprotective potential of selected medicinally important herbs: evidence from ethnomedicinal, toxicological and pharmacological evaluations. Phytochem. Rev. 2022; 21: 1863-1886. https://doi.org/10.1007/s11101-022-09812-5.
11. Aladejana EB, Aladejana AE. Hepatoprotective activities of polyherbal formulations: Asystematic review. J Med. Plants Econ. Devel. 2023;7(1): a206 https://doi.org/10.4102/jomped.v7i1.206.
12. Schuppan D, Jia JD, Brinkhaus B, Hahn EG. Herbal products for liver diseases: A therapeutic challenge for the new millennium. Hepatol. 1999; 30(4):1099-1104. doi: 10.1002/hep.510300437.
13. Balu AVM, Mohammad I, Prakash G, Byeong-Cheol A, Pasupuleti VR, Muhammad DS. Hepatoprotective potential of Malaysian Medicinal plants: A review on phytochemicals, oxidative stress and antioxidant mechanisms. Molecules. 2022; 27(5):1533. doi: 10.3390/molecules27051533.
14. WHO. General guidelines for methodologies on research and evaluation of traditional medicine. Geneva: WHO. 2000; (1): 35.
15. OECD, 2001. Guideline for testing chemicals. Acute toxicity – up and down procedure. 1-35.
16. Guntupalli MMR, Chandana VR, Palpu P, Anine S. Hepatoprotective effects of rubiadin, a major constituent of rubia cordifolia linn. J Ethnopharmacol. 2006; 103(3): 484-490. doi: 10.1016/j.jep.2005.08.073.
17. Reitman S, Frankel SA. Colorimetric method for the determination of serum glutamicoxaloacetic and glutamic pyruvic transaminases. Am. J Clin. Pathol. 1957; 28(1): 56-63. doi: 10.1093/ajcp/28.1.56.
18. Wright PJ, Leathhood PD, Plummer DT. Enzymes in rat urine: Alkaline phosphatase. Enzymologia. 1972; 42(4):317-327.
19. Corcoran RM, Durnan SM. Albumin determination by a modified bromocresol green method. Clin. Chem. 1977; 23: 765-766.
20. Gornall AG, Bardawould CJ, David MM. Determination of serum proteins by means of the biuret reaction. J Biol. Chem. 1949; 177 (2): 751-766.
21. Malloy HT, Evelyn KA. The determination of bilirubin with the photometric colorimeter. J Biol. Chem.1937; 119: 481-490.
22. Szasz G, Persiyn JP. New Substrates for measuring Gamma glutamyl transpeptidases activity. Klinische Chemie Klinische. Biochem. 1974, 12(5): 228.
23. Sinha AK. Colorimetric assay of catalase. Anal. Biochem. 1972; 47(2):389-394. doi: 10.1016/0003-2697(72)90132-7.
24. Ellman GL. Tissue sulfhydryl groups. Arch. Biochem. Biophys. 1959; 82:70-77. doi: 10.1016/0003-9861(59)90090-6.
25. Kakkar P, Das B, Viswanathan PN. A modified spectrophotometric assay of superoxide dismutase. Indian J Biochem. Biophys. 1984; 21(2):130-132.
26. Omaye ST, Turnbull JD, Sauberlich HE. Selected methods for the determination of ascorbic acid in

animal cells, tissues and fluids. Methods Enzymol. 1979; 62:3-11. doi: 10.1016/0076-6879(79)62181-x.
27. Leelavinothan P, Kalist S. Beneficial effect of hesperetin on cadmium induced oxidative stress in rats: an *in vivo* and *in vitro* study. Eur. Rev. Med. Pharmacol. Sci. 2011; 15 (9):992-1002.
28. Desai ID. Vitamin E analysis method for animal tissue. Methods Enzymol. 1984; 105:138-147. doi: 10.1016/s0076-6879(84)05019-9.
29. Edeoga HO, Okwu DE, Mbaebie BO. Phytochemical constituents of some Nigerian medicinal plants. Afr. J Biotechnol. 2005; 4(7): 685-688. doi:10.5897/AJB2005.000-3127.
30. Sofowora A. Medicinal plant and Traditional Medicine in Africa. Lagos-Nigeria. Ibadan: Spectrum books Limited; 1993.
31. Trease GE, Evans WC. Pharmacognosy. 13th Ed. London: Baillиre Tindall; 1989.
32. Harborne JB. Phytochemical methods: A guide to a modern techniques of plant analysis. London: Chapman and Hall LTd; 1973.
33. Taussky HH, Shorr E. A microcolorimetric method for the determination of inorganic phosphorous. J Biol. Chem. 1953; 202(2): 675-685.
