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Original Research Article

ANTIOXIDANT AND HEPATOPROTECTIVE ACTIVITY OF ETHANOL LEAF EXTRACT OF *Setaria megaphylla* IN ACETAMINOPHEN-INDUCED HEPATOTOXICITY IN WISTAR RATS: ANTIDOTAL PERSPECTIVE IN LIVER INJURY

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ABSTRACT

The liver is continuously exposed to a range of chemotherapeutic agents, environmental contaminants, and xenobiotics. Most often, such exposures overwhelm the liver's inherent protective ability, resulting in its injury. This study examined the hepatoprotective potential of *Setaria megaphylla* leaf extract against acetaminophen-induced liver toxicity in Wistar rats. Albino Wistar rats of both sexes, weighing 120 to 150 g, and showing no symptoms of illness, were randomized into nine (9) groups, each with five (5) rats as follows: control, Hepatotoxic (acetaminophen), silymarin (positive control) and *S. megaphylla* leaf extract (120, 240, and 480 mg/kg) groups. Other groups were the *S. megaphylla* leaf extract (120, 240, and 480 mg/kg) plus paracetamol (2000 mg/kg) pretreated groups, respectively. Furthermore, the animals received the extract and silymarin orally for eight (8) days and acetaminophen (per oral) on the eighth day. Biochemical and histological parameters were evaluated 24 hours after acetaminophen administration. Acetaminophen (2000mg/kg) treated rats depicted a statistically significant ($p < 0.001$) increase in liver enzyme levels, including alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), total and combined bilirubin, as well as a decrease ($p < 0.01$-0.001) in albumin and total protein levels. In contrast, pre-treatment with *S. megaphylla* extract (120–480 mg/kg) resulted in a dose-dependent significant decrease ($p < 0.05$-0.001) in these enzyme levels, especially those of total and combined bilirubin, in comparison to the acetaminophen-treated group. Rats treated with *S. megaphylla* leaf extract demonstrated considerable ($p < 0.05$–0.001) and dose-independent elevation of the antioxidant enzyme levels: superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX), compared to the normal and acetaminophen control groups. The chemical and pathological alterations aligned with histopathological findings that suggested the leaf extract of *S. megaphylla* had liver protective ability against acetaminophen injury.

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INTRODUCTION

Hepatotoxicity induced by exogenous agents is still one of the main causes of death worldwide and a major hazard to human life. As a crucial organ, the liver performs many different tasks such as detoxification, plasma protein synthesis, glycogen and vitamin storage, bile secretion, and

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metabolism to protect the body's homeostasis from harmful external influences. Without this crucial detoxification process, exogenous agents could cause excessive production of free radicals that would be detrimental to the liver's normal functions [1,2]. Hence, the liver is constantly exposed to a variety of xenobiotics, environmental pollutants/toxins, and chemotherapeutic agents [3]. Most often, such exposures overwhelm the liver's natural defenses, resulting in hepatic injury.

It is undeniably true that liver diseases pose significant health challenges on a global scale, and it is therefore well known that the conventional medications used to treat liver injuries are occasionally ineffective and may cause serious side effects [4]. While the likelihood of toxic liver damage is frequently associated with taking medications, including those intended to treat such socially significant diseases as cancer [6,7] and tuberculosis [5], the incidence of liver injury of various etiologies has been steadily rising globally.

The current study, which aimed to investigate the hepatoprotective properties of the ethanol leaf extract of *Setaria megaphylla*, used acetaminophen, often referred to as paracetamol (PCM), to induce liver injury. At therapeutic doses, PCM has an excellent safety profile and is an efficient antipyretic-analgesic drug [8], but when used in excess, it can have serious toxic effects, including liver damage in humans, rodents, and mice [9]. According to evidence, the chain of events that results in hepatotoxicity is somehow set off by reactive metabolite formation and glutathione depletion. *The medicinal properties of S. megaphylla,* a tropical and subtropical robust tufted perennial grass, have been extensively explored, particularly by the natives of the Niger Delta in Nigeria for malaria treatment, haemorrhoids, urethritis, inflammation, diabetes, fevers, and various pains [10].

MATERIALS AND METHODS

Chemicals

The acetaminophen utilized in this investigation was acquired from Sigma Aldrich Chemical Company in the United States. The remaining chemicals and biochemical reagents used for this research were of commercial analytical quality.

