Original Article

Paracetamol-induced Liver Damage: Ameliorative Effects of the Crude Aqueous Extract of *Musanga cecropioides*

S. I. Omoruyi[1], A. B. Enogieru[1], O. I. Momodu[1], B. A. Ayinde[2], B. D. Grillo[1]

[1]Department of Anatomy, School of Basic Medical Sciences, University of Benin, Benin City, Edo State, Nigeria, [2]Department of Pharmacognosy, Faculty of Pharmacy, University of Benin, Benin City, Edo State, Nigeria

Abstract

Objective: The protective role of the aqueous stem bark extract of *Musanga cecropioides* against paracetamol-induced liver damage was investigated in Wistar rats using silymarin as a reference drug. **Materials and Methods:** The animals were randomly assigned into five groups of six rats each (A, B, C, D, and E). Rats in group A served as controls and received an equivalent volume of distilled water used to dissolve the extract. To effect liver damage, animals in groups B-E were administered paracetamol at 500 mg/kg body weight orogastrically for 14 days using a metal cannula. Animals in groups C, D, and E were simultaneously pretreated with silymarin at 25 mg, 250 mg, and 500 mg, of the extract, per kg body weight, respectively. The effects of *M. cecropioides* and silymarin were examined on hepatic marker enzymes; aspartate amino-transferases (AST), alanine amino-transferases (ALT), alkaline phosphatase (ALP), and total protein (TP). Antioxidant enzyme activities such as superoxide dismutase (SOD), catalase (CAT), and lipid malondialdehyde (MDA), as well as changes in liver histology, were also evaluated. The animals were sacrificed via cervical dislocation and blood was collected via cardiac puncture into plain bottles. Furthermore, liver tissues were excised and processed for routine hematoxylin and eosin staining. **Results:** *M. cecropioides* and silymarin produced significant ($P < 0.05$) hepatoprotective activity by decreasing the serum levels of AST, ALT, ALP, and lipid peroxidation marker, MDA significantly ($P < 0.05$) increased the levels of TP, SOD, and CAT except for the group administered 250 mg/kg of *M. cecropiodes*. Liver histology revealed the presence of vacuolations and mild chronic infiltrates of inflammatory cells in the livers of paracetamol treated animals. Pretreatment with silymarin and *M. cecropioides* extract produced a remarkable reduction in the severity of vacuolations. **Conclusion:** Crude aqueous extract of *M. cecropioides* protected against paracetamol-induced liver damage perhaps, by its antioxidative effect on hepatocytes, hence eliminating the deleterious effects of toxic metabolites of paracetamol.

Key words: Antioxidant, hepatotoxicity, *Musanga cecropioides*, paracetamol, serum enzymes

How to cite this article: Omoruyi SI, Enogieru AB, Momodu OI, Ayinde BA, Grillo BD. Paracetamol-induced liver damage: Ameliorative effects of the crude aqueous extract of *Musanga cecropioides*. Niger J Health Sci 2015;15:2-7.

Introduction

Liver is the key organ regulating homeostasis in the body. It is involved with almost all the biochemical pathways related to growth, fight against disease, nutrient supply, energy provision, and reproduction.[1] More than 900 drugs have been implicated in causing liver injury[2] and it is the most common reason for a drug to be withdrawn from the market. Drug-induced liver injury is a potential complication of nearly every medication because the liver is central to the metabolic disposition of virtually all drugs and foreign substances.[2,3]

Paracetamol which is safe for use at recommended doses (1000 mg per single dose and up to 3000 mg/day for adults, and up to 2000 mg/day if drinking alcohol). Moreover, it can as well cause potential fatal liver damage in cases of acute overdose and in rare individuals; a normal dose can do the same. The risk is heightened by alcohol consumption. An over dosage of paracetamol is known to be the cause of acute hepatic necrosis in both experimental animals[4,5] and humans.[6,7]

Paracetamol toxicity is caused by excessive use or overdose of the drug. It mainly causes liver injury. It is one of the most common causes of drug poisoning worldwide and the most common cause of acute liver failure in the United States and the United Kingdom.[8,9]