34. Koide H, Oda T. Pathological occurrence of glucose-6-phosphatase in serum in liver diseases. Clin. Chim. Acta. 1959; 554-561. doi: 10.1016/0009-8981(59)90165-2.
35. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the folin phenol reagent. J Biol. Chem. 1951; 193 (1): 265-275.
36. Peterson FJ, Mason RP, Hovsepian J, Holtzman JL. Oxygen-sensitive and- insensitive nitro-reduction by *Escherichia coli* and rat hepatic microsomes. J Biol. Chem. 1979; 254 (10): 4009-4014.
37. Schenkman JB, Remmer H, Estabrook RW. Spectral studies of drug interaction with hepatic microsomal cytochrome. Mol. Pharmacol. 1967; 3(2):113-123. doi:10.1016/S0026-895X(25)14748-2.
38. Fauziaha PN, Maskoen AM, Yuliati T, sWidiarsih E. Optimized steps in determination of malondialdehyde (MDA) standards to diagnostic of lipid peroxidation. Padjadjaran J Dent. 2018; 30(2): 136-139. doi: http://dx.doi.org/10.24198/pjd.vol30no2.18329.
39. Rudel LL, Morris MD. Determination of cholesterol using O-phthalaldehyde. J Lipid Res. 1973; 14: 364-366.
40. Liu RH. Potential synergy of phytochemicals in cancer prevention: mechanism of action. The J Nutr. 2004; 134 (12): 3479S-3485S. doi: 10.1093/jn/134.12.3479S.
41. Jadhav, PA, Thomas AB, Pathan KM, Chaudhari SY, Wavhale RD, Chitlange SS. Unlocking the therapeutic potential of unexplored phyto compounds as hepatoprotective agents through integration of network pharmacology and in-silico analysis. Sci. Rep. 2025; 15: 8425. https://doi.org/10.1038/s41598-025-92868-y.
42. Adetunji OA, Olugbami JO, Adegoke AM, Gbadegesin MA, Odunola OA. Reno-Hepatoprotective and Antidiabetic Properties of Methanol leaf extract of *Laportea aestuans* in Wistar rats. J Evid. Based Integr. Med.2021; Volume 26: 2515690X211017464. doi: 10.1177/2515690X211017464.
43. Zahra M, Abrahamse H, George BP. Flavonoids: Antioxidant powerhouses and their role in Nanomedicine. Antioxidants (Basel). 2024; 13(8):922. doi:10.3390/antiox13080922.
44. Adurosakin OE, Iweala EJ, Otike JO, Dike ED, Uche ME, Owanta JI, et al. Ethnomedicinal uses, phytochemistry, pharmacological activitities and toxicological effects of *Mimosa pudica*- A review. Pharmacol. Research-Modern Chinese Med. 2023;7:100241. https://doi.org/10.1016/j.prmcm.2023.100241.
45. Gong Q, Wang X, Liu Y, Yuan H, Ge Z, Liu Y, et al. Potential hepatoprotective effects of Allicin on Carbon tetrachloride-induced Acute Liver Injury in mice by inhibiting oxidative stress, inflammation, and apoptosis. Toxics. 2024; 12(5):328. doi: 10.3390/toxics12050328.
46. Unsal V, Cicek M, Sabancilar I. Toxicity of carbon tetrachloride, free radicals and role of antioxidants. Rev. Environ. Health. 2021; 36 (2): 279-295. doi:10.1515/reveh-2020-0048.
47. Yaman SO, Ayhanci A. Accenting lipid peroxidation. Intech Open 2021; 95802. http://dx.doi.org/10.5772/intechopen.95802.
48. Johara FT, Hossain S, Jain P, Bristy AT, Emran T, Ahmed R, et al. Amelioration of CCl_4-induced oxidative stress and hepatotoxicity by *Ganoderma lucidum* in long Evans rats. Sci. Rep. 2023; 139(1):9909. doi: 10.1038/s41598-023-35228-y.
49. Marwicka J, Zieba A. Antioxidants as a defence against reactive oxygen species. Aesth. Cosmetol. Med. 2021; 10(6): 271-276. https://doi.org./10.52336/acm.2021.10.6.02.
50. Jamova K, Alomar SY, Alwasel SH, Nepovimova E, Kuca K, Valko M. Several lines of antioxidant defense against oxidative stress: antioxidant enzymes, nanomaterials with multiple enzyme-mimicking activities and low molecular-weight antioxidants. Arch. Toxicol. 2024; 98(5):1323-1367. doi: 10.1007/s00204-024-03696-4.