Experimental animals

Forty-five (45) Wistar rats of both sexes, weighing initially 120 - 150 g, were obtained from the animal house of the University of Uyo, Faculty of Pharmacy, where the study was conducted. The rats were kept in ventilated cages at standard room temperature, with a 12-hour light/dark cycle and then allowed to acclimatize for seven days. They were fed conventional pelleted diets and given unlimited access to water. The Ethics Committee's guidelines for the use and care of experimental animals were followed in the conduct of the experiment. This study's protocols were approved by the University of Uyo's Institutional Ethics Committee (UU/CHS/IHREC/VOL 1/88).

Collection and Identification of plant

Fresh *S. megaphylla* leaves were harvested from the Anwa forest in Uruan L.G.A of Akwa Ibom State, Nigeria. Identification and authentication of the plant was carried out by Prof Margaret Bassey, a taxonomist from the Department of Botany and Ecological Studies, University of Uyo. A voucher specimen (UUPH33F) was preserved at the University of Uyo's Faculty of Pharmacy Herbarium.

Preparation of plant extract

The collected fresh *S. megaphylla* leaves were washed with distilled water and allowed to air dry in the lab for ten days. A Kenwood blender was used to grind the dry leaves into a uniform powder. The 1 kg uniform powder of dry *S. megaphylla* leaves was macerated in 50% aqueous-ethanol for 72 h. A Buchner funnel and Whatman no. 1 filter paper were used to filter the extract, and the filtrate was then evaporated to dryness in a water bath set at 40 °C.

Experimental design

The experimental design comprised forty-five (45) rats, which were randomised into nine (9) groups (n=5). The animals were treated thus:

Group 1 (normal control) animals received normal saline (10 mL/kg) orally for 8 days.

Group 2 (Acetaminophen or PCM-treated group) animals received normal saline (10 mL/kg) once daily for 8 days and were subsequently administered with PCM (2000 mg/kg), orally on the eighth day only.

Group 3 (positive control) animals were pre-treated with 100 mg/kg of silymarin once daily, by oral gavage for 8 days, and subsequently administered with PCM (2000 mg/kg), orally on the eighth day only.

Groups 4, 5, and 6 served as the extract-treated groups. Animals in these groups received only the ethanol extract of *S. megaphylla* leaf at 120, 240, and 480 mg/kg/day, orally for 8 days, respectively, but not PCM.

Groups 7, 8, and 9 served as the test groups (extract + PCM). Animals in these groups were pre-treated with extract of *S. megaphylla* at 120, 240, and 480 mg/kg, respectively, for 8 days and subsequently administered with PCM (2000 mg/kg), orally on the eighth day.

24 hours after receiving PCM, all the animals were sacrificed under a light ethyl ether vapour, and blood was drawn through a cardiac puncture into sterile plain tubes. The complete blood of each sacrificed rat was centrifuged using Nikon Optical Co., Japan at 2500 rpm for 20 minutes to obtain the serum, which was then used for the estimation of liver biochemical parameters.

Assay of liver function parameters

Biochemical markers such as albumin, total and direct bilirubin, total protein, alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), and total cholesterol were estimated using the serum that was obtained. Serum aspartate aminotransferase and alanine

aminotransferase were both calculated at 340 nm [12], and serum alkaline phosphatase activity was measured at 405 nm using established protocols [11]. All other biochemical parameters were estimated using automated analyzers and Fortress Diagnostic Kits, UK, following the manufacturer's protocols.

Assay of oxidative stress markers

After the animals' livers were surgically removed, weighed, and cleansed in ice-cold 0.9% NaCl, they were homogenized using a motor-driven Teflon pestle in a ratio of 1 g of wet tissue to 9 ml of 1.25% KCl. Following a 10-minute centrifugation at 7000 rpm at 4 °C, the supernatants were used for the following assays: reduced glutathione (GSH) activity as outlined by Ellman (1959) [14], glutathione peroxidase (GPX), and catalase (CAT) activities using Fortress Diagnostic Kits, UK, malondialdehyde (MDA) content using the Colorimetric TBARS Microplate Assay Kit, while superoxide dismutase (SOD) activity was according to the protocol outlined by Marklund and Marklund (1974) [13].

Histopathological examination

The livers of the sacrificed rats were washed with saline and then fixed in 10% buffered formalin. The fixed tissues were processed, and serial sections (5 µm thick) were then cut using a Hertz rotary microtome. Each section was stained using the H&E technique, according to Drury (1990) [15]. The sections were examined for histopathological changes using a light microscope, and photomicrographs were taken.

Statistical analysis

The data were analyzed using SPSS version 20.0 software, and the results were displayed as arithmetic means ± standard error of the mean (SEM). Following a one-way analysis of variance (ANOVA), a post hoc test (Tukey-Kramer multiple comparison test) was conducted. Mean differences were considered statistically significant at $p < 0.05$.