Access this article online

Quick Response Code:

Website: www.chs-journal.com

DOI: 10.4103/1596-4078.171381

Address for correspondence: Dr. S. I. Omoruyi, Department of Anatomy, School of Basic Medical Sciences, University of Benin, Benin City, Edo State, Nigeria. E-Mail: sylvester.omoruyi@uniben.edu

Conventional synthetic drugs used in the treatment of liver diseases are sometimes inadequate and can have serious adverse effects. So there is a worldwide trend to go back to traditional medicinal plants.[10-12] *Musanga cecropioides*, R. Brown (Cecropiaceae), the African corkwood tree, also known as the *Umbrella tree*, is found mostly in the tropical forests of Africa stretching from Guinea to Congo. The plant is of high importance to local herbalist because of its diverse medicinal uses which include its use as oxytocic, contraceptive, antihypertensive, antidiabetic, analgesic, and diuretic.[13] Traditionally, the plant is used to induce labor.[14] In some parts of Edo and Delta states of Nigeria, the plant is used as antihelmintic and antidysenteric agent.[15] Among the Yoruba tribe of South-West, Nigeria, hot infusion of stem bark of the plant is also used for the treatment of fever, jaundice, acute gastric poisonings, and liver diseases.

In an earlier study, the mechanism of action involved in the hypotensive properties of the aqueous extract of the leaves of *M. cecropioides* was investigated.[16] Its antidiabetic and hypoglycemic effects have also been investigated.[17]

The aim of the present study was to examine the traditional assumption of the hepatoprotective efficacy of *M. cecropioides* in the treatment of liver diseases resulting from poisonings and to explicate its possible mechanism of action using paracetamol-induced liver damage in experimental rat models.

Materials and Methods

The School of Basic Medical Sciences, University of Benin, granted ethical approval for the study.

Plant material

Fresh bark of stem of the *M. cecropioides* was collected from a deciduous forest around Oluku area within Benin city during the month of September 2011. The plant was identified by Mr. Sunny Nweke of the Department of Pharmacognosy, Faculty of Pharmacy, University of Benin and authenticated at the Forest Research Institute of Nigeria, Ibadan, Nigeria where a herbarium specimen number FHT106428 had been deposited initially.

The sheaths were washed with normal saline, sorted, air-dried at room temperature and protected from direct sunlight and heat for 2 weeks until completely air-dried. They were then pulverized using the laboratory hammer-mill and the powdered samples were stored in air and water-proof containers until required for extraction.

Preparation of aqueous extract

Approximately, 2.0 kg of the powder of bark of stem of *M. cecropioides* was extracted over 24 h using 2 L of distilled water. The mixture was filtered using Whatman filtered paper, and the filtrate evaporated at 60°C using a vacuum rotary evaporator (Buchi, Switzerland). The moist residue was freeze-dried using a vacuum freeze-drier and stored in a desicator. It was preserved in a refrigerator at 4°C until needed. The crude extract was dissolved in double distilled water to make a concentration of 100 mg/ml from which different doses of 250 and 500 mg/kg body weight by oral route were reconstituted.

Preliminary phytochemical screening

Phytochemical screenings were performed using standard procedures by Odebiyi and Sofowora[18] and Trease and Evans.[19] The phytochemicals were screened for anthraquinones, cardiac glycosides, saponins, flavonoids, tannins, alkaloids, phlobotannins, and terpenoids.

Experimental animals

A total of 30 adult male Wistar rats of average weight 250 g were used for this study. The animals were inbred rats obtained from the rat Colony of the Animal House, Department of Anatomy, University of Benin, Benin city. The animals were maintained on grower's marsh manufactured by Bendel Feeds and Flour Mills Limited, Ewu, Edo state, Nigeria and potable water which were made available *ad libitum*. The rats were maintained at an ambient temperature between 28°C and 30°C, humidity of 55% ± 5%, and standard natural photo period of approximately 12 h of light (06:30 h–18:30 h) alternating with approximately 12 h of darkness (18:30 h–06:30 h).

