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Original Research Article

Hepatoprotective effect of the methanol leaf extract of *Lophira lanceolata.* (Ochnaceae): An experimental study

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Abstract

The hepatoprotective effect of methanol leaf extract (ME) of *Lophira lanceolata* (Ochnaceae) was investigated against carbon tetrachloride (CCl_4) induced hepatotoxicity in rats. Rats were randomly divided into 7 groups (n=6) and ME was administered at 100, 200, and 400 mg/kg/day p.o to groups III-V, while groups I, VI and VII received 1 ml/kg Tween 80, Vitamin C and Silymarin at 25 mg/kg/day p.o respectively while group II received only CCl_4. CCl_4 was given every 72 hours to all the groups except group 1. Then serum level of blood samples was assayed for alanine aminotransferase (ALT), alkaline phosphatase (ALP), aspartate aminotransferase (AST), malondialdehyde (MDA), total protein, total bilirubin, albumin and globulin. ME significantly ($p < 0.05$) reduced the serum levels of the liver enzymes when compared with the control group (CCl_4). The levels of ALT, AST and bilirubin at 100 and 400 mg/kg were reduced significantly ($p< 0.05$). Also, ALP levels was lowered at 100 mg/kg but with no significant difference ($p>0.05$). At 100 mg/kg, MDA levels was lowered more significantly ($p<0.05$) than controls (Silymarin and vitamin C). Serum globulin level at 200 mg/kg dose of extract was significantly increased when compared to CCl_4 control group. In conclusion, ME can protect against oxidative stress caused by liver toxicant like CCl_4.

Keywords: Hepato-protective effect, *Lophira lanceolata,* methanol extract, rats, carbon tetrachloride

INTRODUCTION

The liver is the main body organ responsible for the metabolism of xenobiotics. It is an important modulator of lipid metabolism, and it has a critical role in the synthesis of lipoproteins, triglycerides, gluconeogenesis from fatty acids, and cholesterol metabolism (Sherwood, 1997). As the major metabolizing and detoxifying organ in the body, the liver may be subjected to potential damage from an enormous array of pharmaceutical and environmental chemicals. These Injuries result in direct toxicity, due to hepatic conversion of a xenobiotic to an active toxin (Farrel et al., 2002).

Hepatotoxicity is a growing concern for today's modern society. The increasing incidence of industrial pollutions, occupational hazards and unhealthy lifestyle options such as alcoholism, cigarette smoking, substance abuse and consumption of fatty foods have contributed to the morbidity and mortality due to liver disease (Scott, 1998). Many drugs, toxic substances and infectious organisms are associated with hepatotoxicity due to their ability to generate free radicals and to cause a disturbance in hepatocyte biochemistry (Fernandez-Checa and Kaplowitz, 2005). The free radical formation

leading to hepatic damage in the form of jaundice, cirrhosis and fatty liver, which remain one of the serious health problems that can only be removed with natural defensive.

Carbon tetrachloride (CCl_4) is one of the toxic substances to the liver (Al-Shabanah et al., 2000).It is widely used to develop experimental animal models of liver failure (Yoshioka et al., 2016) Excessive production of the reactive species manifests in tissue thiol depletion, lipid peroxidation, plasma membrane damage etc., culminating into severe hepatic injury (Wargovich et al., 2001).

Lophira lanceolata among other folkloric medicinal uses has been used to treat diarrhoea, dysentery, menstrual pain, liver diseases etc. However, these claims are yet to be studied.

In this study, hepato-protective activities of the methanol leaf extract of *Lophira lanceolata* was investigated.