RESULTS

Effects of *S. megaphylla* on liver function indices in PCM-treated rats

The effects of *S. megaphylla* leaf extract on the liver function parameters of rats with and without PCM-induced liver damage are presented in Table 1. PCM administration to rats significantly ($p < 0.001$) increased the levels of ALT, AST, ALP, total and combined bilirubin, and significantly ($p < 0.01$-0.001) decreased the levels of total protein and albumin when compared to control. Pretreatment with *S. megaphylla* extract (120–480 mg/kg) caused a significant ($p<0.05$–0.001) dose-dependent reduction in these enzyme levels, as well as total and combined bilirubin, compared to the PCM-treated group. Significant increases in the levels of albumin ($p < 0.001$) and total protein ($p < 0.01$) were observed in the groups that were pre-treated with the leaf extract of *S. megaphylla* when compared to the PCM-treated group (Table 1). In the normal rats, administration of the leaf extract (120 – 480 mg/kg) had no discernible effects on the levels of ALP, AST, ALT, total and combined bilirubin, as well as albumin and total protein compared to the normal control (Table 1).

Effects of *S. megaphylla* on liver antioxidant markers in PCM-treated rats

The effect of *S. megaphylla* on liver antioxidant markers in rats with PCM-induced liver injuries is shown in Table 2. Administration of PCM was observed to significantly ($p < 0.01$) reduce the levels of GSH, GPx, CAT, and SOD. In contrast, the MDA level was highly increased ($p>0.05$) in comparison to the normal control. Additionally, rats treated with *S. megaphylla* leaf extract exhibited elevated levels of the antioxidant enzymes like SOD, CAT, and GPX in both normal and PCM-injured rats, in a significant ($p < 0.05$-0.001) and dose-independent manner, compared to the normal and PCM control groups, respectively. Similarly, following the extract administration, the GSH level was considerably ($p < 0.001$) higher than in the normal and PCM-treated groups. However, when compared to the PCM-treated group, pretreatment with the leaf extract significantly ($p < 0.05$-0.01) decreased the levels of MDA (Table 2).

Histopathological studies of liver sections of normal, PCM, and PCM plus ethanol leaf extract of *S. megaphylla*-treated rats

The photomicrographs of liver sections of normal, PCM, and PCM plus ethanol leaf extract of *S. megaphylla*-treated rats are shown in Figures 1 - 3. Histopathological analysis of liver sections from normal control rats revealed a clear integrity of the hepatic histoarchitecture, evidenced by normal hepatocytes, central veins, and average-sized sinusoidal spaces (Figure 1). In contrast, the PCM alone treated rats' liver sections showed areas with severe structural damage, characterized by necrosis around the central vein and some showing ballooning degeneration, confirming the induction of liver damage (Figure 1). PCM (2000 mg/kg)+ silymarin (100 mg/kg) treated rats showed liver sections with wide multiple areas of necrotic hepatocytes. Higher magnification showed haemorrhagic parenchyma (Figure 1). Extract (120, 240, and 480 mg/kg) alone treated rats showed normal liver architecture, an array of normal hepatocytes, central vein, and average-sized sinusoid with no pathological lesion seen (Figure 2). The liver section from Group 7, pre-treated with extract 120 mg/kg, showed arrays of normal and hydropic hepatocytes, as well as a focal area of interstitial inflammation and degeneration (Figure 3). The liver section obtained from Group 8, pre-treated with extract 240 mg/kg, showed normal parenchyma with arrays of hepatocytes, but with a mildly inflammatory infiltrated sinusoid with mononuclear inflammatory cells as well as congested blood vessels (Figure 3.1c). The liver section from Group 9, pre-treated with extract 480 mg/kg, showed normal parenchyma with multiple lobular glycogen-accumulated hepatocytes (Figure 3).