Chemicals and reagents

Normal saline was manufactured by Unique Pharmaceuticals, Sango-Otta, Nigeria and Paracetamol tablets by Emzor Pharmaceuticals Industries Ltd., Isolo, Lagos, Nigeria. Other reagents were all of analytical grade.

Induction of paracetamol hepatotoxicity

The animals were divided into five groups of A, B, C, D, and E. Group A animals served as the controls and were given 10 ml/kg of normal saline. Animals in groups B, C, D, and E were given paracetamol at 500 mg/kg of body weight through orogastric route using a metal cannula. The animals in group C were pretreated with 25 mg/kg of body weight of silymarin before the administration of paracetamol during the period of the experiment. Similarly, animals in groups D and E were pretreated with 250 and 500 mg/kg of body weight of the aqueous crude stem bark extract of *M. cecropioides* via oral route 1 h before the administration of paracetamol. The chosen dose for paracetamol was based on previous research by Gupta *et al*.[20] All treatments were given daily for 14 days and animals were sacrificed on day 15 by cervical dislocation after anesthesia.

Assay for hepatic marker enzymes

The blood samples were collected via cardiac puncture and stored in plain bottles. The blood samples were centrifuged at 3000 revolutions/min using a table-top centrifuge (Shanghai Surgical Instrument Factory, Shanghai, China) at 37°C for 15 min to separate the sera. Serum alanine (ALT), aspartate (AST) amino-transferases, alkaline phosphatase (ALP) as well as total protein (TP) were assayed spectrophotometrically, using Randox colorimetric assay diagnostic kits (Randox, Northern Ireland).

Assay for antioxidant enzymes

After sacrifice, the liver tissues were excised and a part chopped off to be homogenized in a mortar and pestle with a pinch of acid washed sand and a total of 5 ml of normal saline (0.9% saline) added sequentially during the homogenization process. The homogenates were centrifuged at 3500 rpm for 5 min with the aid of a centrifuge. The clear supernatants were collected using a micropipette and transferred into an empty specimen container and refrigerated until needed for the assays. Assay for superoxide dismutase (SOD) was done using the method of Misra and Fridovich[21] while the method of Cohen *et al.*[22] was used for catalase (CAT) activity. Finally, assay for lipid peroxidation and malondialdehyde (MDA) was done using the method of Varshney and Kale.[23]

Histopathological studies

The liver tissues of the rats were carefully dissected out and freed from the supporting adipose tissue and ligaments. After rinsing the liver in normal saline, different sections were taken from each lobe and the tissues were processed for routine hematoxylin and eosin (H and E) staining using the method of Drury *et al*.[24] Briefly, the excised tissues were fixed in 10% formal saline for 24 h, processed by paraffin embedding method and sections of 5 μ thickness were cut. The sections were for H and E demonstration of liver histoarchitecture under a light microscope. Photomicrographs of the desired area of liver sections were captured using the research microscope domiciled in the Department of Anatomy, School of Basic Medical Sciences, University of Benin, Benin city.

Data analysis

Weights and biochemical assay values were presented as mean (standard error of means). Data analysis was carried out using Statistical Package for Social Sciences, version 17, Manufactured by International Business Machine Corporation. The significance of difference in the means of all parameters was determined using one-way analysis of variance with 95% confidence interval. Least square difference and *posthoc* test was carried out for all groups in comparison with the control group. Statistical significance was set at $P < 0.05$.

Results

Phytochemical screening

Results of the phytochemical screening are shown in Table I. The bark of stem of *M. cecropioides* was fond to contain cardiac glycosides, saponins, flavonoids, tannins, plobatanins, and terpenoids (+). Alkaloids and anthraquinones were not observed to be absent (−).

