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HEPATOPROTECTIVE POTENTIALS OF ACTIVE FRACTIONS OF *Lawsonia inermis (Lythraceae)* AGAINST ACETAMINOPHEN-INDUCED LIVER DAMAGE IN RATS

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ABSTRACT

Lawsonia inermis (henna) is widely distributed across African countries. It has been used by herbal doctors in treating oxidative stress-related diseases in humans. Its leaves contain valuable antioxidants and protective molecules, which can protect humans against hepatic damage from toxic effects. This study analyzed the hepatoprotective ability of *L. inermis* leaf fractions against acetaminophen-induced liver damage in rats. Fifty-four (54) rats were divided into nine (9) groups, and each group allocated six (6) rats. Group 1, 2, 3, 4, 5, 6, 7, 8 and 9 were given 2 mL/kg/day of normal saline, normal control (NCN), 600 mg/kg/day of acetaminophen alone, negative control (NCA), 600mg/kg/day of acetaminophen and 200 mg of n-hexane fraction (AH200), 600mg/kg/day of acetaminophen and 400 mg of n-hexane fraction (AH400), 600 mg/kg/day of acetaminophen and 200 mg of ethyl acetate fraction (AE200), 600 mg/kg/day of acetaminophen and 400 mg of ethyl acetate fraction (AE400), 600mg/kg/day of acetaminophen and 200 mg of methanol fraction (AM200), 600mg/kg/day of acetaminophen and 400 mg of methanol fraction (AM400), 600mg/kg/day of acetaminophen and 25 mg/kg/day of silymarin (AS25), respectively. The serum chemistry, lipid profiles, hepatic enzyme markers and oxidative stress markers were analyzed using standard methods. Histopathological examination of hepatocytes of the treated rats was determined. The results revealed lower significant values of total protein, albumin, globulin, HDL, SOD, CAT, and GPx, and higher significant values of serum bilirubin, glucose, creatinine and urea, triglyceride, cholesterol, LDL, ALT, AST, ALP, LDH and MDA in NCA-treated rats than in the others. Interestingly, higher insignificantly levels of total protein, albumin, globulin, HDL, SOD, CAT, and GPx, and lowered insignificantly values of bilirubin, glucose, creatinine, urea, triglyceride, cholesterol, LDL, asnd ALT, AST, ALP, LDH, and MDA were noted among *L. inermis* fractions treated rats. Abnormal hepatic cell architectures were observed in the livers of NCA-treated rats than in other groups, however, reduced hepatic dysfunction/normal liver cell architectures were restored among the rats treated with *L. inermis* leave fractions. These results have suggested that all levels of *L. inermis* leave fractions in this study possessed hepatoprotective potential against acetaminophen-induced hepatic cell damage,

Keywords: Acetaminophen, active fractions, hepatoprotective, *L. inermis*, liver damage, rats

INTRODUCTION

The fundamental area of herbal medicine that deals with the preparations of plants to combat/improve health and the management of diseases is called herbal therapy (Atangwho, 2008). In traditional societies especially, in African continents, preparations of herbs and natural plants are used to

enhance the healing of different ailments (Bussmann & Sharon, 2006). A lot of people are moving toward liking traditional treatment for these reasons; 1) natural treatment with higher potent and minimal side effects, 2) the treatments are cheap and easily accessed by individuals, and 3) the treatment of ailments with herbs/natural plants preparations use to be lasting and permanent (WHO, 2003). Frequent exposure to certain chemical toxicants and pathogens caused some complicated health issues in humans. These chemicals or drugs that are prepared to be healthy and friendly are turned to become serious health problems with various adverse effects on health (Effiong *et al*., 2014).

A good example of these toxic chemicals is acetaminophen. Acetaminophen (AC) is the synthetic drug commonly available at the pharmacy counter, mostly used to reduce fever and body pains. It is an analgesic and antipyretic drug and its safety has been considered under approved therapeutic doses. However, their overdose causes serious hepatic dysfunction in humans and animals (Effiong *et al*., 2014; Hira *et al*., 2019). Long-time intake of acetaminophen (AC) mega doses may generate kidney abnormalities (Nwanjo et al., 2006). Liver diseases have become a problem worldwide and the synthetic drugs used for their treatment are not reliable due to their serious negative side effects (Ndatsu *et al*., 2013). However, nature is the source of medicinal products, as shown by the many convectional drugs synthesized from medicinal plants (Cragg & Newman, 2013). In the African continent, there are many available plants and their materials have been reported to possess hepatocyte protective potentials. Medicinal plants contain valuable antioxidants and protective molecules, which can protect humans against hepatic damage from toxic effects (Atangwho, 2008).

Nowadays, in the pharmaceutical industry, there are lot of interests in developing medicines that possess the hepatic protective ability from herbs and plant materials (Sanchez, 2017; Florence et al., 2020). Among these plants, one is *Lawsonia inermis. Lawsonia inermis* (Lythraceae), commonly called henna (English), Laili (Hausa), and Mehendi, in India, is widely distributed across African countries (Malekzudeh et al., 1968). It is a multi-branched and glabrous tree of 2-6m tall with greyish-brown bark. The leave of *L. inermis* has been reported to be used by herbal doctors in treating oxidative stress-related diseases like rheumatoid arthritis, ulcers, diarrhoea, leprosy, fever, diabetes, and cardiac disease (Sastri et al., 1962; Malekzudeh et al., 1968). Traditionally, different parts of henna are being utilized in the management of bleeding disorders, ulcers, eczema, and skin disorders. The anti-hyperglycemia (Mikhaeil e al., 2004), immuno-stimulatory (Hemalatha et al., 2004), and memory-enhancing (Arayne et al., 2007) activities were the biological properties of *L. inermis* reported. It has been demonstrated to be an anti-inflammatory, antipyretic, and analgesic agent (Ali et al., 1995).