Table 1: Effects of ethanol leaf extract of *S. megaphylla* on liver function indices in PCM-treated rats

Treatment	AST(IU/L)	ALT(IU/L)	ALP(IU/L)	T.P (g/dL)	ALB(g/dL)	T.B (mg/dL)	C.B (mg/dL)
Control	81.00±7.02	33.67±4.26	33.33±2.40	54.67±2.03	42.67±1.45	10.60±0.67	5.60±0.42
PCM	281.67±9.21^{c}	98.00±6.03^{c}	98.00±4.36^{c}	21.00±0.58^{b}	12.67±1.45^{c}	41.23±1.24^{c}	26.40±1.78^{c}
PCM+Silymarin	87.00±9.01^{f}	37.00±3.67^{e}	34.75±3.22 ^{f}	52.75±1.38 ^{e}	39.50±1.32^{f}	11.70±0.84^{f}	5.95±0.84^{f}
SM (120 mg/kg)	82.00±5.21^{f}	32.25±3.22^{f}	28.50±1.94^{f}	61.00±1.08^{f}	36.00±2.08^{f}	10.45±0.40^{f}	5.58±0.34^{f}
SM (240 mg/kg)	83.50±3.43^{f}	35.25±3.20^{e}	31.25±2.69^{f}	55.00±1.78^{e}	39.25±2.17^{f}	13.20±0.57^{f}	5.75±0.39^{f}
SM (480 mg/kg)	85.00±6.34^{f}	27.50±1.50^{f}	26.00±2.48^{f}	53.50±1.94^{e}	44.50±1.66^{f}	11.23±0.87^{f}	6.33±0.62^{f}
SM (120 mg/kg) +PCM	124.00±2.08^{a,f}	57.67±8.84^{a,d}	59.00±1.15^{a,d}	42.33±1.20^{e}	39.33±1.76^{f}	20.57±0.30$^{b\,e}$	13.90±0.49$^{b\,e}$
SM (240 mg/kg) +PCM	106.50±7.50^{e}	48.50±8.50^{d}	48.00±2.00^{d}	52.00±1.00^{e}	44.00±1.00^{f}	17.15±0.65^{ae}	9.80±0.70^{af}
SM(480 mg/kg) +PCM	93.50±1.50^{f}	34.50±3.50^{f}	39.50±3.50^{e}	52.50±2.50^{e}	38.50±1.50^{f}	16.95±0.25^{ae}	10.65±0.15^{be}

Data are expressed as mean ± SEM. Significant at $^{a}p < 0.05$, $^{b}p < 0.01$, $^{c}p < 0.001$ when compared to normal control; $^{d}p < 0.05$, $^{e}p < 0.01$, $^{f}p < 0.001$ when compared to PCM control. n = 5. AST = Aspartate aminotransferase, ALT = Alanine aminotransferase, ALP = alkaline phosphatase, T.P = total protein, ALB = albumin, T.B = total bilirubin, C.B = conjugated bilirubin, PCM = Paracetamol.

Table 2: Effects of ethanol leaf extract of *S. megaphylla* on liver antioxidants in PCM-treated rats

Treatment	**GSH** (µmol/L)	**GPX** (U/mg)	**CAT** (U/mL)	**SOD** (U/mg)	**MDA**(nmol/mL)
Control	0.88±0.08	0.11±0.01	4.53±1.30	0.49±0.04	0.53±0.07
PCM	0.46±0.07^{b}	0.07±0.01^{b}	1.74±0.63^{b}	0.22±0.08^{b}	0.80±0.08
PCM+Silymarin	1.11±0.08^{e}	0.15±0.01$^{b\,e}$	1.53±0.54^{b}	0.53±0.03^{d}	0.43±0.05^{d}
SM (120 mg/kg)	1.21±0.12^{f}	0.14±0.01^{ad}	1.58±0.34^{b}	0.67±0.06^{d}	0.30±0.07$^{a\,e}$
SM (240 mg/kg)	1.16±0.06^{e}	0.15±0.01$^{b\,e}$	1.10±0.18^{c}	0.71±0.02^{f}	0.24±0.03$^{b\,f}$
SM (480 mg/kg)	1.17±0.04^{e}	0.15±0.01$^{b\,e}$	1.11±0.23^{c}	0.77±0.03^{ae}	0.16±0.03$^{c\,f}$
SM (120 mg/kg) + PCM	1.43±0.04$^{a\,f}$	0.16±0.01$^{b\,e}$	1.20±0.56^{b}	0.83±0.04$^{a\,f}$	0.14±0.04$^{c\,f}$
SM (240 mg/kg) + PCM	1.47±0.21$^{a\,f}$	0.17±0.01$^{b\,e}$	0.75±0.10^{ce}	0.96±0.02$^{b\,f}$	0.11±0.01$^{c\,f}$
SM(480 mg/kg) +PCM	1.31±0.16^{f}	0.17±0.01$^{b\,e}$	0.90±0.19$^{c\,d}$	0.95±0.03^{bf}	0.10±0.00$^{c\,f}$

Data are expressed as mean ± SEM. Significant at $^{a}p < 0.05$, $^{b}p < 0.01$, $^{c}p < 0.001$ when compared to normal control; $^{d}p < 0.05$, $^{e}p < 0.01$, $^{f}p < 0.001$ when compared to PCM control. n =5. GSH=Reduced glutathione, GPX=Glutathione peroxidase, CAT=Catalase, SOD=superoxide dismutase, MDA=Malondialdehyde.

