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HEPATOPROTECTIVE ACTIVITIES OF METHANOLIC EXTRACT OF *NAUCLEA LATIFOLIA*

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ABSTRACT

The hepatoprotective activity of the root bark extract of Nauclea latifolia was tested in vivo. The root bark was defatted with diethyl ether and then extracted with methanol and the methanolic extract was recovered on a 9.3 % w/w yield. The LD_{50} of the methanolic extract in rats was 300 mg/kg (i.p). It showed no significant effect on pentobarbital-induced sleep in rats after i.p. injection. The extract significantly reduced pentobarbital-induced sleep in paracetmol and carbon tetrachloride (CCl_4) intoxicated rats. The elevataion of Aspartate aminotransferase (AST), Alanine aminotransferase (ALT) and alkaline phosphatase (AP) induced by paracetamol and CCl_4 intoxication in rats was also significantly ($P<0.05$) attenuated by the extract. The methanolic extract reduced the leakage of lacte dehydrogenase (LDH) in isolated rat heptocytes but had no significant effect on lipid peroxidation. It is suggested that the methanolic extract of N. latifolia might contain hepatoprotective principles that support its use in the treatment of liver diseases traditionally.

Keywords: Nauclea latifolia, Folk medicine, hepatoprotective, Tashi ya igiya, Ubiri agu.

INTRODUCTION

Nauclea latifolia Sm. (Rubiaceae) known as "Ubiri Agu" in Igbo (meaning "the breath of a lion") and Tashi ya igiya" in Hausa (meaning "the medicinally useful bark"), which are two of the three major Nigerian languages enjoys a good folk reputation in Nigerian ethnomedicine. Alcoholic extracts from the root of this plant have been used extensively by herbalists and native doctors for treating human ailments including malaria, wounds, coughs, gonorrhea, stomach aches and gastrointestinal tract disorders (Dalziel, 1984), ulcer and liver ailments (Iwu, 1982). The fruit is also used to cure headache and menstrual pain (Githens, 1948). The bark has been reported to contain resins, bitter principles, tannins and indoquinolizodine alkaloids (Hotelier and Delaveau, 1975). The present study reports the hepatoprotective effects of the methanolic extract of the root bark of *N. latifolia* against liver damage in experimental models of hepatic lesions induced by paracetamol, CCl_4 and tert-butylhydroperoxide, respectively.

MATERIALS AND METHODS

Plant material

The plant material (root bark) was collected in January 1990, from Obukpa in Nsukka Local Government Area, Enugu State (Nigeria) and identified as *Nauclea latifolia* Sm. (Rubiaceae) by Mr. J.M. Ekekwe of the Department of Botany, University of Nigeria, Nsukka. A voucher specimen (# 303) has been deposited in the department's herbarium.

Preparation of extract

The fresh root bark of *N. latifolia* was dried at room temperature and then reduced to a coarse powder. The powder (240.5 g) was initially defatted with diethyl ether using soxhlet extraction for 9 h. The dried marc was then extracted with 70 % methanol at room temperature for 72 h. Subsequently, the extract was concentrated to dryness in vacuum at 40°C, using rotary evaporator buchii and stored at 4 °C, until used for the study. The yield was 29.3 %.

Animals

Male and female Wister (120 – 150 g) rats were obtained from the stock maintained in the laboratory animals unit of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka. They were kept in wire mesh cages, allowed free access to water and fed *ad libitum* with chicken grower mash containing 16 % crude protein.

Acute toxicity

Thirty rats of both sexes were randomly selected into six equal groups of five, 3 males and 2 females. Groups 1 – 5 were injected intraperitoneally with varying doses (100; 200; 300; 400 and 800 mg/kg) of the methanolic extract in normal saline while group 6, which served as the control, received not more than 3 ml/kg of normal saline by the same route. After treatment the animals were observed for clinical signs over a period of 24 h. Deaths within this period were recorded and the LD_{50} was determined by the method of (Miller and Tainter, 1937).