In Asia countries, leaf preparations are used to manage blood glucose levels (Behzad et al., 2018). The phytochemical analysis carried out showed the presence of triterpenes, steroids in the n-hexane extract (Raja et al., 2013), and flavonoids, steroids, tannins, saponins, triterpenes, and polyphenols (Garba et al., 2015). The ethanol extract of *L. inermis* leaf at the dose (0.8mg/Kg) reduced the level of blood glucose from 194 to 75 mg in alloxan-induced mice Syamsudin et al., 2008). In *an in-vitro* study, the *L. inermis* leaf methanol extract has excellent antihyperglycemic activities Arayne et al., 2007). Blood glucose level reductions (39.08%) with ethanol extract at 400mg of *L. inermis* for 21 days have been reported (Sigh et al., 2015). Chikaraddy *et*

al. (2012) and Amuzat et al. (2021) have demonstrated that an aqueous leaf extract of *L. inermis* is safe as an anti-diabetic preparation at the effective doses; of 200 mg/kg, 400 mg/kg, and 600 mg/kg. Oral administration of n-hexane, ethyl acetate and ethanol serial extraction of *L. inermis* leaves didn't cause or showed a toxic effect in rats (Widyawati et al., 2019). To date, studies on active fractions of *L. inermis* leaves extracts have not been found. This work focused on the use of *L. inermis* leaf active fractions in protecting acetaminophen's adverse effect on the hepatocytes. The current work described the hepatoprotective potentials of active fractions of *L. inermis* on acetaminophen-induced liver damage in rats.

MATERIALS AND METHODS

Collection, Identification, And Preparation of Plant Materials

Fresh leaves of L. inermis were collected from Lapai town, Lapai Local Government, Niger State, Nigeria in July 2021. The identification and authentication were done by a botanist in the Department of Biological Sciences, Ibrahim Badamasi Babangida University Lapai, Niger state (Voucher number IBB/BS/00007). The leaves on the stalk were carefully removed, dried under shade, and ground using a grinder (Lab mill, serial No. 4745, Christy and Norris Ltd, England) into powder. The powdered leaves were weighed in an airtight container and kept for future use.

Ethanol Extract and Fractions of *L. inermis* Leaves

The extraction was done using the method of Hira *et al*. (2021) with slight modification. This was done by soaking 600g of powdered leaves in 3 L of ethanol for 7 days at 0 ^{o}C. The resultant extract was filtered using Whatman Number 4 filter paper. The extracted material was evaporated using a rotary evaporator (Buchi R-200 New Castle, DE) under high pressure at 40 ^{o} C to obtain the dried ethanol extract (4.8%). A portion of ethanol *L. inermis* extract was weighed (20 g) into 250 mL n-hexane in a separating funnel to separate the soluble portion from the insoluble portion and at 35^{o} C, the n-hexane soluble portion was evaporated using a rotary evaporator that yielded 2.5% of n-hexane fraction of *L. inermis* (HFLI). The insoluble portion of n-hexane was extracted with ethyl acetate, which gave a 6.5% ethyl acetate fraction of *L. inermis* (EFLI). The insoluble portion of ethyl acetate was further extracted with 91% methanol (MFLI) to give 8.4%. All the *L. inermis* fractions collected were kept in airtight containers at 0^{o} C for future use.

Experimental Animals

Briefly, about forty-eight (48) albino rats of different sexes (220±6.25g) were used for this study. The rats were bought from the animal Laboratory, Department of Biochemistry, Federal University of Technology, Minna (FUTMIN), kept, acclimatized in standard laboratory conditions, and fed with livestock pellet and water ad libitum in the Animal House of the Department of Biochemistry, Ibrahim Badamasi Babangida University Lapai, Niger State. The animal experiments were performed following the University principles for laboratory animal use and animal caring procedures and guidelines for good laboratory practices (OECD, 2001).

Hepatotoxicity Induction

This was performed using the method of Hira et al. (2019) with slight modification. The Acetaminophen (Sigma Aldrich, U.S.A.) was dissolved in normal saline with constant stirring and the induction was done orally (p.o.) with a single dose of acetaminophen at 600mg/kg, body weight (b.w), p.o.

Experimental design and treatment

At the end of 7 days of acclimatization, the fifty-four (54) rats of different sexes were randomly shared into nine (9) groups with six (6) animals in a group. Initially, animals in groups (3, 5 & 7) were administered 200 mg/Kg of HFLI, EFLI, and MFLI, respectively, while groups (4, 6 & 8) were given 400 mg/kg doses of HFLI, EFLI and MFLI respectively, to protect their livers against expecting damages induced by the mega dose of acetaminophen. Then, the normal experimental treatment was commenced after 24 hrs of fasting for 14 days.

Table 1: Experimental design

Group	NO. of animals	Treatment
1	6	NCN
2	6	NCA
3	6	AH200
4	6	AH400
5	6	AE200
6	6	AE400
7	6	AM200
8	6	AM400
9	6	AS25

NCA: Negative control (Acetaminophen; 600 mg/kg/day); NCN: Normal control (Normal saline; 2 mL/Kg/day); AH200: Acetaminophen (600 mg/kg/day) + n-hexane fraction of *L. inermis* (200 mg/kg/day); AH400: Acetaminophen (600 mg/kg/day) + n-hexane fraction of *L. inermis* (400 mg/kg/day); AE200: Acetaminophen (600 mg/kg/day) + acetyl acetate fraction of *L. inermis* (200 mg/kg/day); AE400: Acetaminophen (600 mg/kg/day) + acetyl acetate fraction of *L. inermis* (400 mg/kg/day); AM200: Acetaminophen (600 mg/kg/day) + methanol fraction of *L. inermis* (200 mg/kg/day); AM400: Acetaminophen (600 mg/kg/day) + methanol fraction of *L. inermis* (400 mg/kg/day); AS25: Acetaminophen (600 mg/kg/day) + Silymarin (25 mg/kg/day).

On the 15th day of treatment, the animals were allowed to fast for 24 hrs, sacrificed by cervical dislocation for blood collection. The blood collected was allowed to clot, centrifuged at 3000 rpm for 2 minutes and serum was collected for the analysis of liver functions. The livers were also harvested for histopathological analysis.