Interest in herbal medicines is increasing due to their effectiveness, minimal side effects in their clinical experience and relatively low cost. Herbal medicines have been used traditionally worldwide for the prevention and treatment of various diseases. According to the WHO, 80 % of the world population use plant-based remedies for their primary form of healthcare (Evan et al., 1998). Herbal drugs play a role in the management of various liver disorders most of which speed up the natural healing processes of the liver (Subramoniam et al., 1998). Recent studies have reported the hepatoprotective activity of plants such as *Murrayakoenigii, Citrus lemon, Citrus aurantium* and *Tephrosia purpur* (Gupta, 2009). Several other medicinal plants have also shown potential hepatoprotective effects include the rhizomes of *Zingiber officinale* (Saad et al., 2017), *Alchornea cordifolia* leaves (Kouakon et al., 2017), *Thymus vulgaris* (Nagwa and Manar 2017), *Tylophora indica* and *Hoslundia opposite (*Rachna, 2018), *Viola canescens* (Abdullah et al., 2017)*, Stachys pilifera* (Esmaeel et al., 2017)*, Sphaeranthus amaranthiodes* (Somnath et al., 2017)*, Spondias mombin* (Luky et al., 2017)*, Polygonum amplexicaule* (Faiza et al., 2017)*, Homalium letestui* (Jude et al., 2017), *Garcinia Morella* (Nabajyoti et al., 2017), *Pseudocedrala kotschyi* (Moise et al., 2017).

MATERIALS AND METHODS

Materials

Chemicals, reagents, solvents and Standard Drugs

Carbon tetrachloride (CCl_4) (JHD, China), liquid paraffin (Quilikems, India), tween 80 ((JHD, China)), ethylene diamine tetra acetic acid (EDTA), ALT substrate solution, NaOH solution, AST substrate solution, trichloroacetic acid, ALP substrate solution, distilled water (Lion water, UNN), Silymarin, Vitamin C (Vinco Pharmaceutical, Nigeria) were used.

Instruments

Electrical animal weighing balance (B. Bran Scientific and Instruments Co., England), analytical weighing balance, spatula, beakers, measuring cylinders, test tubes, centrifuging tubes, incubator, electrical centrifuges (B. Bran Scientific and Instruments Co., England), UV-Visible spectrophotometer (Easy-Way Medical England 752 W, England), a milling machine (Lab mill, serial No. 4745, Christy and Norris Ltd., England), Soxhlet apparatus, large extraction vessels and rotary evaporator (B. Bran Scientific and Instruments Co., England).

Plant collection and identification

The fresh leaves of *L. lanceolata* were collected from Nsukka, Enugu state, Nigeria in the month of May, in 2016, identified and authenticated by Mr Alfred Ozioko of the International Centre for Ethno medicine and Drug Development (Inter CED), Nsukka, Enugu state. A dried voucher specimen was preserved at the Pharmacognosy Herbarium, Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka (specimen number: PCG/UNN/0311) thereafter.

Preparation of plant extract and fraction

The leaves of *L. lanceolata* were air-dried at room temperature and ground into powder with a grinder (ADDIS, Nigeria). The powdered material (2370 g) was macerated with 4.5 L of 70% methanol for 72 h with constant shaking. The resultant mixture was filtered using Whatman (No. 1) filter paper and the filtrate was concentrated to dryness under vacuum at 40^{o}C using rotary evaporator.

Animals

Adult Swiss albino rats (150 - 200 g) were used for the study. Rats were obtained from the animal laboratory facility of the department of Pharmacology and toxicology, University of Nigeria, Nsukka. The animals were maintained freely on standard pellets and water. The animal use ethical approval was obtained from the Institutional Ethics committee of the University of Nigeria, Nsukka and was in compliance with the National Institute of Health Guide for Care and Use of Laboratory Animals (Pub No. 85 – 23, revised 1985).

Methods

Pharmacological Tests

Carbon Tetrachloride Induced Toxicity

The carbon tetrachloride induced biologic oxidation model was used as described by Suja et al., (2004). After seven days of acclimatization, the 35 rats of both sexes were divided into seven groups (n=5). Group I received Tween 80 (Negative control), group II received CCl_4 (CCl_4: liquid paraffin (1:2); (1 ml/kg/day p.o), the animals in the test groups (III to V) received 100, 200 and 400 mg/kg of the methanol extract respectively. Group VI received vitamin C (25 mg/kg p.o), and group VII were given Silymarin (25 mg/kg). The treatments lasted for seven days and 1 ml/kg of carbon tetrachloride (CCl_4) was given to groups II to VII once every 72 hours to induce the liver damage. On the 8^{th} day, blood sample was collected for the assay of serum enzymes alanine aminotransferase (ALT) and serum aspartate aminotransferase (AST) activities (Reitman et al., 1957), serum alkaline phosphatase (ALP) activity (Kleinet al., 1960; Babson et al., 1966), malondialdehyde (MDA) determination by Thiobarbituric acid method (Plaser et al., 1966), determination of serum total proteins (Lubran, 1978), determination of serum albumins (Doumas et al., 1971; Doumas and Peters, 1997), calculation of serum globulin, determination of total bilirubin (Stone, 1954).

