ORIGINAL ARTICLE

Hepatoprotective effect of the solvent fractions of the stem of *Hoslundia opposita* Vahl (Lamiaceae) against carbon tetrachloride- and paracetamol-induced liver damage in rats

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The hepatoprotective potentials of the stem solvent fractions of *Hoslundia opposita* Vahl were investigated. The fractions were prepared and tested for hepatoprotective effect against carbon tetrachloride (CCl_4) and paracetamol-induced liver damage in rats. Changes in the levels of biochemical markers of hepatic injury viz; -aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and bilirubin were determined in both treated and control groups of rats. The effects of the extracts were compared with that of sylimarin (100 mg/kg). Phytochemical analysis and acute toxicity studies of the extract were also performed. The results showed that CCl_4 and paracetamol (2 g/kg) elevated the levels of AST, ALT, ALT and bilirubin. Treatment with the methanol extract and methanol and ethyl acetate fractions of *Hoslundia opposita* (100 mg/kg) ameliorated the effects of the hepatoxins and significantly ($P>0.05$) reduced the elevated levels of the biochemical marker enzymes, while the chloroform and hexane fractions showed no significant ($P<0.05$) hepatoprotective effect. The extracts showed good toxicity profile with an LD_{50} value above 5000 mg/kg for the methanol extract. Phytochemical analysis showed the presence of resins, flavonoids, sterols/triterpenes, tannins, saponins, alkaloids, reducing sugars, cardiac glycosides and proteins in the solvent fractions. These results suggest that the stem of *Hoslundia opposita* contains bioactive principles with hepatoprotective effect.

Key words: Carbon tetrachloride, hepatic marker enzymes, hepatoprotective activity, *Hoslundia opposita*, liver damage, paracetamol

INTRODUCTION

The liver plays a major role in metabolism and has a number of functions in the body, including glycogen storage, decomposition of red blood cells, plasma protein synthesis and detoxification. It performs and regulates a wide variety of high-volume biochemical reactions requiring very specialized tissues.[1]

Some toxic chemicals or compounds known as hepatotoxins produce varying degrees of damage to the liver. They may produce a variety of morphological changes, which may be typical of the various agents. Liver damage is usually associated with elevation in the serum levels of many biochemical markers such as AST, ALT, ALP, bilirubin, triglycerides and cholesterol.[2]

The advancements in modern medicine notwithstanding, there are no synthetic drugs for the management of liver disorders. Herbs play a critical role in the management of many liver disorders.[3,4] In the absence of a reliable and effective hepatoprotective agent in modern medicine, a number of medicinal plant preparations have been recommended for the treatment of liver problems.[5] There are avalanche of scientific support on the efficacy of medicinal plants in the management of drug-induced and other liver disorders.[6-18]

Hoslundia opposita Vahl (Lamiaceae) known locally as 'Oke ota' by the Ibo tribe of South-eastern Nigeria is traditionally acclaimed to be very effective in the management of liver diseases. The plant has a wide natural distribution, occurring both in the tropical and subtropical open woodlands such as Namibia, Botswana, Swaziland and in the coastal areas of Kwazulu-Natal extending to Mpumalanga and Limpopo. The plant is also common in other African countries such as Senegal, Sudan and Ethiopia.[19] *H. opposita* is a medium-sized, tender, shrub, growing up to 5 m high. The leaves of the plant are oval in shape, about 10 cm long and 4 cm broad and oppositely arranged at the nodes. The plant possesses minute, white or creamy green coloured flowers, starting from October to February, and fruits are fleshy, berry-like in shape and attractively orange-red in colour.[20]

Locally, the plant is used in the treatment of cough, chest

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pain, fever, hookworm, stomach disorders, wounds, liver diseases and mental disturbances.

Although the plant is widely used in the Eastern parts of Nigeria for the treatment of liver diseases, there has not been any scientific investigation on the hepatoprotective properties of the plant. The high cure rates acclaimed in the use of *H. opposita* stimulated our interest in investigatiing the hapatoprotective effects of the stem extracts of the plant in experimental drug-induced hepatic injury models.

