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Research Article
Hepatoprotective Effect of *Senna mimosoides* Aqueous Leaf Extract Against Carbon Tetrachloride Induced Hepatotoxicity in Albino Rats

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Abstract

Background and Objective: Most medicinal plants presently employed in traditional medicine are used without scientific evaluation. In this study, the hepatoprotective potential of the aqueous extract of *Senna mimosoides* leaves was investigated on Wistar albino rats to ascertain its efficacy and to scientifically validate its traditional use. **Materials and Methods:** A total of 30 Wistar Albino rats of the same sex were used and grouped as follows: Group A (control) were administered 0.2 mL of normal saline only, other rats in groups B, C, D were treated with 50, 100 and 250 mg kg^{-1} of aqueous extract of *Senna mimosoides*, respectively, group E received 25 mg kg^{-1} of silymarin, a standard hepatoprotective drug while group F served as the positive control. Carbon tetrachloride was used to induce hepatotoxicity in all the groups except group A. **Results:** There was a significant dose-dependent decreases ($p<0.05$) in the enzymatic activities of AST aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP). However, result showed significant dose-dependent increases ($p<0.05$) in the enzymatic activities of glutathione peroxidase (GPx), superoxide dismutase (SOD), catalase (CAT) and glutathione-S-transferase (GST). In addition, enzymatic effects (whether increases or decreases) observed in the group that was treated with 250 mg kg^{-1} of extract were more than that of the group that was treated with 25 mg kg^{-1} of silymarin a standard hepatoprotective drug. **Conclusion:** Based on the results obtained from this study, it was concluded that the leaves of *Senna mimosoides* possessed antihepatotoxic effect.

Key words: *Senna mimosoides*, hepatoprotective potential, antioxidant enzymes, carbon tetrachloride, hepatoprotective drug, antihepatotoxic effect

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The leaves of *S. mimosoides* are used in folklore medicine to treat diseases. Previous scientific investigation showed that the leaves of the plant possess immunomodulatory and antinflammatory potential[1]. According to WHO, 25 million people die from liver disorder and over 400 million people around the globe are exposed to the risk of hepatotoxicity. Continuous exposure of the liver to environmental toxins, abuse by poor drug habits and excessive alcohol can lead to various ailments like hepatitis, cirrhosis and alcoholic liver disease[2,3]. Hepatotoxicity refers to liver dysfunction or liver damage that is associated with an overload of drugs or xenobiotics[4]. Hepatotoxicity can be caused by a wide variety of pharmaceutical agents, natural products, chemicals and environmental pollutants and dietary constituents[5-7]. Though there are lots of antihepatotoxic orthodox drugs, they have little to offer for reduction of liver disorders and it is chiefly the plant-based preparations that are employed for the treatment of liver disorders[8]. The drugs offered by orthodox medicine for the treatment of liver diseases are corticosteroids and immunosuppressants which provide only symptomatic relief mostly without influencing the disease process and their use is associated with the risk of relapse and danger of side effects[9]. The increasing efficacy, availability and low cost of herbal drugs have necessitated the screening of plant based material for antihepatotoxic effect. Hepatic diseases or injuries are induced experimentally by administration of carbon tetrachloride, CCl_4 since it is known to produce acute hepatocellular injury with centrilobular necrosis and steatosis[10].

Senna mimosoides formerly known as *Cassia mimosoides* belongs to the family Caesalpiniaceae and the genus Senna. It is a weed common in wastelands, roadsides and fallows in Savannah zone and widespread in West Africa[11]. *Senna mimosoides* leaves are used in Nsukka folklore medicine, Ukehe Enugu State, Nigeria to treat oedema in pregnant women and breast milk toxicity in neonates[11,12]. The aqueous leaf extract of *Senna mimosoides* exhibits anti-inflammatory effects by stabilizing membrane, inhibiting phospholipase A_2 activity and prostaglandin synthase activity[11,12], it has also been found to possess immunomodulatory potential especially in boosting innate and adaptive immunity[1]. However no study has been carried out to determine the possible hepatoprotective effect of the *S. mimosoides* leaves. The increasing rate of diseases associated with liver disorder and increasing cost and side effect of orthodox drug necessitated the screening of plant materials for more effective, less toxic and cost effective hepatoprotective drug. In this study, the hepatoprotective potential of the aqueous leaf extract of *Senna mimosoides* was accessed using rat models of carbon tetrachloride induced liver damage.