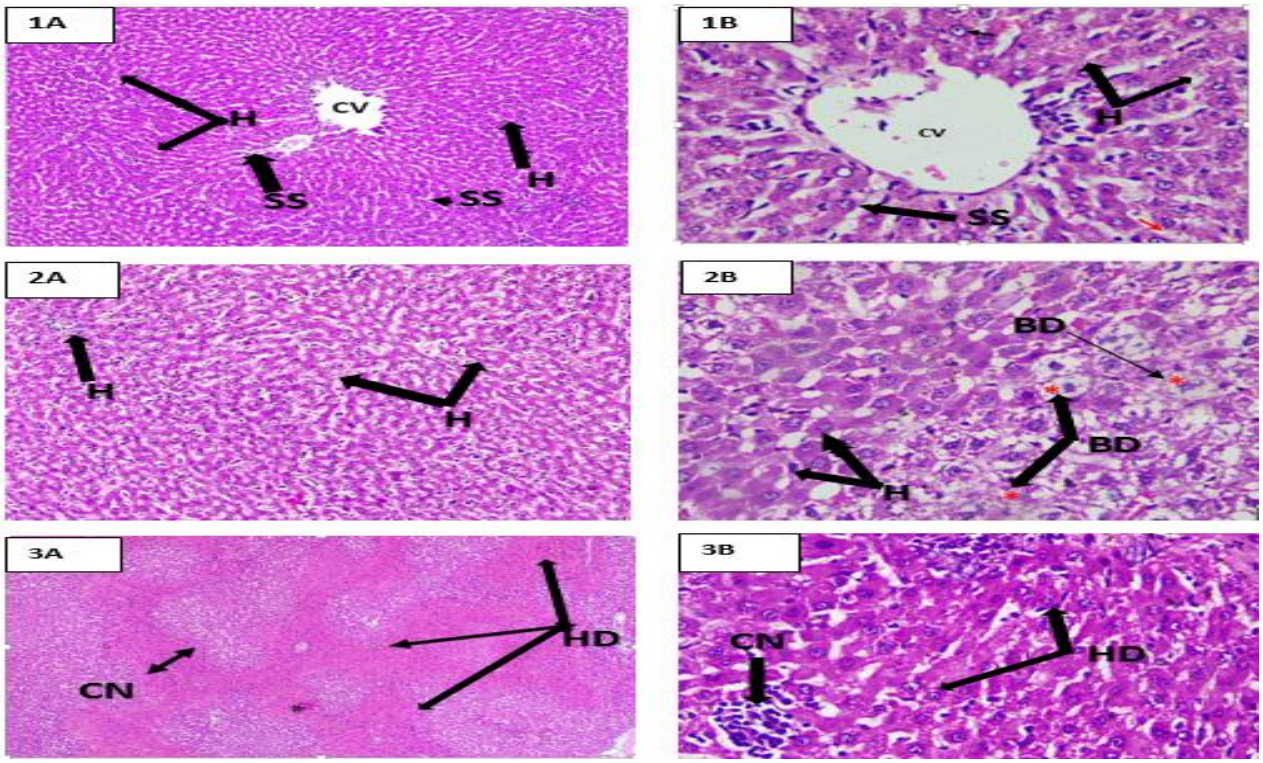

Figure 1: Histological sections of livers of rats treated with normal saline 10 mL/kg (1), PCM 2000 mg/kg (2), and silymarin 100 mg/kg and PCM 2000 mg/kg (3) at magnification A (x100) and B (x400), stained with H&E technique; hepatocyte (H), central vein (CV), sinusoidal space (SS), ballooning degeneration (BD), hepatocytes degeneration (HD), confluence necrosis (CN).