Liver Damage Assessment

In this, serum was used for the biochemical analysis of ALT. AST, ALP, and total bilirubin, conjugated (direct) bilirubin, creatinine, and total protein (Reitman & Frankel, 1957); (Jendrassik & Golf, 1938). Lipid profiles (triglycerides, cholesterol, HDL, and LDL were also estimated (Sahu et al., 2005; Nigam, 2011). Superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and malondialdehyde (MDA) were estimated using the method of Nebot et al. (1983), Aebi, (1984), Paglia and Valentine, (1967) and Al-Rasheed et al. (2016), respectively.

Liver Histopathological Examination of Rats

Histopathological examinations of the harvested livers of the experimental rats were performed by preserving the rat liver tissues in a 10% formaldehyde solution. The processed liver tissues were separately embedded in paraffin wax and about 4 - 6µm thick sections were made from each fixed tissue on the different slides. Hematoxylin and Eosin (H &E) dyes were used to stain the sections on the slides (Slaoui & Fiete, 2011).

Examination and Photomicrograph of the Slides

A light microscope with ×4, ×10, and ×40 was used for the observation of slides, and the megapixels microscope camera (MoticTM 90 at ×160 magnification was used to take the photomicrographs.

Statistical analysis

The data were presented in mean±SEM (standard error of the mean). One-way analysis of variance and least significant difference (LSD) test was performed to evaluate the significant difference. Probability levels of less than 0.05 were considered significant.

RESULTS

The results of the mean serum chemistry of the studied rats are presented in table 2. It reveals a significant reduction (P≤0.05) of serum total protein (8.34 mg/dL), albumin (4.56 mg/dL), and globulin (3.78 mg/dL) in rats treated with NCA (negative control group) than in the others.

Contrarily, the NCN-treated rats had a higher significant value (P≤0.05) of total protein (21.25 mg/dL), albumin (13.78 mg/dL), and globulin (7.47 mg/dL) as compared to other groups. Indirectly, the serum bilirubin (0.173 mg/dL), glucose (153.25 mg/dL), creatinine (68.45 mg/dL) and urea (121.16 mg/dL) contents in rats treated with NCA (negative control group) are higher significantly (P≤0.05) than the other groups, while in groups treated with NCN (normal control) demonstrated lower significant values (P≤0.05) of bilirubin (0.110 mg/dL), glucose 93.24 mg/dL), creatinine 29.12 mg/dL) and urea (59.34 mg/dL) (Table 2). Inversely, all groups of rats treated with different levels of *L. inermis* fractions had higher significant values (P≤0.05) of total protein, albumin, and globulin and lower significant values (P≤0.05) of serum bilirubin, glucose, creatinine, and urea than NCA- and AS25-treated rats (Table 2).

Table 2: Serum chemistry level of rats treated with *L. inermis* leave fractions

Treatment	Serum chemistry levels (mg/dL)						
	Total protein	Albumin	Globulin	Bilirubin	Glucose	Creatinine	Urea
NCA	8.34±1.04^{d}	4.56±1.11^{d}	3.78±2.23^{d}	0.173±1.43^{a}	153.25±2.64^{a}	68.45±2.56^{a}	121.16±1.65^{a}
NCN	21.25±1.23^{a}	13.78±1.35^{a}	7.47±2.54^{a}	0.110±1.24^{d}	93.24±2.34^{c}	29.12±2.11^{d}	59.34±1.12^{c}
AH200	16.45±1.23^{b}	12.03±1.21^{b}	4.42±2.21^{b}	0.125±1.12^{c}	96.34±2.32^{c}	37.46±2.32^{c}	64.34±1.25^{c}
AH400	16.48±1.21^{b}	12.11±1.12^{b}	4.37±2.13^{b}	0.124±1.12^{c}	96.12±2.30^{c}	37.32±2.21^{c}	64.21±1.32^{c}
AE200	16.65±1.34^{b}	12.21±1.42^{b}	4.44±2.01^{b}	0.125±1.32^{c}	95.54±2.31^{c}	37.26±2.23^{c}	64.02±1.24^{c}
AE400	16.63±1.31^{b}	12.32±1.36^{b}	4.31±2.15^{b}	0.124±1.21^{c}	95.23±2.43^{c}	37.23±2.21^{c}	64.11±1.31^{c}
AM200	16.74±1.42^{b}	12.45±1.14^{b}	4.29±2.31^{b}	0.120±1.11^{c}	94.45±2.20^{c}	37.12±2.14^{c}	63.56±1.21^{c}
AM400	16.80±1.36^{b}	12.50±1.21^{b}	4.30±2.24^{b}	0.121±1.14^{c}	94.24±2.14^{c}	37.21±2.16^{c}	63.48±1.32^{c}
AS25	14.56±1.14^{c}	10.76±1.10^{c}	3.80±2.43^{c}	0.132±1.34^{b}	102.56±2.56^{b}	43.21±2.23^{b}	72.26±1.54^{b}

Values with the same superscript letter(s) along the same column are not significant different ($P \leq 0.05$). NCA: Negative control (Acetaminophen; 600 mg/kg/day); NCN: Normal control (Normal saline; 2 mL/Kg/day); AH200: Acetaminophen (600 mg/kg/day) + n-hexane fraction of *L. inermis* (200 mg/kg/day); AH400: Acetaminophen (600 mg/kg/day) + n-hexane fraction of *L. inermis* (400 mg/kg/day); AE200: Acetaminophen (600 mg/kg/day) + acetyl acetate fraction of *L. inermis* (200 mg/kg/day); AE400: Acetaminophen (600 mg/kg/day) + acetyl acetate fraction of *L. inermis* (400 mg/kg/day); AM200: Acetaminophen (600 mg/kg/day) + methanol fraction of *L. inermis* (200 mg/kg/day); AM400: Acetaminophen (600 mg/kg/day) + methanol fraction of *L. inermis* (400 mg/kg/day); AS25: Acetaminophen (600 mg/kg/day) + Silymarin (25 mg/kg/day).