MATERIALS AND METHODS

Animals

Adult Wistar rats (90-260 g), adult Swiss albino mice (15-36 g) of either sex obtained from the animal house of the Department of Pharmacology and Toxicology, University of Nigeria, Nsukka, Enugu State, Nigeria, were used for the study. The animals were housed under standard conditions of temperature (28±2°C) and 12-h light/dark cycle with free access to standard livestock feeds and clean drinking water. All the studies were approved by the University of Nigeria Animal Ethical Committee.

Plant Material

Fresh stems of *H. opposita* were collected from Umu-Osigide, a village in Enugu State, Nigeria. The plant material was authenticated by Mr. Ozioko of the Bioresources Development and Conservation Programme (BDCP), Nsukka.

Extraction and Differential Solvent Fractionation

The fresh stems of *H. opposita* were washed with water, sun-dried for 8 days and then pulverized using a grinding machine. The stem powder (2000 g) was extracted with a total of 6 L of methanol in soxhlet extractor at 80°C. The filtrate was evaporated at 50°C with a rotary evaporator and completely dried over water bath to give 124.84 g (6.24%) of the crude methanol extract (ME). The ME (80 g) was subjected to differential solvent fractionation by the cold maceration method using hexane (HF), chloroform (CF), ethyl acetate (EF) and methanol (MF) to obtain 1.40 g (HF), 1.45 g (CF), 0.71 g (EF) and 43.23 g (MF) fractions. In the preliminary screening, the ME at 100 mg/kg showed significant hepatoprotective properties; hence, the solvent fractions were studied for hepatoprotective effects at this dosage. The solvent fractions were also subjected to phytochemical screening.[21] The acute toxicity of ME was also estimated in mice by the intraperitoneal route using the method described by Lorke.[22]

Evaluation of Hepatoprotective Activity

The crude ME and the solvent fractions (HF, CF, EF and MF) were investigated for the hepatoprotective activities using two models of experimental hepatic injury. All rats were allowed free access to food and water. Silymarin (100 mg/kg) and liquid paraffin (1 mg/kg) were used as reference hepatoprotective agent and negative control treatment, respectively.

Carbon Tetrachloride-Induced Hepatotoxicity Model

The animals were divided into seven groups of six rats each and subjected to the following treatments.[23] Groups 1 and 2 served as the controls and received liquid paraffin (1 mL/kg) for 9 days. Group 3 received silymarin (100 mg/kg) for 9 days. Groups 4, 5, 6 and 7 received 100 mg/kg of ME, EF, CF and HF, respectively, for 9 days. All administrations were by oral route. On the ninth day of the experiment, the animals in groups 2-7 were administered CCl_4 in liquid paraffin (1:1) at the dose of 1 mg/kg intraperitoneally. Blood samples were collected from the animals 24 hours after CCl_4 administration through retro orbital puncture for the assay of liver enzymes.

Paracetamol-Induced Hepatotoxicity Model

Animals divided into seven groups of six rats each were used for the study.[2] Groups 1 and 2 received normal saline (1 mL/kg, orally) for 7 days. Groups 3, 4, 5, 6 and 7 received 100 mg/kg of silymarin, MF, EF, CF and HF orally, respectively, once a day for 7 days. On the fifth day, after the administration of the respective treatments, all the animals in groups II, III, IV, V, VI and VII were administered paracetamol (PCM) 2 g/kg orally. On the seventh day, the blood samples were collected via ocular puncture for the estimation of biochemical marker enzymes.

Biochemical Analysis

The blood samples were allowed to coagulate for 30 min and the clear serum was separated by centrifuging at 2500 rpm for 10 min and was then used for the analysis of biochemical hepatic markers-total bilirubin,[24] aspartate aminotransferase (AST),[25] alanine aminotransferase (ALT)[25] and alkaline phosphatase (ALP)[26]

Statistical Analysis

The results are expressed as mean±SEM, (N=5). Statistical significant was determined by one-way analysis of variance (ANOVA) and subjected to LSD *post hoc* tests. Significant differences between mean were accepted when $P<0.05$.