Statistical analysis

Results obtained were expressed as Mean ± SEM. The data were analyzed using one way ANOVA, followed by DUNNET post hoc using Graph pad Prism version 5.03 software. $p<0.05$ was considered statistically significant.

RESULTS

The percentage yield

The percentage yield of the ME was calculated to be 4.59 % w/w.

Phytochemical analysis

The ME of *Lophira lanceolata* gave positive test for flavonoid, alkaloid, glycoside, saponins, and terpenoids, reducing sugar, oils and carbohydrates as reported by Onyeto et al., (2014).

Pharmacological test

Acute toxicity test

There was no mortality recorded in the mice upon oral administration at 5000 mg/kg (Onyeto et al., 2014).

Effect of methanol extract of *Lophira lanceolata* on serum ALT activity induced carbon tetrachloride rats

The serum ALT activity of the group treated with 100 and 400 mg/kg was significantly ($p<0.05$) lower than that of the untreated (CCL_4) control. The effects of the 100 and 400mg/kg was comparable ($p>0.05$) to that of Silymarin (Table 1).

Effect of methanol extract of *Lophira lanceolata* on serum AST activity induced carbon tetrachloride rats.

The serum AST activity of the group treated with 100 and 400 mg/kg was also significantly ($p<0.05$) lower than that of the untreated (CCL_4) control, and comparatively better than Silymarin (Table 1).

Effect of methanol extract of *Lophira lanceolata* on serum ALP activity induced carbon tetrachloride rats

Animal groups treated with various doses of methanol extract had lower serum ALP activity but the difference was not significant ($p>0.05$) when compared to the untreated CCL_4 control. Silymarin treatment had a significantly ($p<0.05$) better effect on serum ALP than the extracts (Table 1)

Effect of methanol extract of *Lophira lanceolata* on MDA activity induced carbon tetrachloride rats.

Treatment with methanol extract of 100 mg/kg significantly lowered ($p<0.05$) the MDA levels (Products of Oxidative stress) far better than all other treatments including Silymarin (Table 1)

Effect of methanol extract of *Lophira lanceolata* on serum protein, globulin and albumin activity induced carbon tetrachloride rats

Liver damage using CCL_4 and treatment with methanol extract or vitamin C or Silymarin had no significant ($p>0.05$) effect on serum proteins and albumin levels. However, damage using CCL_4 led to higher albumin levels in animal groups given CCL_4 and treated with

Table 1. The serum enzyme activity and malondialdehyde levels in rats given carbon tetra chloride (CCL_4) and treated with varied doses of methanol extract of *Lophira lanceolata*