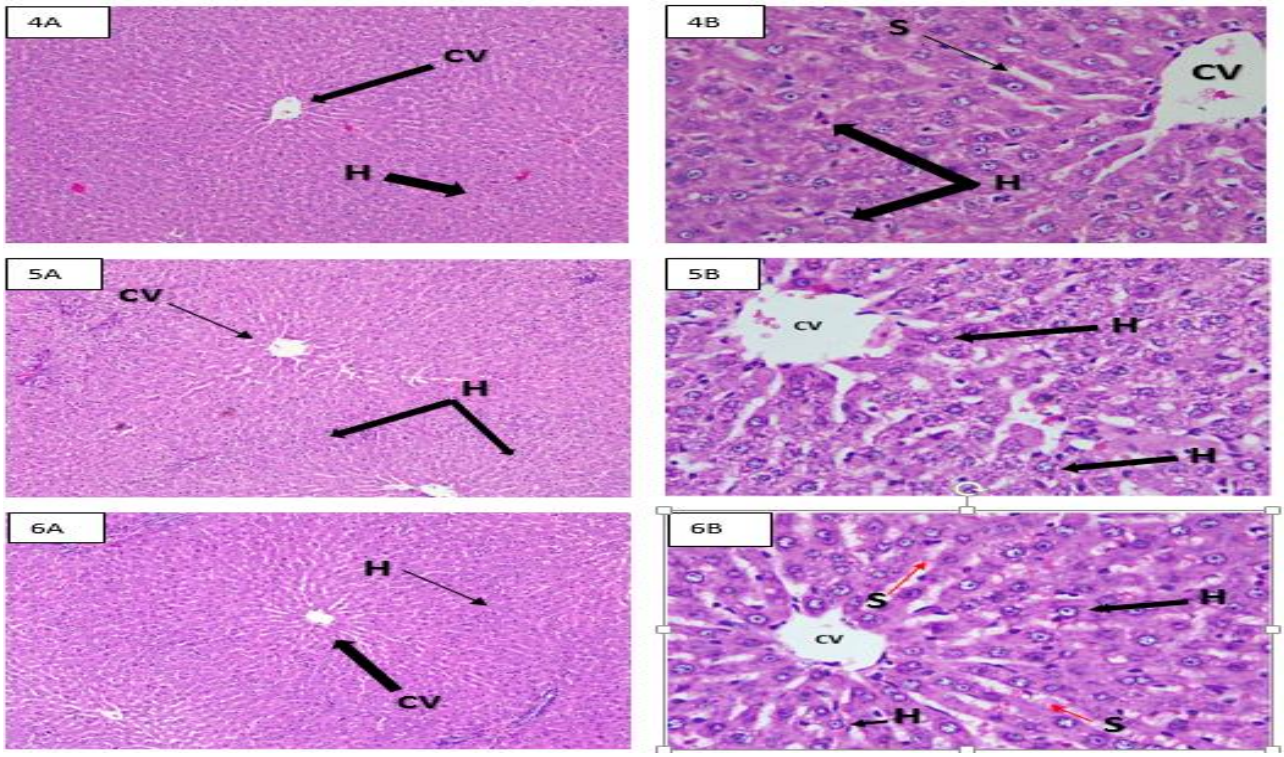

Figure 2: Histological sections of livers of rats treated with 120 mg/kg (4), 240 mg/kg (5), and 480 mg/kg (6) *S. megaphylla* at magnification A (x100) and B (x400), stained with H&E technique; hepatocytes (H), central vein (CV), sinusoid (S).