MATERIALS AND METHODS

Plant material: The leaves of *Senna mimosoides* were collected from Ibagwa Roadside, Nsukka in Enugu State of Nigeria, during the months of July and August, 2011. The plant characterization and identification was carried out by a taxonomist, Mr. P.O. Ugwuozor, in the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka and the sample was kept in the Herbarium Voucher of the Department.

Aqueous extraction: A known amount, 2000 g, of *Senna mimosoides* leaves was extracted with 8400 mL of distilled water using cold maceration. It was filtered first with calico and subsequently with glass wool and finally Whatman No.4 filter paper. The filtrate was concentrated by lyophilisation. A brown slurry-like substance was obtained and stored in the refrigerator for further investigation.

Animal and husbandry: Wistar albino rats weighing between 130-250 g were obtained from the Animal House, Faculty of Biological Sciences, University of Nigeria, Nsukka. These animals were given standard feeds (Vital) for at least one week after purchase to acclimatize them to their new environment before use.

Ethical clearance: The procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation and with the Helsinki Declaration of 1975 as revised in 2000 and as stipulated by the faculty of Biological Sciences, University of Nigeria, Nsukka (Pub No. 85-23, revised 1985).

Experimental design: In this study, a total of thirty Wistar albino rats used were grouped as follows:

Group A : Rats were treated with normal saline only (normal control)

Group B : Rats were administered with CCl_4 and treated with 50 mg kg^{-1} b.wt., of extract

Group C : Rats were administered with CCl_4 and treated with 100 mg kg^{-1} b.wt., of extract

Group D : Rats were administered with CCl_4 and treated with 250 mg kg^{-1} b.wt., of extract

Group E : Rats were administered with CCl_4 and treated with 25 mg kg^{-1} b.wt. of silymarin a standard hepatoprotective drug

Group F : Rats were administered with CCl_4 and served as the negative control

Carbon tetrachloride, CCl_4 induction of hepatotoxicity: Wistar albino rats of either sex weighing 180-220 g were used for this study. Animals were divided into 6 groups of 5 animals each and treated orally with the extract for 7 days. Carbon tetrachloride CCl_4 was used to induce hepatotoxicity on day 3 and 5. Animals were sacrificed under light ether anaesthesia 24 h after the last dose. Blood was collected by cardiac puncture in plain tubes and liver was removed, rinsed in cold saline, blotted with filter paper and weighed. Liver homogenate was prepared in 0.25 M sucrose solution and centrifuged at 7000 rpm for 10 min at 4°C. The supernatant was used for various biochemical assays. Serum was separated by centrifugation at 3000 rpm at 4°C for 10 min and used for measurement of various biochemical markers.

Biochemical analysis

Assay of key liver marker enzymes: The total homogenate was used in assaying for liver marker enzymes such as serum alanine aminotransferase (ALT)[13], serum aspartate aminotransferase (AST)[13], serum Alkaline phosphatase (ALP)[13] and also in determining serum bilirubin concentration[14].

Assay of antioxidant enzymes and non-enzymatic antioxidants: Total homogenate was also used in assaying for antioxidant enzyme such as catalase[15], superoxide dismutase (SOD)[16], glutathione S-transferase (GST)[17] and glutathione peroxidase[13] and non-enzymatic antioxidants such as reduced glutathione concentration (GSH)[18] and malondialdehyde (MDA)[19] concentration.

Histological assays: The tissue pieces taken from the liver of the rats were fixed by neutral buffered formalin (10%) and subsequently embedded in paraffin. The sections (5 µm thick) were stained by haematoxylin and eosin to study the histopathological changes in the liver.

Statistical analysis: The results were expressed as mean±SD and test of statistical significance was carried out using one way analysis of variance (ANOVA). The data obtained were analysed using Statistical Product and Service Solutions (SPSS), version 18. $p<0.05$ was considered significant.