The results of hepatic enzyme markers level of rats treated with *L. inermis* fractions are shown in table 4. It reveals that the normal control (NCN) group had mean serum ALT not significantly different (P≤0.05) as compared to the groups treated with various fractions of *L. inermis*. The NCN groups had smaller mean serum ALT and AST values of 51.56 and 125.24 IU/L, respectively, and the values do not differ significantly compared to the groups treated with AH200 (54.56 and 126.56 IU/L), AH400 (53.56 and 125.56 IU/L), AE200 (54.26 and 126.12 IU/L), AE400 (52.56 and 123.25 IU/L), AM200 (52.14 and 121.35 IU/L) and AM400 (51.67 and 121.56 IU/L), respectively. These values are significantly reduced (p≤0.05), as compared to that of the positive control (AS25) (61.45 and 136 IU/L) and the negative control (NCA) (97.45 and 231.45 IU/L) respectively (Table 4). In contrast, the levels of serum ALP and LDH in the normal control (NCN), positive control (AS25) groups, and the groups served with different concentrations of *L. inermis* fractions are not significantly different (P≤0.05), but significantly elevated in the negative control group (Table 4). The negative control group had a high value of ALP (87.24 IU/L) and LDH (112.24 IU/L), which differed significantly (P≤0.05), as compared to the normal, positive controls and all fractions treated groups (Table 4).

Table 3: Serum liver enzyme marker levels of rats treated with *L. inermis* leave fractions

	Hepatic enzyme markers (IU/L)			
Treatment	ALT	AST	ALP	LDH
NCA	97.45±3.21^{a}	231.45±3.34^{a}	87.24±1.56^{a}	112.244±2.56^{a}
NCN	51.56±1.60^{c}	125.24±1.68^{c}	42.60±1.20^{b}	81.45±1.04^{b}
AH200	54.45±2.01^{c}	126.56±2.11^{c}	44.54±1.54^{b}	84.82±1.53^{b}
AH400	53.56±1.84^{c}	125.56±2.05^{c}	43.64±1.26^{b}	84.63±1.50^{b}
AE200	54.26±1.82^{c}	126.12±2.01^{c}	44.23±1.40^{b}	84.52±1.34^{b}
AE400	52.56±1.56^{c}	123.25±1.46^{c}	43.45±1.18^{b}	82.65±0.68^{b}
AM200	52.14±1.45^{c}	121.35±0.84^{c}	43.26±1.08^{b}	82.06±0.24^{b}
AM400	51.67±0.43^{c}	121.56±1.24^{c}	42.83±0.68^{b}	81.68±0.21^{b}
AS25	61.45±2.01^{b}	136.34±2.56^{b}	48.23±1.42^{b}	88.56±1.66^{b}

Values with the same superscript letter(s) along the same column are not significant different (*P* ≤ 0.05). NCA: Negative control (Acetaminophen; 600 mg/kg/day); NCN: Normal control (Normal saline; 2 mL/Kg/day); AH200: Acetaminophen (600 mg/kg/day) + n-hexane fraction of *L. inermis* (200 mg/kg/day); AH400: Acetaminophen (600 mg/kg/day) + n-hexane fraction of *L. inermis* (400 mg/kg/day); AE200: Acetaminophen (600 mg/kg/day) + acetyl acetate fraction of *L. inermis* (200 mg/kg/day); AE400: Acetaminophen (600 mg/kg/day) + acetyl acetate fraction of *L. inermis* (400 mg/kg/day); AM200: Acetaminophen (600 mg/kg/day) + methanol fraction of *L. inermis* (200 mg/kg/day); AM400: Acetaminophen (600 mg/kg/day) + methanol fraction of *L. inermis* (400 mg/kg/day); AS25: Acetaminophen (600 mg/kg/day) + Silymarin (25 mg/kg/day).

The results of lipid profile rats treated with different concentrations of *L. inermis* are shown in table 4. It reveals that increase significantly (P≤0.05) of triglyceride (434.12 mg/dL), cholesterol (934.12 mg/dL), LDL (94.34 mg/dL) and LDL/HDL (1.67 mg/dL) were recorded in NCA-treated rats as compared to others. Lower significant value of triglyceride (120.24 mg/dL), cholesterol (551.23 mg/dL), LDL (46.12 mg/dL) and LDL/HDL (0.41 mg/dL) were detected in normal control (NCN-treated) group than the

other groups. However, lower HDL (56.42 mg/dL) and higher HDL (112.20 mg/dL) levels were demonstrated in NCA- and NCN-treated rats, respectively. Contrarily, the lipid profile levels recorded in various levels of *L. inermis* fractions-treated rats did not differ significantly P≤0.05) from each other, but were lower significantly than the negative and positive control groups. In contrast, the HDL (56.42 mg/dL) value in NCA-treated rats is lower than the values recorded in *L. inermis* fractions-treated rats (Table 4).

Table 4: Serum lipid level of rats treated with *L. inermis* leave fractions

Treatment	Serum lipid levels (mg/dL)				
	Triglyceride	Cholesterol	LDL	HDL	LDL/HDL ratio
NCA	434.12±1.12^{a}	934.12±2.53^{a}	94.34±0.84^{a}	56.42±1.54^{d}	1.67±0.11^{a}
NCN	120.24±1.02^{e}	551.23±2.14^{d}	46.12±0.54^{d}	112.20±1.45^{a}	0.41±0.23^{d}
AH200	135.23±1.23^{c}	645.34±2.24^{c}	51.68±0.65^{c}	104.61±1.26^{b}	0.55±0.24^{c}
AH400	133.40±1.21^{c}	644.36±2.14^{c}	54.32±0.45^{c}	104.45±1.23^{b}	0.52±0.25^{c}
AE200	136.65±1.32^{c}	644.12±2.32^{c}	54.46±0.35^{c}	104.55±1.32^{b}	0.52±0.12^{c}
AE400	136.81±1.22^{c}	643.65±2.34^{c}	54.43±0.46^{c}	104.46±1.43^{b}	0.52±0.22^{c}
AM200	136.15±1.14^{c}	624.78±2.25^{c}	55.21±0.26^{c}	104.12±1.32^{b}	0.53±0.11^{c}
AM400	134.56±1.20^{c}	623.65±2.22^{c}	55.32±0.21^{c}	104.14±1.28^{b}	0.53±0.12^{c}
AS25	213.43±1.43^{b}	682.21±2.45^{b}	64.68±0.43^{b}	96.35±1.52^{c}	0.67±0.24^{b}