Table I: Results of phytochemical screening are shown

Phytochemicals	Results
Anthraquinones	–
cardiac glycosides	+
Saponins	+
Flavonoids	+
Tannins	+
Alkaloids	–
Phlobatannins	+
Terpenoids	+

The results of AST, ALT, and ALP in control rats are presented in Table II. The means of levels of AST, ALT, and ALP in control rats were 34.33 (1.45), 25 (1.53), and 70.00 (1.88) IU/L, respectively, whereas in paracetamol treated rats, these levels were elevated to 83.50 (1.50), 87.50 (1.50), and 151.67 (2.33) IU/L, respectively. *M. cecropioides* pretreatment at the dose 250 mg/kg significantly reduced the paracetamol-induced rise in the AST, ALT, and ALP ($P < 0.05$). The mean values in the treated were minimized to 53.33 (2.56), 63.00 (2.21), and 93.50 (2.06) IU/L, respectively, when compared with paracetamol treated group. With a higher dose of 500 mg/kg *M. cecropioides* further reduction mean values of AST, ALT, and ALP to 42.83 (1.80), 47.47 (2.12), and 88.17 (2.68) IU/L, were recorded, respectively. Pretreatment with Silymarin at 25 mg/kg also prevented the paracetamol-induced rise in the means of AST, ALT, and ALP with mean values of 38.67 (1.28), 63.00 (2.21), and 83.83 (2.259) IU/L recorded, respectively. The same trend was observed in TP values [Table II].

The liver SOD and CAT observed in control rats were 30.17 (1.51) and 29.00 (1.34) IU/L, respectively, whereas MDA was 14.17 (1.96) IU/L. However, significant changes were noted in paracetamol treated groups, as SOD and CAT were reduced to 18.83 (2.99) and 17.33 (1.80) IU/L, respectively, whereas MDA was increased to 25.33 (2.17) IU/L. *M. cecropioides* pretreatment (250 mg/kg and 500 mg/kg) also significantly reversed paracetamol-induced changes in SOD (21.67 [1.26] and 25.33 [1.17] IU/L), CAT (19.33 [1.45] and 23.67 [1.78] IU/L); and MDA (22.40 [1.60]; 19.97 [1.74] IU/L, respectively. Similar findings were observed with silymarin [Table II].

Histopathological findings are shown in Figure 1 for controls and exposed rats. Histopathological findings revealed that the administration of paracetamol resulted in necrosis of hepatocytes as well as deposition of fats in the tissues [Figure 1, plate 2] when compared with controls [Figure 1, plate 1], but the severity was reduced in those groups of animals pretreated with 25 mg/kg of silymarin, 500 mg/kg and 250 mg/kg of the crude aqueous extract of bark of stem of *M. cecropioides* [Figure 1, plates 3-5]. The fatty deposition appeared as empty spaces or vacuoles as fat deposits were not stained for during routine H and E staining.

Discussion

This study evaluated the potential hepatoprotective role of *M. cecropioides* in paracetamol-induced liver damage in rats. Damage to the liver or hepatotoxicity did not result from paracetamol itself, but from one of its metabolites, N-acetyl-*p*-benzoquinone imine (NAPQI). NAPQI is known to deplete the liver's natural antioxidant glutathione and directly damage cells in the liver, leading to liver failure.[25] Many hepatoxins could induce liver injury through oxidative stress,

Table II: Effect of Musanga cecropioides aqueous extract of bark of stem on biochemical parameters in paracetamol-induced hepatotoxicity in rats