RESULTS

The *H. opposita* extract showed positive reactions for proteins, cardiac glycosides, reducing sugars, alkaloids, saponins, tannins, acidic compounds, sterols/triterpenes, flavonoids and resins. The methanol stem extract (ME) administered intraperitoneally up to 5000 mg/kg did not produce lethality or signs of acute toxicity in mice after 24 h, an indication of relative safety. Administration of

CCl_4 and PCM significantly caused both hepatocellular and cholestatic liver damage leading to elevation in the levels of liver enzymes-AST, ALT and ALP and total bilirubin. These biochemical markers were significantly elevated in rats that received CCl_4 and PCM alone than the normal rats (liquid paraffin control). The effect of the solvent fractions of the ME of *H. opposita* on the biochemical markers in CCl_4- and PCM-intoxicated rats are summarized in Tables 1 and 2. CCl_4- and PCM-induced hepatic injuries were remarkably ameliorated by MF and EF (100 mg/kg), while CF and HF (100 mg/kg) gave mild-to-moderate hepatoprotection against these hepatotoxins [Tables 1 and 2]. There was a significant reduction (P<0.05) in the biochemical marker enzymes in the rats treated with the fractions and silymarin.

DISCUSSION

Because *H. opposita* is widely used in folk medicine for the treatment of liver diseases, we investigated the anti-hepatotoxic properties of the stem extract of the plant using two experimental models. Various mechanisms may be associated with the damage done to the liver by different hepatotoxins. It has been postulated that CCl_4 is biotransformed by the cytochrome P_{450} system to produce highly reactive trichloromethyl free radicals, which in turn covalently binds to cell membranes and organelles to elicit lipid peroxidation. This causes functional and morphological changes in the cell membrane, disturbed calcium homeostasis and finally cell death.[27,28] CCl_4 is also known to significantly increase liver tissue weight; total lipid content is significantly elevated with a concomitant inhibition of total protein synthesis, manifesting as decreased tissue total protein.[29] The hepatotoxin is also known to interfere with the phospholipids synthesis,[28] and to decrease liver plasma membrane phospholipids content resulting in fatty liver.[30-32]

In the PCM model, the drug is said to be eliminated mainly as sulfate and glucuronide. Only a small amount (5%) is metabolized via the cytochrome P_{450} enzyme system to the alkylating metabolite *N*-acetyl-*p*-benzoquinone imine (NAPQI), which is responsible for the toxic side effects of PCM.[33] However, upon administration of toxic doses of PCM, the sulfation and glucuronidation routes become

Table1: Effect of *H. opposita* extracts on biochemical parameters in CCl_4- induced hepatic injury in rats

Treatment	Total bilirubin (mg/dL)	AST (IU/L)	ALT (IU/L)	ALP (IU/L)
Control (liquid paraffin 1 mL/kg only)	0.562±0.04 (100.00)	145.540±7.29 (100.00)	50.726±5.12 (100.00)	57.142±1.96 (100.00)
Control (liquid paraffin 1 mL/kg)+CCl_4	1.339±0.10 (0.00)	242.916±5.39 (0.00)	125.208±1.94 (0.00)	132.856±4.57 (0.00)
Positive control (silymarin, 100 mg/kg)+CCl4	0.8424±0.09* (63.96±11.58)	152.872±5.34* (92.47±0.05)	53.034±2.83* (96.90±0.16)	72.858±1.82* (79.24±2.40)
MF (100 mg/kg)+CCl_4	0.907±0.07* (55.60±9.01)	169.624±7.52* (75.27±0.08)	60.210±5.26* (87.27±0.12)	88.578±3.07* (58.48±4.05)
EF (100 mg/kg)+CCl_4	1.123±0.03* (27.80±3.86)	176.950±13.18* (67.74±0.14)	66.396±9.38* (78.96±0.08)	100.002±4.79* (43.39±6.33)
CF (100 mg/kg)+CCl_4	1.296±0.08* (5.53±10.30)	210.460±5.59* (33.33±0.60)	104.280±8.91* (28.10±0.06)	113.572±1.34* (25.47±1.77)
HF (100 mg/kg)+CCl_4	1.426±0.08 (−11.20±10.30)	232.448±14.30 (10.75±0.15	102.072±7.12* (31.06±0.18)	120.710±5.91* (16.04±7.81)