RESULTS

Effect of aqueous extract of *Senna mimosoides* on antioxidants enzymes: In Table 1 below, intoxication with CCl_4 depleted the plasma levels of SOD, CAT, GST, GPx activity of the Wistar Albino rats (group F) as compared to those of the control (group A). However, there was a dose-dependent significant increase ($p<0.05$) in the plasma levels of SOD, CAT, GST, GPx activity and in glutathione concentration of the Wistar Albino rats (groups B, C and D) on administration of aqueous extract of *Senna mimosoides*. Also at the highest dose of 250 mg kg^{-1}, results showed that the hepatoprotective potential of *Senna mimosoides* extract outweighed that of silymarin (a standard hepatoprotective drug). MDA values from the table showed a dose- dependent significant decreases ($p<0.05$) in test groups A, B, C, D and E (0.62±0.055, 0.75±0.400, 0.64±0.045, 0.56±0.041 and 0.66±0.088) respectively when compared to group F (0.90±0.054).

Effect of the extract on serum level of AST, ALT, ALP and bilirubin: As depicted in Table 2, there was a significant ($p<0.05$) increase in AST (71.80±1.3 IU L^{-1}), ALT (81.20±0.84 IU L^{-1}), ALP (96.80±0.84 IU L^{-1}) and bilirubin (0.792±0.008) serum level of rats in group F after CCl_4 administration as compared to the normal control A (48.20±0.84 IU L^{-1}), (53.00±1.00 IU L^{-1}), (58.00±1.00 IU L^{-1}) and (0.588±0.008 IU L^{-1}), respectively. However, treatment

Table 1: Effect of various concentration of aqueous extract of *Senna mimosoides* on enzymatic antioxidants and lipid peroxidation

Groups	GPx (mM min^{-1} μg^{-1})	SOD (%)	CAT (mg protein mL^{-1})	MDA (mg Mal kg^{-1})	GST (mM min^{-1} μg^{-1} protein)	Glutathione (Mmol g^{-1} tissue)
A	472±6.41	82.1±0.742	0.47±0.017	0.62±0.055	24.04±0.547	5.46±0.316
B	301±6.22[a]	61.7±1.037[a]	0.27±0.009[a]	0.75±0.400[a]	21.80±0.507[a]	3.08±0.093[a]
C	379±13.19[a]	74.9±0.418[a]	0.31±0.019[a]	0.64±0.045[a]	22.48±0.510[a]	4.17±0.241[a]
D	470±5.23[a]	81.0±0.791[a]	0.42±0.022[a]	0.56±0.041[a]	23.68±0.537[a]	5.16±0.193[a]
E	460±8.17[a]	76.4±0.652[a]	0.39±0.029[a]	0.66±0.088[a]	23.10±0.314[a]	4.97±0.273[a]
F	237±4.61*	45.5±0.500*	0.19±0.014*	0.90±0.054*	13.97±0.449*	2.21±0.239*

Values are represented as mean±standard deviation (n = 5), values having significant value of $p<0.05$ are considered significant, *Significant when compared with group A, [a]Significant when compared with group F