Treatments	Means of serum enzyme activity and malondialdehyde levels, with standard error in bracket.							
	Alanine aminotransferase (IU/L)		Aspartate aminotransferase (IU/L)		Alkaline phosphatase (IU/L)		Malondialdehyde (µmol/L)	
	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment
Tween 80 control	45.58 (2.08)	47.25 [a] (2.07)	72.08 (1.90)	74.34 [a] (2.77)	318.10 (17.96)	327.20 [a] (19.59)	1.43 (0.06)	1.42 [acd] (0.05)
CCL_4 only	45.90 (1.59)	88.71 [b] (0.56)	72.39 (2.22)	107.80 [b] (1.89)	313.7 (16.24)	504.80 [b] (9.87)	1.41 (0.05)	1.85 [b] (0.10)
CCL_4 + 100 mg/kg b.w. extract	47.35 (2.13)	63.47 [c] (8.69)	71.54 (2.20)	94.11 [c] (6.99)	322.20 (16.77)	456.00 [b] (21.40)	1.40 (0.08)	1.27 [c] (0.05)
CCL_4 + 200 mg/kg b.w. extract	45.03 (2.22)	83.43 [b] (1.20)	73.16 (2.25)	109.50 [b] (3.59)	319.30 (17.76)	478.00 [b] (14.56)	1.44 (0.08)	1.50 [d] (0.09)
CCL_4 + 400 mg/kg b.w. extract	47.40 (2.49)	67.07 [c] (5.61)	72.31 (3.06)	95.21 [c] (5.01)	300.80 (18.02)	434.40 [b] (54.44)	1.42 (0.07)	1.51 [d] (0.11)
CCL_4 + Vitamin C	46.96 (1.53)	78.45 [b] (3.71)	72.85 (2.33)	102.80 [bc] (4.16)	320.30 (17.53)	462.40 [b] (23.04)	1.45 (0.06)	1.50 [d] (0.07)
CCL_4 + Silymarin	44.45 (1.95)	69.20 [c] (4.96)	75.51 (2.64)	97.53 [bc] (6.74)	336.20 (20.82)	317.90 [a] (2.07)	1.46 (0.05)	1.40 [acd] (0.05)

[a, b, c, d] Different alphabetical superscripts in a column indicate significant difference between the means, $p < 0.05$

Table 2. The serum protein and bilirubin levels of rats given carbon tetra chloride (CCL_4) and treated with varied doses of methanol extract of *Lophira lanceolata*

Treatments	Means of serum proteins and bilirubin, with standard error in bracket.							
	Total protein (g/dl)		Albumin (g/dl)		Globulin (g/dl)		Bilirubin (mg/dl)	
	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment
Tween 80 control	6.43 (0.14)	6.67 (0.05)	3.38 (0.09)	3.75 (0.17)	3.05 (0.12)	2.92 [a] (0.17)	0.22 (0.03)	0.23 [a] (0.02)
CCL_4 only	6.56 (0.27)	6.93 (0.18)	3.47 (0.08)	3.60 (0.24)	3.09 (0.22)	3.33 [ab] (0.26)	0.25 (0.02)	0.51 [b] (0.05)
CCL_4 + 100 mg/kgb.w. extract	6.31 (0.19)	6.95 (0.13)	3.41 (0.08)	3.82 (0.13)	2.91 (0.15)	3.13 [ab] (0.13)	0.26 (0.04)	0.28 [ac] (0.02)
CCL_4 + 200 mg/kg b.w. extract	6.64 (0.11)	6.98 (0.23)	3.42 (0.09)	3.47 (0.23)	3.22 (0.12)	3.51 [b] (0.31)	0.27 (0.03)	0.31 [c] (0.02)
CCL_4 + 400 mg/kg b.w. extract	6.69 (0.21)	6.90 (0.30)	3.76 (0.08)	3.82 (0.17)	3.22 (0.24)	3.36 [ab] (0.15)	0.25 (0.03)	0.30 [ac] (0.01)
CCL_4 + Vitamin C	6.42 (0.16)	6.77 (0.17)	3.50 (0.12)	3.77 (0.12)	2.91 (0.19)	3.00 [ab] (0.08)	0.27 (0.04)	0.28 [ac] (0.03)
CCL_4 + Silymarin	6.49 (0.19)	6.61 (0.18)	3.49 (0.15)	3.48 (0.16)	2.70 (0.14)	3.12 [ab] (0.16)	0.23 (0.03)	0.27 [ac] (0.02)

[a, b, c] Different alphabetical superscripts in a column indicate significant difference between the means, $p < 0.05$

varied doses of methanol extracts. The effect on the globulin levels was most severe on the group treated with 200 mg/kg extract (Table 2)

Effect of methanol extract of *Lophira lanceolata* on serum bilirubin activity induced carbon tetrachloride rats

The serum bilirubin levels of the animal groups treated with the extracts, vitamin C, and Silymarin were all significantly lower ($p<0.05$) than that of the CCL_4 untreated control. The serum bilirubin of animal groups treated with 100 and 400 mg/kg, vitamin C and Silymarin were comparable to that of the tween 80 control (Table 2).