*P values <0.05 considered significant compared to untreated group (CCl_4 group). Percentage hepatoprotection is shown in parenthesis

Table 2: Effect of the *H. opposita* extracts on biochemical parameters in paracetamol-induced hepatic injury in rats

Treatment	Total bilirubin (mg/dL)	AST (IU/L)	ALT (IU/L)	ALP (IU/L)
Control (normal saline only)	0.562±0.04 (100.00)	145.540±7.29 (100.00)	50.726±5.12 (100.00)	57.142±1.96 (100.00)
Control (normal saline)+PCM	1.750±0.09 (0.00)	247.104±9.28 (0.00)	106.984±4.48 (0.00)	105.712±1.82 (0.00)
Positive control (silymarin, 100 mg/kg)+PCM	0.864±0.08* (74.58±6.73)	152.868±3.47* (92.78±3.42)	53.212±4.653* (95.58±8.27)	65.714±3.31* (82.35±6.81)
MF (100 mg/kg)+PCM	1.058±0.09* (58.25±7.58)	161.248±8.34* (84.53±8.21)	57.936±5.78* (87.18±10.27)	69.980±3.67* (73.57±7.56)
EF (100 mg/kg)+PCM	1.296±0.10* (38.22±8.42)	171.718±8.51* (74.23±8.38)	64.766±6.29* (75.04±11.18)	79.284±5.46* (54.41±11.24)
CF (100 mg/kg)+PCM	1.706±0.16* (3.70±13.47)	203.130±6.50* (43.30±6.40)	82.816±8.39* (41.21±14.91)	103.570±1.60* (4.41±3.29)
HF (100 mg/kg)+PCM	1.836±0.14 (-7.24±11.78)	217.788±11.06* (28.86±10.89)	96.744±7.05* (18.20±12.53)	91.428±4.87* (29.41±10.03)

*P values <0.05 considered significant compared to the untreated group (PCM group). Percentage hepatoprotection is shown in parenthesis

saturated, and hence, higher percentage of PCM molecules are oxidized to highly reactive NAPQI. Higher dose of PCM and NAPQI can alkylate and oxidize intracellular glutathione (GSH) and protein thiol groups, which result in the depletion of liver GSH pool and subsequently lead to increased lipid peroxidation and liver damage.[34]

In this study, *H. opposite* stem demonstrated significant (P<0.05) liver protection against the hepatic injuries caused by the two hepatotoxins. It is evident that several phytoconstituents have the ability to induce microsomal enzymes either by accelerating the excretion of the hepatotoxin or by inhibition of lipid peroxidation induced by it.[35] Phytoconstituents like flavonoids,[36,37] triterpenes,[38] saponins[39] and alkaloids[40] are known to possess hepatoprotective activities. Flavonoids, tannins and microelements have been suggested to act as antioxidants and exert their antioxidant activity by scavenging the free radicals, which cause lipid peroxidation.[41] A good number of naturally occurring compounds have been shown to protect the liver and other organs from damage.[3,42] There is every possibility that these active principles alone or in combination may be responsible for the hepatoprotection demonstrated in this study. Recently, total flavonoids were reported to protect animals from liver injury and liver fibrosis.[43-45] The protective effect exhibited by the fractions could be due to the protection of hepatic drug metabolizing enzymes. The hepatic injury caused by CCl_4 is associated with damage to the endosplasmic reticulum and any agent capable of ameliorating CCl_4 -induced liver toxicity must have some direct or indirect effect on the liver.[46] It is therefore concluded that the stem extracts of *H. opposita* has promising hepatoprotective potentials.

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