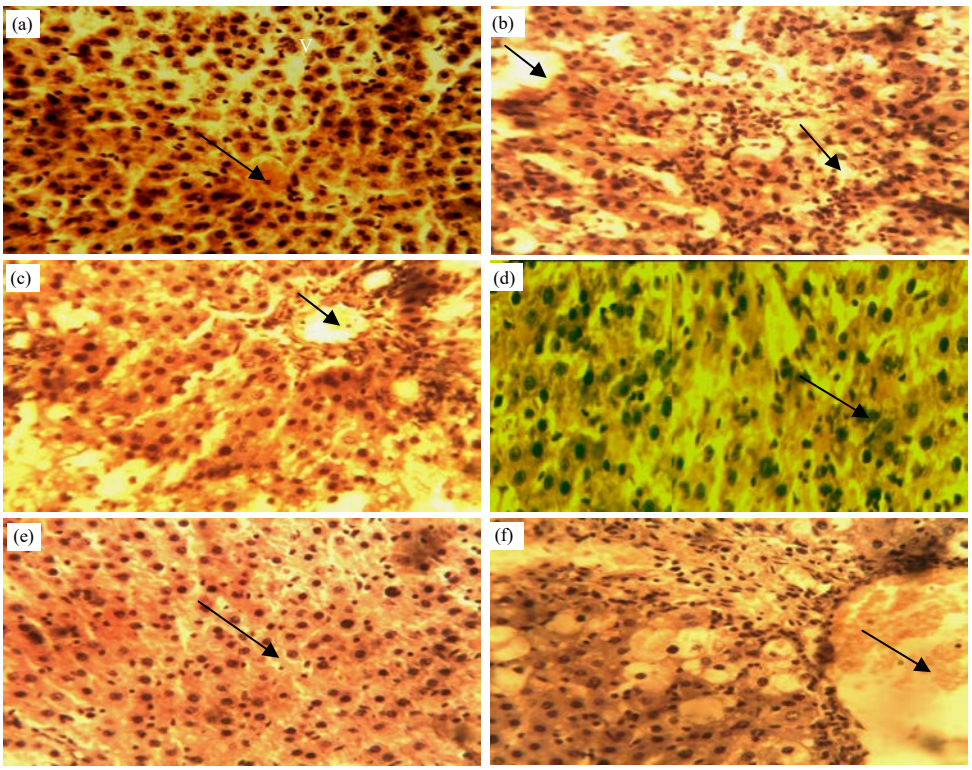

Fig. 1(a-f): Photomicrograph of liver section from (a) Group A rats given normal saline and olive oil, showing the central vein (V) with normal trabeculae structure of hepatocytes, (b) Group B rats treated with 50 mg kg^{-1} of S.m extract, olive oil and CCl_4, → shows multifocal areas of hepatocyte degeneration and necrosis, (c) Group C rats treated with 100 mg kg^{-1} of extract, olive oil and CCl_4, → showing some areas of central zonal vacuolation and necrosis of hepatocytes, (d) Group D rats treated with 250 mg kg^{-1} of S.m. extract, olive oil and CCl_4, → showing minimal vacuolation of hepatocytes and proliferation of Kupffer cells, (e) Group E rats treated with silymarin, olive oil and carbon tetrachloride with no remarkable histologic change → indicates the normal trabeculae structure of hepatocytes and (f) Group F rats given normal, olive oil and carbon tetrachloride, → shows widespread centrilobular degeneration of hepatocytes (A)
H and E x400

Table 2: Effect of various concentrations of aqueous extract of *Senna mimosoides* on liver function enzymes and bilirubin

Groups	AST (IU L^{-1})	ALT (IU L^{-1})	ALP (IU L^{-1})	Bilirubin
A	48.20±0.84	53.00±1.00	58.00±1.00	0.588±0.008
B	60.40±1.14[a]	65.00±1.58[a]	72.40±1.14[a]	0.736±0.005[a]
C	55.40±1.14[a]	59.20±0.84[a]	67.00±1.00[a]	0.698±0.008[a]
D	50.60±1.14[a]	55.20±1.34[a]	59.20±0.84[a]	0.626±0.011[a]
E	50.40±1.14[a]	57.00±1.00[a]	62.80±0.84[a]	0.658±0.025[a]
F	71.80±1.30*	81.20±0.84*	96.80±0.84*	0.792±0.008[a]

Values are represented as mean±standard deviation (n = 5), values having significant value of $p<0.05$ are considered significant, *Significant when compared with group A, [a]Significant when compared with group F

with S. *mimosoides* aqueous extract caused a significant ($p<0.05$) decrease in the activity of these enzymes and bilirubin in groups B, C and D rats of AST (60.40±1.14, 55.40±1.14 and 50.60±1.14 IU L^{-1}), ALT (65.00±1.58 IU L^{-1}, 59.20±0.84 and 55.20±1.30 IU L^{-1}), ALP (72.40±1.14 IU L^{-1}, 67.00±1.00 and 59.20±0.84 IU L^{-1}), bilirubin (0.736±0.005, 0.698±0.008 and 0.626±0.011) when compared to group F. This decrease by the extract was concentration dependent which was why there was a maximum decrease in group D rats which received the highest dose of the extract i.e., 250 mg kg^{-1}. The same effect was observed, in group E rats treated with silymarin. The effect of 250 mg kg^{-1} of extract is almost synonymous to that of 25 mg kg^{-1} of silymarin.