Values with the same superscript letter(s) along the same column are not significant different ($P \leq 0.05$). ; NCA: Negative control (Acetaminophen; 600 mg/kg/day); NCN: Normal control (Normal saline; 2 mL/Kg/day)AH200: Acetaminophen (600 mg/kg/day) + n-hexane fraction of *L. inermis* (200 mg/kg/day); AH400: Acetaminophen (600 mg/kg/day) + n-hexane fraction of *L. inermis* (400 mg/kg/day); AE200: Acetaminophen (600 mg/kg/day) + acetyl acetate fraction of *L. inermis* (200 mg/kg/day); AE400: Acetaminophen (600 mg/kg/day) + acetyl acetate fraction of *L. inermis* (400 mg/kg/day); AM200: Acetaminophen (600 mg/kg/day) + methanol fraction of *L. inermis* (200 mg/kg/day); AM400: Acetaminophen (600 mg/kg/day) + methanol fraction of *L. inermis* (400 mg/kg/day); AS25: Acetaminophen (600 mg/kg/day) + Silymarin (25 mg/kg/day).

The results of oxidative stress markers in rats treated with *L. inermis* fractions are shown in table 5. It reveals that the NCN-treated group had the greatest level of SOD (97.34 IU/L), CAT (231.21 IU/L) and GPx (134.48 IU/L), which is higher significantly (P≤0.05) than other groups. The negative control group had the lowest SOD (31.25 IU/L), CAT (25.54 IU/L), and GPx (34.68 IU/L) levels than the AS25-treated group (51.68 IU/L (Table 5). The various levels of AH, AE, and AM-treated groups have not differed significantly from each other, but are significantly lower (P≤0.05) than the NCA-treated group (Table 5). However, the negative control group (NCA) had the highest serum MDA value of 6.75 IU/L, which differed significantly (P≤0.05) compared to the others. In addition, the MDA values in normal (1.62 IU/L), positive control groups (1.69 IU/L), and other groups treated with various levels of fractions were not significantly different from each other (P≤0.05), but lower than that of the NCA-treated group (Table 5).

Table 5: Serum antioxidant enzyme levels of rats treated with *L. inermis* leave fractions

	Serum antioxidant enzymes (IU/L)			
Treatment	SOD	CAT	GPx	MDA
NCA	31.25±1.56^{c}	25.54±1.02^{d}	34.68±0.24^{d}	6.75±3.20^{a}
NCN	97.34±1.43^{a}	231.21±2.14^{a}	134.48±1.56^{a}	1.62±2.10^{b}
AH200	56.24±1.54^{b}	80.46±1.54^{b}	91.21±1.34^{c}	1.67±1.45^{b}
AH400	54.56±1.64^{b}	81.66±1.32^{b}	93.23±1.45^{c}	1.66±1.46^{b}
AE200	51.45±1.56^{b}	83.23±1.62^{b}	96.57±1.46^{c}	1.65±1.23^{b}
AE400	54.78±1.53^{b}	83.68±1.45^{b}	97.78±1.53^{c}	1.66±1.35^{b}
AM200	56.65±1.42^{b}	84.56±1.24^{b}	98.45±1.65^{c}	1.68±1.12^{b}
AM400	53.56.±1.56^{b}	85.04±1.46^{b}	98.65±1.61^{c}	1.65±1.21^{b}
AS25	51.68±1.35^{c}	66.76±1.34^{c}	96.26±1.04^{c}	1.69±1.32^{b}

Values with the same superscript letter(s) along the same column are not significant different ($P \leq 0.05$). NCA: Negative control (Acetaminophen; 600 mg/kg/day); NCN: Normal control (Normal saline; 2 mL/Kg/day); AH200: Acetaminophen (600 mg/kg/day) + n-hexane fraction of *L. inermis* (200 mg/kg/day); AH400: Acetaminophen (600 mg/kg/day) + n-hexane fraction of *L. inermis* (400 mg/kg/day); AE200: Acetaminophen (600 mg/kg/day) + acetyl acetate fraction of *L. inermis* (200 mg/kg/day); AE400: Acetaminophen (600 mg/kg/day) + acetyl acetate fraction of *L. inermis* (400 mg/kg/day); AM200: Acetaminophen (600 mg/kg/day) + methanol fraction of *L. inermis* (200 mg/kg/day); AM400: Acetaminophen (600 mg/kg/day) + methanol fraction of *L. inermis* (400 mg/kg/day); AS25: Acetaminophen (600 mg/kg/day) + Silymarin (25 mg/kg/day).

The results of the liver sections of rats treated with different levels of *L. inermis* fractions are shown (Figure 1). The adverse effects of acetaminophen intoxication were observed in rats served with acetaminophen alone (NCA group) as compared to other groups. In NCA-treated rats, severely abnormal hepatic cell architectures (cytoplasmic vacuoles with dense vascular congestions, nuclear debris, dilated hepatocyte, sinusoid, and swelling of the liver cells i.e. neutrophilic infiltration)) were noticed (Figure A). In contrast, the liver sections of rats treated with normal saline (NCN-treated rats) recorded intact hepatic architecture with normal hepatocytes (Figure B). Interestingly, the histopathological lesions (centrilobular, swollen, degenerated, and necrotic hepatocytes) observed in the hepatic cells of NCA-treated rats were considerably reduced or almost reversed to normal in the hepatic cells of AS25-, AH-, AE-, and AM-treated rats (Figure C – I). The reversed to almost normal necrosis and the neutrophilic infiltration were observed in the hepatic cells of rats treated with all levels of *L. inermis* fractions and the positive control groups compared to that of NCA-treated rats.