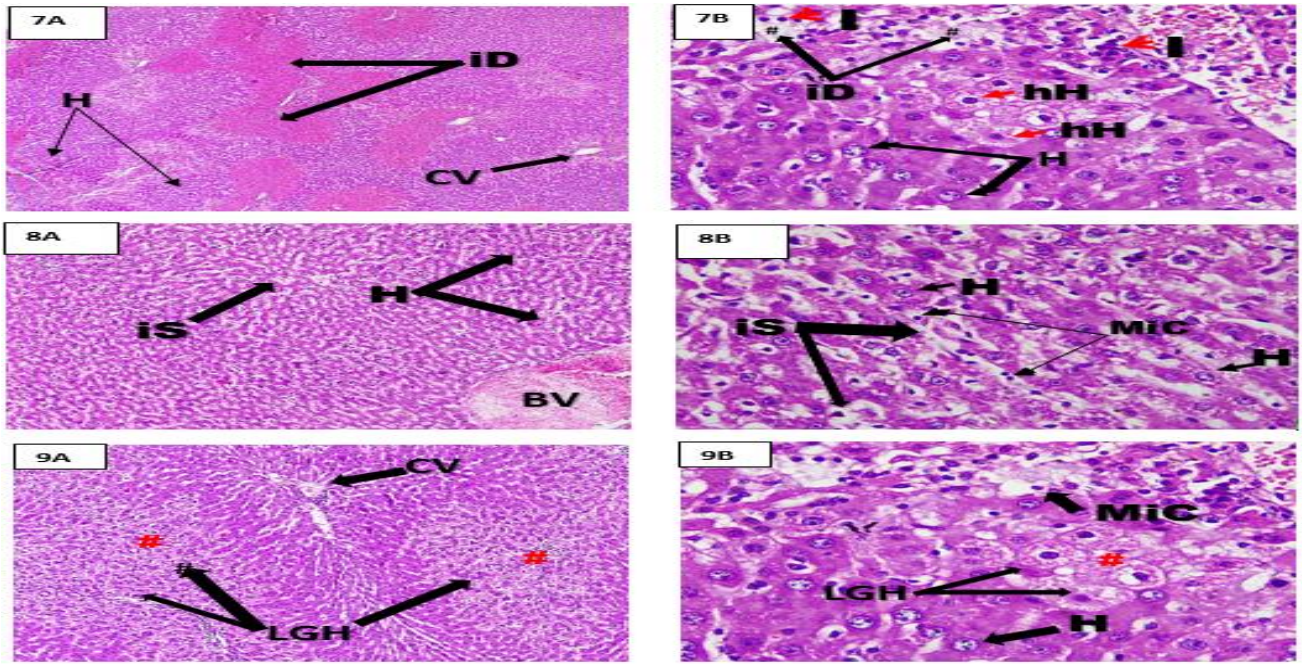

Figure 3: Histological sections of livers of rats treated with SM 120 mg/kg and PCM 2000 mg/kg (7), SM 240 mg/kg and PCM 2000 mg/kg (8), and SM 480 mg/kg and PCM 2000 mg/kg (9) at magnification A (x100) and B (x400) stained with H&E technique; hepatocytes (H), hydropic hepatocytes (hH), interstitial inflammation (I), interstitial degeneration (iD), inflammatory infiltrated sinusoid (iS), mononuclear inflammatory cells (MiC), blood vessel (BV), central vein (CV), SM=*S. megaphylla*

DISCUSSION

Exogenous agents that induce organ toxicity serve as standardized models to study the beneficial as well as the toxic effects of natural products, chemicals, and many potential drugs and organ function.

In the present research, PCM was used to induce liver injury. At therapeutic doses, PCM has an excellent safety profile and is an efficient antipyretic-analgesic drug [8] but can produce severe toxic effects such as hepatic damage in humans, rats, and mice when administered in toxic doses [9]. According to evidence, the chain of events leading to hepatotoxicity may be somehow set off by reactive metabolite formation and glutathione depletion.

The homeostatic role of the liver is a composite of several functions. Elevated serum enzyme levels, which are signs of cellular leakage and the degradation of cell membrane integrity in the liver, are reflections of damage to the liver's structural integrity [16]. This damage is depicted by a rise in the serum transaminases' levels, such as alkaline phosphatase (ALP), aspartate aminotransferase (AST), and alanine aminotransferase (ALT), due to their cytoplasmic location and the fact that cellular damage causes them to be released into the circulation. The activities of serum ALP, ALT, AST, total and direct bilirubin, total cholesterol, total protein, and albumin (ALB), which are initially located in the cytoplasm, can be used to assess liver function [17]. These molecules and enzymes leak into the bloodstream during liver damage, serving as a sign of liver damage [18]. The increase in the activity of ALT is typically accompanied by an increase in AST, and it is always specific to hepatocellular damage [19]. ALT is therefore a better parameter for identifying liver injury because it is more specific to the liver. However, serum levels of total protein, bilirubin, and ALP are linked to hepatic cell function. A pathological alteration in biliary flow is indicated by an elevation in the serum level of ALP, which is brought on by elevated synthesis in the presence of increasing biliary pressure [20;21]. The concentration of these cellular enzymes in the serum indicates the alterations in the plasma membrane's permeability and/or integrity [22].

In the present investigation, PCM-dosed rats depicted significant elevation in the levels of AST, ALP, ALT, total bilirubin (TB), and conjugated bilirubin (CB) as well as lower levels in albumin and total protein in serum, compared to control, an indication of acetaminophen-induced liver injury, which denotes injury to the liver cells. These findings corroborate the result of previous studies [18], indicating abnormally high levels of serum ALP, AST, ALT, total/direct bilirubin, and total cholesterol as well as decreases in albumin and total protein, following the toxic dose of PCM. Histopathological evaluation of the PCM-treated groups confirmed the induction of liver injury by PCM. The present result is, however, in line with the previous studies, an indication of PCM [9] induced necrotic damage of the hepatic cells and leakiness of the plasma membrane when administered in supramaximal doses.