Histological result: The Fig. 1a-f show the photomicrograph of liver sections of the different treatment groups. The Fig. 1a

shows that the liver section from rats given normal saline had a central vein with normal trabeculae structure of hepatocytes while Fig. 1f which is the liver section from rats induced hepatocellular damage with CCl_4 there showed a widespread centrilobular degeneration of hepatocytes and severe passive congestion around the central vein. The Fig.1b-d represented the liver sections from rats that were induced with hepatotoxicity and treated with different dosage of the extract while Fig. 1e was also induced but treated with silymarin (standard drug).

DISCUSSION

In Table 1, the observed decrease in glutathione concentration and in the activity of GPx, CAT, SOD, GST in CCl_4 treated group suggested an oxidative type of injury corresponding with the findings of Lv *et al.*[20], Mansour *et al.*[21], Al-Olayan *et al.*[22], Noori *et al.*[23], Achudume *et al.*[24], Sarkar *et al.*[25] and Singh and Gandhi[26], who reported oxidative type of injury with CCl_4-induced hepatotoxicity. This is further substantiated by an elevation in the levels of lipid peroxidation as depicted by the significant elevation in the levels of MDA in the liver of rats treated with CCl_4 correlating with previous reports by researchers who reported the increased lipid peroxidation in CCl_4 treated rats[27-30]. The decrease in the activity of these enzymes in the present study could be attributed to the excessive utilization of these enzymes in inactivating the free radicals generated during the metabolism of CCl_4[25,26]. As reported in previous study the plant is rich in flavonoids and tannins and this could possibly accounts for its anti-oxidant property which enabled it to protect the liver against the disastrous effects of free radicals and reactive oxygen species (ROS) produced during the metabolism of CCl_4[30]. This is in agreement with former findings of Yadav and Agarwala[31], Middleton *et al.*[32] and Palanisamy *et al.*[33] which reported that plant secondary metabolites exert physiological effects in animals.

The Table 2 demonstrated that CCl_4 induces degeneration in hepatocytes by causing a significant increase in hepatic enzymes and bilirubin level. The marked elevation is in conformity with earlier independent reports of the deleterious biochemical effect of CCl_4 on hepatic integrity[34,35]. Increase in the liver enzyme and bilirubin induced by CCl_4 was remarkably reduced by the administration *S. mimosoides* extract which is in agreement with the histopathological result. Moreover, according to literature, the plant extract has the ability to stabilize membrane and this might also be the reason why there was decrease in hepatic enzyme and bilirubin level[2,10]. This finding correlates with a previous report that the *Solanum nigrum* reduced the level of hepatic enzymes in CCl_4-induced hepatotoxicity[36].

The active compounds of the *Senna* extract responsible for the observed effects have not been identified in the present study. Isolation, purification and structural elucidation of the active constituent involved in the hepatoprotective effect and evaluation of the potential usefulness of this extract in clinical conditions associated with liver damage should be investigated in further studies.

CONCLUSION

The result of this study demonstrated that aqueous extract of *S. mimosoides* leaves had a protective effect against CCl_4-induced liver injury in rat. Its free radical scavenging capabilities and antioxidant activity is due to its high polyphenols, tannins and flavonoids contents. Hence, it may be used in treating acute liver injury. This gives scientific evidence to the claims in the use of this plant in folklore medicine. Therefore, the present work provides conclusive evidence for the hepatoprotective effect of S. *mimosoides* against CCl_4-induced hepatotoxicity.

SIGNIFICANCE STATEMENT

This study discovered the hepatoprotective effect of the leaves of *S. mimosoides* that can be beneficial in the treatment of diseases related to the liver. The study will help researchers to uncover the active component of the plant responsible for this effect.

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