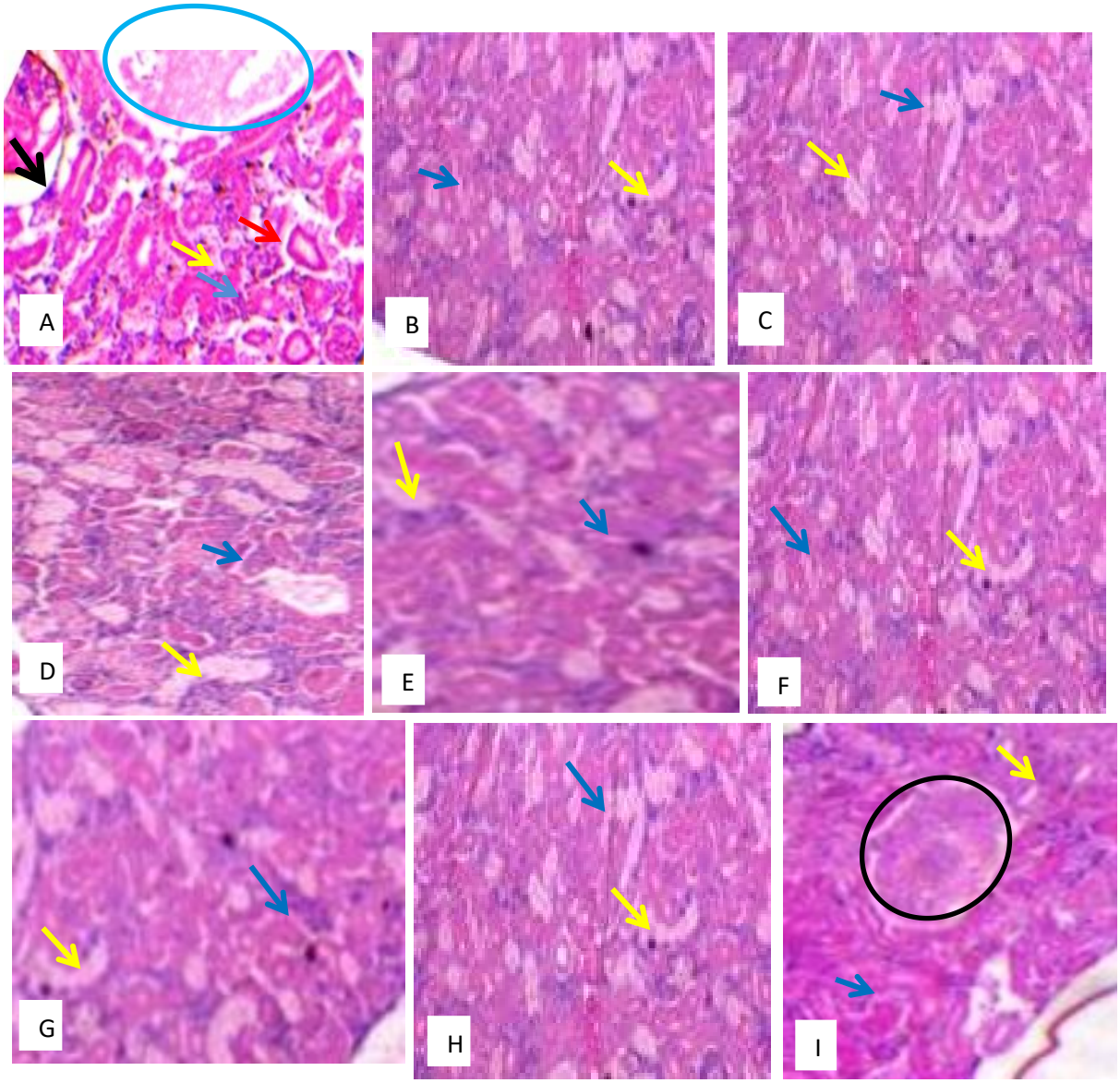

Figure 1: The hepatic cells assessment of healthy rats treated with *L. inermis* leaf fractions at 21 days of the experiment (40×). (A) Negative control (600 mg/kg of acetaminophen alone); (B) Normal control (2 mL/Kg of normal saline); (C) 600 mg/kg of acetaminophen + 200 mg/kg of n-hexane fraction of *L. inermis*; (D) 600 mg/kg of acetaminophen + 400 mg/kg of n-hexane fraction of *L. inermis*; (E) 600 mg/kg of acetaminophen + 200 mg/kg of acetyl acetate fraction of *L. inermis*; (F) 600 mg/kg of acetaminophen + 400 mg/kg of acetyl acetate fraction of *L. inermis*; (G) 600 mg/kg of acetaminophen + 200 mg/kg of methanol fraction of *L. inermis*; (H) 600 mg/kg of acetaminophen + 400 mg/kg of methanol fraction of *L. inermis*; (I) 600 mg/kg of acetaminophen + 25 mg/kg of Silymarin. Black arrow: dilated sinusoid, Red arrow: dilated hepatocyte, Blue outline: Cytoplasmatic vacuole with dense vascular congestions and nuclear debris in the liver hepatocyte, Yellow arrow: normal sinusoid, Blue arrow: normal hepatocyte, Black outline: normal central vein.

DISCUSSIONS

In this study, the hepatoprotective ability of active fractions of *L. inermis* against acetaminophen-induced hepatic injuries in rats was investigated. The extensive utilization of the acetaminophen-induced hepatic toxicity model is performed to ascertain the protective measures of plant extracts and conventional drugs (Avijeet et al., 2008). Lower significant ($P \leq 0.05$) levels of total protein, albumin, and globulin observed in NCA-treated rats as compared to others (Table 2) are an indication of hepatocyte damage generated by higher dosing of acetaminophen. This alteration in liver tissue may result from functional endoplasmic reticulum failure, failing the synthesis of protein and triglyceride bioaccumulation (Ravikumar et al., 2010). The mega-dosing of acetaminophen could negatively affect the metabolism of protein probably through protein synthesis, such as albumin and globulin inhibition in the hepatocytes.

In contrast, the higher significant level of bilirubin in NCA-treated rats than in the other (Table 2) may attribute to the process of compensation/retaliation in response to alterations in peroxidative cells, which causes biliary gland injury. In vivo, bilirubin acts as a powerful antioxidant, anti-mutagen, and a strong protector of endogenous tissue (Ndatsu et al., 2013). Likewise, a higher significant level of blood glucose detected in NCA-treated rats than in other groups is a sign of hepatocytes damage (Table 2). This finding supported what was reported by Ndatsu et al. (2013), and Amuzat et al. (2021) that continuous dosing of acetaminophen elevated the blood glucose in rats. The metabolism of glucose may be inhibited by hepatotoxicity by down-regulating the production and secretion of insulin from the pancreas or by down-regulation of blood glucose to tissues.