	Mean values of the enzymes Mean (SD)				
	Group A Control	Group B (paracetamol 500 mg/kg)	Group C (Silymarin + paracetamol 500 mg/kg)	Group D (*Musanga* 250 mg/kg + paracetamol 500 mg/kg)	GroupE (*Musanga* 500 mg/kg + Paracetamol 500 mg/kg)
Alkaline Phospatase (IU/L)	70.00 (1.88)	151.67 (2.33)[1]	83.83 (2.59)[2]	93.50 (2.06)[2]	88.17 (2.68)[2]
Aspartate Amino-transferase (IU/L)	25.00 (1.53)	87.50 (1.50)[1]	46.50 (2.06)[2]	63.00 (2.21)[2]	47.47 (2.12)[2]
Alanine Amino-transferase (IU/L)	34.33 (1.45)	83.50 (1.500)[1]	38.67 (1.28)[2]	53.33 (2.56)[2]	42.83 (1.80)[2]
Total Protein (IU/L)	7.83 (0.60)	5.33 (0.420)[1]	8.17 (0.48)[2]	6.17 (0.31)	7.17 (0.70)[2]
Superoxide Dismutase (IU/L)	30.17 (1.51)	18.83 (2.99)[1]	28.83 (1.40)[2]	21.67 (1.26)	25.33 (1.17)[2]
Catalase (IU/L)	29.00 (1.34)	17.33 (1.80)[1]	25.83 (1.54)[2]	19.33 (1.45)	23.67 (1.78)[2]
Malon Dialdehyde (IU/L)	14.17 (1.96)	25.33 (2.17)[1]	17.83 (1.89)[2]	22.40 (1.60)	19.97 (1.74)[2]

[1]Represents significant difference (P<0.05) of mean values when compared with group A, [2]Represents significant difference (P<0.05) of mean values when compared with group B. SD: Standard deviation

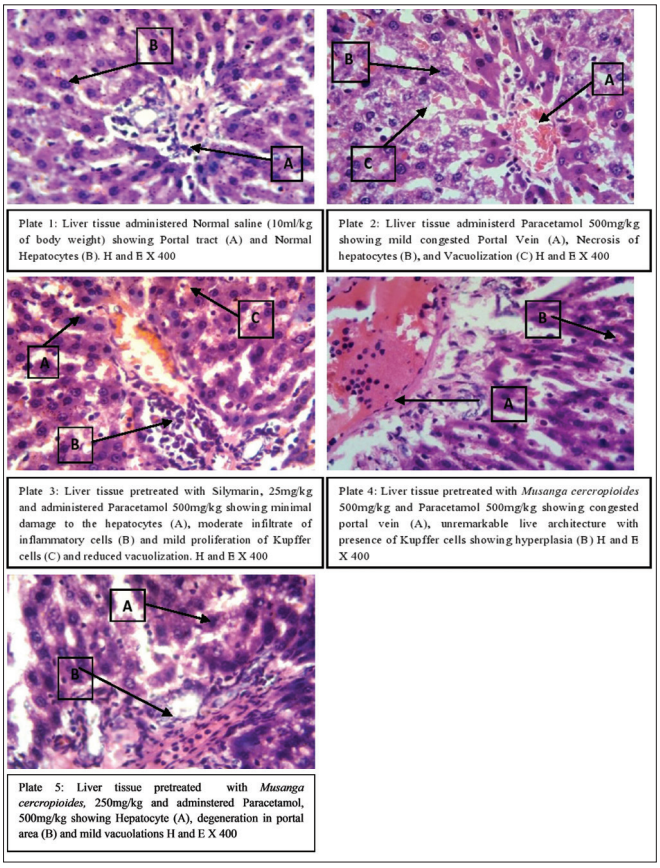

Figure 1: The histopathological findings in the controls and the exposed rats.

inflammation, fibrogenesis and liver necrosis.[26] Acute injury to hepatocytes alters their transport function and membrane permeability, leading to leakage of marker enzymes from the cells.[27]

In the assessment of liver damage caused by paracetamol, the determination of enzyme levels such as AST, ALT in the serum is largely used. Necrosis or membrane damage releases the enzyme into circulation and, hence, it can be measured in the serum. A high level of AST is a marker of liver damage as AST catalyses, the conversion of alanine to pyruvate and glutamate and is released in a similar manner. Therefore, ALT is more specific for the liver, and is thus a better parameter for detecting liver injury. Elevated levels of serum enzymes are indicative of cellular leakage and loss of functional integrity of cell membrane in the liver.[28] Serum ALP and TP levels on other hand are related to the function of hepatic cells. Increase in serum level of ALP is due to increased synthesis, in the presence of increasing biliary pressure.[29] Thus, an effective control of ALP activity is necessary and this points toward an early improvement in the secretory mechanism of the hepatic cell.