DISCUSSION AND CONCLUSION

Discussion

The efficacy of any hepatoprotective drug is essentially dependent on its capacity of either reducing the harmful effects or maintaining the normal physiologic function which has been disturbed by hepatotoxic agents, (Tripathi et al., 1999). Mechanisms of drug induced hepatotoxicity includes: Interference with bilirubin transport and conjugation; cytotoxic injury; cholestasis; mixed cytotoxic/cholestatic injury; fatty liver (steatosis); chronic active hepatitis, cirrhosis and sub-acute necrosis; phospholipidosis; liver tumours; and non-specific changes, (Davis et al., 1977). Mechanisms of hepatoprotectective effect includes: Glycine-mediated cytoprotection which protects the liver, kidney and other cells against cell death in various models of hypoxia and ATP depletion, (Lemasters et al., 1981); estradiol and ethinylestradiol reduce the degree of liver injury caused by hepatotoxicants as well as ischemia-reperfusion.

In liver damage, total protein, albumin and globulin synthesis are impaired leading to their decrease in serum concentrations (Grant, 1987).

Carbon tetrachloride was reported to produce free radicals, which affect the cellular permeability of hepatocytes leading to elevated levels of serum biochemical parameters like ALT, AST and ALP (Vinoth et al., 2009). The levels of serum aspartate amino transaminase (AST), Alanine amino transaminase (ALT), Alkaline Phosphate (ALP) and Malondialdehyde (MDA) were taken as an index for oxidative stress induced by CCl_4.

The results of this study revealed that daily oral supplementation of the methanol extract of *Lophira lanceolata* significantly ($p<0.05$) reduced the elevation of mean serum concentration of AST and ALT induced by CCl_4 in a dose dependent manner. The administration of 100 and 400 mg/kg of the extract produced more reduction in serum AST and ALT as compared to the standard drug, Silymarin. This reduction suggest the protection of the structural integrity of hepatocyte cell membrane or regeneration of damaged liver cells caused by CCl_4. ALT is a better index of liver injury, as liver ALT activity represents 90 % of total enzymes present in the body (Moss and Butterworth, 1974).

Serum levels of ALP were reduced by the methanol extracts of *Lophira lanceolata* though not significant. A more significant reduction was seen with the standard drug, Silymarin. Administration of vitamin C did not reduce the serum level of the enzyme ALP. Overall, the progressive decrease in the levels of serum liver enzymes implies that the free radicals, which were generated due to the CCl_4 induction, were being mopped up by the plant extract treated with ME of *Lophira lanceolata* after seven days post treatment and also indicates an early improvement in the cellular membrane integrity of the hepatic cell.

The reduction of the serum level of MDA by the ME (100 mg/kg) was very high. This confirms the antioxidant mediated mechanism incorporation.

In liver diseases, there is elevation of conjugated and unconjugated bilirubin concentrations (Hass, 1999). The extract reduced total bilirubin concentration relative to the control groups. However, these reductions were not significant when compared to any of the control groups. Suppression of increased activity of ALP correlating with a decrease in raised bilirubin level indicates the stability of the biliary dysfunction in the rat liver during hepatic injury with CCl_4 (Gole et al., 1997).

One of the major functions of the liver is the synthesis of serum protein and its metabolism. Hepatotoxicity can impair the protein synthesis function of the liver (David, 1999). The extract showed an increase in the serum total protein, albumin and globulin concentrations, relative to the negative control and CCL_4 control group. The extract also showed sufficient increase in albumin and total protein concentrations, relative to the positive control group. There is sufficient increase in globulin concentrations at 200 mg/kg, relative to the positive control group. This implies that the extract is effective in reversing liver damage caused by carbon tetrachloride at different dose levels.

CONCLUSION

The results of this study suggest that the ME of *Lophira lanceolata*, possesses potent hepatoprotective effects against carbon tetrachloride induced toxicity in rats. However, further work is required to elucidate the phytochemical constituents of *Lophira lanceolata*, in order to verify any other

advantages, the extract may possess over other hepatoprotective agents.

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