Surprisingly, the insignificant decreased levels of blood glucose observed in all rats served with *L. inermis* fractions confirm its hepatoprotection ability and liver healing. This mechanism of action exhibited by *L. inermis* fractions could be due to its ability to increase insulin production and secretion by the liver. Amuzat et al. (2021) have narrated that *L. inermis* contained some bioactive compounds that can activate the insulin production gland to release insulin. The insulin released could trigger proper regulation of carbohydrate metabolizing and the establishment of normal blood glucose (Sigh et al., 2015). This result conforms to what was reported by Ndatsu et al. (2013) that a low level of blood glucose was recorded in hepatotoxic rats served with fermented products of soybeans (tofu).

The crude extracts of *L. inermis* leave at 200, 400 and 500 mg/kg were able to reverse the increased level of glucose in rats (Amuzat et al., 2021). The reduction of the negative effects of concurrent dosing of acetaminophen was reported in rats (Taj et al., 2011). The ethanol extract of *S. ilicifolium* was demonstrated to have hepatoprotective and nephroprotective potentials in rats induced with drug hepatotoxicity and nephrotoxicity (Sohail et al., 2019). In addition, a higher significant value of creatinine and urea found in drug-induced rats could signify liver and kidney dysfunction. Acetaminophen-induced hepatocyte injury may result in nephrocyte damage (Sohail et al., 2019). Yousef et al. (2010) have narrated that higher levels of creatinine and urea are signs of kidney malfunction. However, the insignificant reversed order of higher levels of creatinine and urea to lower levels among the rats treated with *L. inermis* leaves fractions confirms the hepatoprotective and nephroprotective potential of *L. inermis* leaf fractions. This finding is supported by the report of Yousef et al. (2010). The healing

effects of hepatocytes and nephrocytes in drug intoxicated rats by *S. Indica* ethanol extract were confirmed (Taj et al., 2011).

Furthermore, a significant increase ($P \leq 0.05$) of the serum hepatic marker enzymes (ALT, AST, ALP, and LDH) observed in NCA-treated rats compared to others is a clear indication of hepatocytes injury caused by ROS induced as a result of acetaminophen continuous dosing during the experimental period (Table 4). That is cellular damage and dysfunctional arrangement of the molecular cell membranes of the liver caused by acetaminophen toxicity. The finding is similar to what was reported by Rajesh and Latha, (Rajesh & Latha, 2004) that serum hepatic enzymes increase is an indication of cell membrane leakage and malfunction integrity of molecular cell membranes of the liver.

Bartlett, (2004) has reported evidence of hepatocyte necrosis and damage to cell membranes, which resulted in higher values of serum hepatic marker enzymes in rats. Similar reports were given by Anis *et al*. (2013), Effiong et al. (2014), and Florence et al. (2020) in rats. Thus, the lower values of the serum hepatic markers experienced in normal control rats, which are not significantly different ($P \leq 0.05$) from other groups given various levels of *L. inermis* leave fractions, signify no suspect/occurrence of any sign of liver dysfunction (Table 3). Interestingly, the higher hepatic dysfunction caused by acetaminophen intoxication was reduced insignificantly ($P \leq 0.05$) among the rats treated with all levels of *L. inermis* leave fractions, suggesting the higher hepatoprotective ability of the fraction against acetaminophen toxicity in the rats. That is, the reduction/normality of hepatocyte dysfunction by *L. inermis* leave fractions and their active principles suggest improved functional integrity of the hepatic cells. In the previous works, Florence et al. (2020) reported the protective potential of active fractions of *Lannea barteri* against acetaminophen toxicity in rats, and combined leaf extracts of *Gongronema latifolium* and *Nauclea latifolia* reduced acetaminophen toxicity in rats (Effiong *et al*., 2014) and protective potential of the ethyl acetate fraction of *L. inermis* fruits extract against carbon tetrachloride-induced oxidative damage in rat liver (Anis *et al*., 2013). Anis *et al*, (2013) have confirmed the hepatoprotective, nephroprotective, and cardio-protective potentials of *L. inermis* fruit extract in rats.

Subsequently, higher significant values ($P \leq 0.05$) of triglyceride, cholesterol, LDL, and the ratio of LDL to HDL and lower significant value (≤ 0.05) of HDL were observed in NCA-treated rats (rats served 600 mg/kg) acetaminophen only) compared to others indicated hepatic tissue damage (Table 3), which may translate to cardiovascular-related diseases. Cardiovascular ailments include atherosclerosis and hypercholesterolemia. This may also signify the adverse effects of conventional drug intoxication on the activity of the hormone-sensitive enzyme (lipase) due to insulin deficiency/absence. Lipase converts triglyceride to fatty acids and glycerol while insulin inhibits the activity of lipase in the adipose tissue and thereby increases the plasma level of free fatty acids. The free fatty acids in the liver are broken down to yield acetyl CoA and the excess of it is converted into cholesterol, triglyceride, and ketone bodies (Hauwa et al., 2014). These contradict the finding of Ghadir et al. (2011) that liver injury is correlated with total cholesterol, HDL and LDL, but not with triglyceride levels. The decreased levels of serum total cholesterol, LDL, and HDL signify progress in liver damage. These findings are in the shoulder with the report of Ndatsu et al. (2013) that acetaminophen

intoxication elevated the serum levels of cholesterol and LDL in rats.

Elevated levels of triglyceride, cholesterol, and LDL are true signs of hypercholesterolemia and atherosclerosis (Oboh, 2006; Adeyemi & Akanji, 2011). Oluyomi et al. (2014), and Ravikumar et al. (2010) have narrated that CCl4 intoxication in rats increased the levels of cholesterol and LDL. In contrast, decreased levels of serum HDL found in drug intoxicated rats may be linked with a higher risk of the atherogenic index, which is a prediction of cardiovascular abnormalities. Increased and low plasma levels of LDL and HDL respectively, observed in rats exposed to Fijk were narrated by Oluyomi et al. (2014). Conversely, the insignificant reduction ($P \leq 0.05$) of triglyceride, cholesterol, and LDL levels and increased levels of HDL observed among rats served *L. inermis* fractions indicated the protection potential of *L. inermis* leave fractions against tissue damage caused by high dosing of acetaminophen. That is *L. inermis* possesses beneficial activity against coronary risk caused by drug intoxication hyperlipidemia. Singh et al. (2015) have reported that crude leaf extract of *L. inermis* possessed protective effects against coronary dysfunction caused by drug intoxication. Low levels of triglyceride, cholesterol, and LDL and a higher level of HDL suggest repair of coronary risk (Alam et al., 2013).