Results from the present study showed a significant elevation of ALP, AST, and ALT levels in paracetamol treated rats when compared to controls, but on pretreatment with graded doses of *M. cecropioides,* induced rises in ALP, AST, and ALT from paracetamol treatment was much reduced and this was statistically significant ($P < 0.05$) when compared with paracetamol treated group pretreatment with graded doses of *M. cecropioides*. TP levels of paracetamol treated animals dropped significantly, but there was a significant increase in animals on pretreatment with graded doses of *M. cecropioides* ($P < 0.05$) as TP levels were found to be approaching the normal value. Silymarin at 25 mg/kg of body weight also prevented the induced rises of ALP, AST, and ALT levels as a result of paracetamol treatment and also resulted in a significant increase in TP levels when compared with animals treated with only paracetamol.

Biological systems protect themselves against the damaging effects of activated species by several means. These include free radical scavengers and chain reaction terminators; enzymes such as SOD, CAT, and glutathione peroxidase (GPx) system.[30] It is known that SOD removes superoxide by converting it to H_2O_2, which can be rapidly converted to water by CAT and GPx.[31] However, oxidative stress results in toxicity when the rate at which the reactive oxygen species are generated exceeds the cell capacity for their removal and this leads to lipid peroxidation which is an autocatalytic process, and a common

consequence of cell death. One of the end products in the lipid peroxidation process is MDA.[32] Decrease in enzyme activity of SOD is a sensitive index in hepatocellular damage and is the most sensitive enzymatic index in live injury.[33] Liver is a major site with its highest activity of CAT, an enzymatic antioxidant which is also widely distributed in all animal tissues. A function of CAT is to decompose hydrogen peroxide and protect the tissues from highly reactive hydroxyl radicals.[34] It is, therefore, worthy of note that a reduction in the activity of CAT may result in a number of harmful effects due to the assimilation of superoxide radical and hydrogen peroxide. Result from the present study showed that administration of a higher dose of *M. cecropioides* (500 mg/kg) increased the level of CAT activity to almost that produced by silymarin, the standard hepatoprotective drug. This prevented the accumulation of excessive free radicals and protected the liver from toxicity initiated by paracetamol intoxication.

The increase in MDA levels in liver suggests enhanced lipid peroxidation leading to tissue damage and failure of antioxidant defense mechanisms to prevent the formation of excessive free radicals. Lipid peroxidation has been postulated to be the basis of destructive process of liver injury due to paracetamol intoxication. In the present study, the elevations in the levels of end products of lipid peroxidation in the liver of rat treated with paracetamol were observed to be high but pretreatment with *M. cecropioides* significantly reversed these changes. Hence, it is possible that the mechanism of hepatic protection by *M. cecropioides* is due to its antioxidant effect.

Results of histopathological studies support the results of biochemical parameters analyzed in the present study as the control group of animals showed normal architecture of the liver and integrity of the hepatocytes. However, histological analyses of livers of rats treated with paracetamol showed significant hepatotoxicity, characterized by necrosis, vacuolization of hepatocytes, inflammatory hepatic tissues, including the presence of moderate infiltration of inflammatory cells. Furthermore, pretreatment with Silymarin and *M. cecropioides*, reduced the severity of these damages. The empty spaces or vacuoles in the paracetamol treated groups were as a result of fat deposition in the liver parenchyma and this indicated early stage of fatty liver.

From preliminary phytochemical studies, it was shown that *M. cecropioides* contains flavonoids, triterpenoids, and steroids and these were in consonance with the results of the work reported by Kadiri and Ajayi.[35] A number of scientific reports indicated certain flavonoids, triterpenoids, and steroids have protective effect on liver cells due to its antioxidant properties.[36-38] The presence of these compounds in the extract may be responsible for its protective role in paracetamol-induced liver damage in rats. Hence, the results from the present study support the use of *M. cecropioides* in folklore practice in the treatment of liver infection and other liver diseases as it exerts significant hepatic protection against paracetamol-induced liver toxicity.

Financial support and sponsorship

Nil

Conflicts of interest

There are no conflicts of interest.

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