Pentaobarbital sleeping time in rats

This study was conducted using a known method (Mcleod, 1970). Four groups of five Wister rats of both sexes each were kept in separate stainless steel cages for one week before the commencement of the experiment. Group 1 which served as the control was injected with pentobarbital sodium (35 mg/kg, i.p). Groups 2 – 4 were similarly treated 30 min after they were respectively injected with increasing doses (50; 100; 200; mg/kg) of the extract. The time of injection, time of sleep (when righting reflex was lost) and the time of awakening (when righting reflex was regained) were recorded and the mean sleeping time calculated.

Paracetamol and CCl_4-induced hepatotoxicity in rats

Seven groups of (6 rats per group) of mixed sexes were used in this study. Group 1 served as the control and was given normal saline every 12 h for 4 days. Two groups, 2 and 3 received 100 mg/kg of the methanolic extract (p.o) reconstituted in normal saline as in 1 above. Another two groups, 4 and 5 received 100 mg/kg (p.o) of silibinin (Max Planck Institute, FRG) suspended in water as in 1 above. One hour after the last dose, all the animals in groups 2 and 3 were given orally a single dose of 650 mg/kg of paracetamol and CCl_4 diluted with liquid paraffin to give a dose of 0.15 ml/kg respectively. At the same time all the animals in groups 4 and 5 were given the same treatment. Groups 6 and 7 that served as the negative controls were given normal saline for 4 days, then I h after the last dose, they were given paracetamol and CCl_4 as in groups 2 and 3 above. Twelve hours later, all the animals in all the groups were given a single dose of pentobarbital sodium (35 mg/kg i.p.) and the duration of sleep for each animal recorded.

Paracetamol and CCl_4-induced elevation of serum enzymes in rats

The grouping was as in the last experiment above. The procedure and the treatment regimen were also the same. One h after the last dose, each animal in their respective groups was challenged with paracetamol and CCl_4 respectively as in the previous experiment. Two other control groups just like in the previous experiment also received paracetamol and CCl_4 respectively. Twelve hours later the animals were anesthetized with ether and blood samples obtained by cardiac puncture. Serum levels of aspartate amino-transferase (AST) and alanine amino-transferase (ALT) were measured in the serum by a standard method (Reitman and Frankel, 1957). The serum levels of alkaline phosphatase were also measured (Bessey and Lowry, 1946).

Isolation and culture of hepatocytes from rat liver

Liver parenchymal cells were isolated using a known method of (Seglen, 1976).

Tert-Butyl Hydroperoxide-induced elevation of malonaldialdehyde and lactate dehydrogenase in isolated rat hepatocytes

Hepatocytes were incubated in Minimum Eagle's Medium at a final concentration of 5 x 10^6 cells/ml in 25 ml Erlenmeyer at 37°C and gassed with O^2/CO^2 (95 %, 5 %). Tert-butyl hydroperoxide (tBH) was added in 20 µl of DMSO to hepatocytes suspension at a final concentration of 1.5 mM. The methanolic extract was added simultaneously. The control flasks received the same volume (0.4) % v/v) of DMSO alone. Lipid peroxidation in isolated hepatocyte was monitored by production of malondialdehyde (MAD). Thirty minutes after intoxication, 1 ml cell suspension was deproteinated with 2 ml 10 % TCA and centrifuged. The supernatant (2ml) and an equal volume of 1 % thiobarbituric acid (TBA) were heated in a boiling water bath for 10 min and allowed to cool. The absorbance was measured at 532 nm and compared to standards.

Disturbance of cell membranes was estimated by asuring the leakage of LDH into the medium following a standard method (Joyeux *et al.*, 1990).

Statistical Analysis

The data collected were subjected to statistical analysis using the procedure outline by (Steel and Torrie, 1980) for a completely randomized design.

RESULTS

Acute toxicity

The methanolic extract produced dose–related death in rats (10%, 100 – 200 mg/kg; 50%, 300 mg/kg; 100 %, 400 – 800 mg/kg. The LD_{50} was calculated to be 300 (129 – 453) mg/kg. Starting from 400 – 800 mg/kg, poisoned rats showed clinical signs as severe as depression, crawling gait, inability to move the hind limbs, muscular spasm similar to that observed in strychnine poisoning and death.