Furthermore, significantly decreased levels of oxidative stress markers (CAT, SOD, and GPx) observed in NCA-treated rats compared to other groups suggest hepatic cell damage through oxidative stress produced by drug intoxication (Table 5). This result is supported by the work of Anis et al. (2013) that CCl_4 toxicity elevates the levels of the antioxidant system in rats. A reduction in serum antioxidant enzymes is a sign of hepatic risk (Hira et al., 2021), and the decreased levels of oxidative stress markers in kidneys and lungs of rats intoxicated by CCl_4 were reported by Taye and Abdel-Raheem (2012). Hira et al, (2021) reported low levels of oxidative stress markers in rats intoxicated with acetaminophen. This is also supported by Effiong et al, (2014) that drug toxicity significantly lowered the antioxidant capacity in rats. In the previous study, acetaminophen intoxication was able to cause oxidative stress in mitochondrial and the formation of peroxyl nitrite in rats (Knight et al., 2001).

In livers of murine, acetaminophen toxicity generated a critical mediator called peroxyl nitrite (Knight et al., 2002). In contrast, higher levels of MDA observed in NCA-treated rats as compared to others signify liver injury by acetaminophen intoxication. That is the *L. inermis* fractions were able to stop/prevent the generation of ROS and are also free radical neutralizer boosters, which may reduce the caused hepatocyte damage. A similar result to those of Anis et al, (2013) and Hira et al. (2021), is that CCl_4 and acetaminophen toxicity in rats generate a higher level of serum MDA, respectively, and elevation in MDA are signs of hepatic risk (Hira et al., 2021). Conversely, a significant increase in levels of oxidative stress markers among rats treated with all levels of *L. inermis* leave fractions compared to NCA-treated rats indicates fractions hepatoprotective against acetaminophen toxicity, which may be probably due to possible attenuation of oxidative stress. It may also be attributed to the reduction activities of the *L. inermis* leave fractions on hepatic cell damage. These hepatoprotective effects of *L. inermis* fractions could be probably due to their ability to elevate the levels of some bioactive compounds that are antioxidants. These fractions may also act directly as ROS scavengers, as a result of antioxidant compounds present or by elevating the

production of antioxidant molecules (Gupa et al., 2002).

The antioxidant potentials of these fractions could be possible due to the presence of phenol compounds (Anis et al., 2013). The presence of bioactive compounds (alkaloids, tannins, flavonoids, phenols, etc.) in *L. inermis* aqueous leaf extract has been reported (Amuzat et al., 2021). The presence of phenols flavonoids and others has been reported to influence the biological potentials of plant extracts ((Gupa et al., 2002). A similar report was narrated by Anis et al. (2013) that *L. inermis* fruit extract increased the level of oxidative stress markers in rats. Effiong et al. (2014) revealed the protective potentials of combined leaf extracts of *Gongronema latifolium* and *Nauclea latifolia* by elevating the antioxidant enzyme levels against continuous dosing of acetaminophen in rats. The hepatoprotective potentials of any conventional drug to minimize the effect of liver injury caused by liver intoxication are an indication of its protective abilities (Effiong et al., 2014). In animals, SOD, CAT, and GPx are the three major components of antioxidant systems and the function of these enzymes is to neutralize the activities of superoxide and peroxide ions in the molecular cells (Khan et al., 2021). The antioxidant enzyme system plays an important role in the defense of cells against oxidative stress.

Finally, in this study, the results obtained from microscopic observations of the liver cell are in line with the results of serum liver enzymes and markers of oxidative stress. The massive levels of abnormal hepatocytes observed in ANC-treated rats (acetaminophen-induced rats) imply the hepatic cell damage generated by acetaminophen intoxication. These intoxications are generally oxidative stress-related ailments due to the production of reactive oxygen species (ROS). These findings are in order with the works demonstrated by Rajesh and Latha (2004), Efiong et al. (2014), and Khan et al. (2021) that concurrent administration of acetaminophen-induced severe abnormal hepatocytes architecture. The higher production of acetaminophen metabolites by the activity of cytochrome P450 activity causes hepatic necrosis during overdose intake of acetaminophen (Nwanna & Oboh, 2007; (Ndatsu et al., 2013). The normal hepatic cell architecture observed in NCN-treated rats was an indication of no hepatotoxic caused by acetaminophen intoxication. This result is following those reported by Taj et al. (2011), and Efiong et al. (2014). However, the decreased levels of hepatic cell dysfunction observed in AS25-, AH-, AE-, and AM-treated rats than those of NCA-treated rats imply a high protective ability of Silymarin and various concentrations of *L. inermis* leave fractions against the hepatotoxic caused by acetaminophen. Decreased adverse effects of acetaminophen on liver and kidney functions and the metabolism of blood glucose in rats by *Stokeyia indica* extract (Taj et al., 2019), *Sargassum ilicifolium* extract [(Khan et al., 2021), and *Gongronema latifolium* and *Nauclea latifolia* extract (Effiong et al., 2014), *Lannea barteri* extract (Florence et al., 2020) and *L. inermis* fruits extract [45]. Sohail et al. (2019) have demonstrated the hepatic and nephritic protective potentials of *S. ilicifolium* extracts in rats.

CONCLUSION

The results from this study have suggested that the n-hexane, acetyl acetate and methanol active fractions at all levels of *L. inermis* leaf possessed hepatoprotective potentials against acetaminophen-induced hepatic cell damage, thus suggesting promising hepatic toxicity amelioration. Further research on the isolation and characterization of active compounds that

are responsible for its hepatic protection potential and their mechanism of action are already on the way.

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