The extract-alone treated groups depicted a non-insignificant change in the liver function indices of the treated animals (AST, ALT, ALP, T.B, C.B), which is also confirmed by the histological findings, evidenced by normal histo-architecture of the liver section with no pathological lesions seen.

However, this implies that there was no liver damage brought on by the extract. The results showed that the extract-pretreated groups had their liver function parameters (AST, ALT, ALP, T.B, C.B) significantly reduced, suggesting the liver protective potential of the leaf extract. This was further confirmed by the histopathological examination, which showed a fairly intact histo-architecture of the liver, especially at a high dose of the extract, suggestive of an ameliorative effect. However, the levels of total protein and albumin did not significantly change in the extract-alone treated groups as well as the pre-treatment groups, indicating that the liver's capacity to synthesize proteins was not altered. This may be attributable to the fact that serum bilirubin which is an index of hepatocyte's ability to perform synthetic function was not affected by the extract pretreatment but was rather reduced in PCM-treated groups, as was previously reported by Oyewole et *al* (2012) [23], and also corroborates earlier findings by Udobang and Okokon (2017) [24].

Reactive oxygen species (ROS) are important contributors to the development of oxidative stress, which can cause a number of diseases, including cancer, anemia, ischaemia, diabetes, inflammation, degenerative diseases, cardiovascular diseases, and liver disease [25]. ROS associated with lipid peroxidation have been suggested as one of the primary causes of these diseases [26]. The generation of ROS as a result of the rigorous oxidative processes occurring in human beings is one of the most effective precursors to systemic cell and tissue damage. Free radicals, however, can pose toxic risks to a variety of biological molecules when they are present in excess through protein synthesis inhibition, DNA damage, and lipid peroxidation. Antioxidant defense system enzymes like Superoxide dismutase (SOD), glutathione reductase (GSH), glutathione peroxidase (GPx), catalase (CAT), and nonenzymatic antioxidants, malondialdehyde (MDA), render these free radicals ineffective [27]. Since antioxidants prevent oxidation, they eliminate these free radical intermediates by being oxidized, even at very low concentrations. As a result, antioxidants perform a variety of physiological functions in the body that prevent these oxidation reactions, ultimately shielding the body from dangerous cascade reactions [28].

MDA has been utilized in numerous studies as a marker of oxidative stress, where a reduction in first-line antioxidants raises free radicals, which cause lipid peroxidation or result in the removal of electrons from nearby molecules, resulting in abnormal metabolism. Oxidative stress is typically confirmed by a rise in MDA levels, which is followed by a decrease in first-line antioxidants such as SOD, GSH, GPx, and CAT. The antioxidant marker enzymes represent protection against oxidative tissue damage.

The result of this study revealed that PCM treatment significantly lowered the activities of GSH, GPx, CAT, and SOD, while increasing the level of MDA in liver tissues, which denotes depletion of the antioxidant capacity of the cells. The decreased GSH, GPx, CAT, and SOD, as well as the increase in the level of MDA, may have been caused by an increase in ROS as a result of oxidative stress due to PCM intoxication. The decreased antioxidant capacity of the cells following increased MDA levels in the liver is indicative of increased lipid peroxidation, which may lead to tissue damage [29]. This, however, lends credence to the result obtained from liver function tests as well as the histopathological findings, which showed damage to the liver tissues. ROS accumulation occurs when cells are unable to efficiently scavenge free radicals due to elevated ROS production or a compromised antioxidant system [30].

In conclusion, the administration of the leaf extract of *S. megaphylla* to rats restored the activities of GSH, GPX, SOD, and MDA in PCM-induced liver toxicity, thus exerting a hepatoprotective effect.

CONCLUSION

The ethanol leaf extract of *Stachytarpheta megaphylla* significantly ameliorated PCM-induced liver damage in rats. It reduced elevated liver enzymes (ALP, ALT, AST), bilirubin levels, and restored protein and albumin concentrations. The extract also enhanced antioxidant defence by increasing SOD, CAT, GPx, and GSH levels while reducing MDA. These effects were dose-dependent for liver enzymes and dose-independent for antioxidants. No adverse effects were observed in normal rats treated with the extract. Overall, *S. megaphylla* exhibits promising hepatoprotective and antioxidant properties.

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AUTHORS' CONTRIBUTION

All authors contributed substantially to the success of this work. **IKU** conceived the idea, co-planned the work, and revised the experimental design with **JEO** and **JAU**. The final work was re-vetted by all authors.

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CONFLICT OF INTEREST

The authors report that they have no conflicts of interest.

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