Pentobarbital induced sleep in rats

The methanolic extract of *N. Latifolia* root bark did not influence the duration of sleep following pentobarbital administration in rats at the dose range of 50 – 200 mg/kg (Table 1).

Table 1: The effects of the methanolic extract *of N. latifolia* root bark on pentobarbitone-induced sleep in rats

Groups	Dose of the extract (mg/kg)	Mean sleeping time (min ± S.e.m)
1	Control (Normal saline)	86.25 ± 13.41
2	50	90.40 ± 4.10
3	100	88.30 ± 2.98
4	200	84.20 ± 0.96

Paracetamol and CCl_4-induced hepatotoxicity in rats

After treatment with paracetamol and CCl_4, the duration of the pentobarital-induced hypnosis in rats increased significantly ($p<0.01$) in the groups that were given normal saline. On those pretreated with the methanolic extract of *N. latifolia* root bark and silibinin, the duration of pentobarbital effect was significantly ($p<0.01$) lower in comparison with the above groups. The normal control group that was not pretreated with any thing, had the lowest sleeping time. The difference was also significant ($p<0.05$) when compared with the groups treated with the extract and Silibinin respectively (Figure 1).

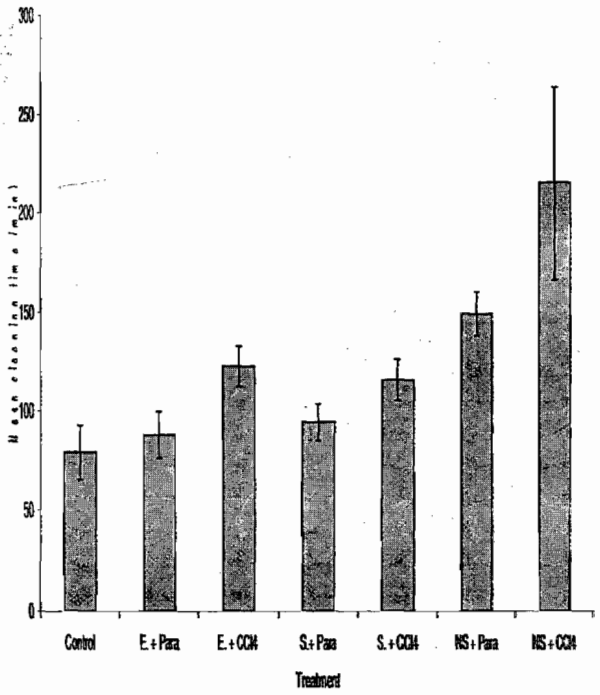

Figure 1: The effects of the methanolic extract of *N. latifolia* root bark extract on pentobarbitone-induced sleep in rats challenged with paracetamol and carbon tetrachloride

E = Extract; S = Silibinin; Para = Paracetamol, NS = Normal saline, CCl_4 = Carbon tetrachloride

Paracetamol and CCl_4-induced elevation of serum enzyme

Paracetamol and CCl_4 caused liver damage as manifested by the remarkable increases in the activities of the transaminases (AST, ALT) and alkaline phosphatase (ALP) in the serum of the experimental animals twelve hours after they were given to the animals. In the experimental animals pretreated with the methanolic extract of *N. latifolia* root bark and silibinin, there were significant ($p<0.05$; 0.01) decreases in the level of the transaminases when compared with the groups that were given normal saline. The normal control showed the least level of these enzymes (Table 2).

Table 2: The effects of the methanolic extract of *N. latifolia* root bark on paracetamol and carbon tetrachloride-induced elevation of serum enzymes in rats

Treatment	ALP (IU/L)	AST (IU/L)	ALT (IU/L)
Control	41.0 ± 0.7 * ++	69.8 ± 8.9 ** ++	21.4 ± 1.4 ** ++
Extract + paracetamol	74.5 ± 2.2*	87.3 ± 1.2*	18.2 ± 1.8**
Extract + CCl_4	65.6 ± 0.8++	90.4 ± 1.5+	25.1 ± 0.9++
Silbinin + paracetamol	82.0 ± 4.0	100.7 ± 5.4 *	23.9 ± 2.9 **
Silibinin + CCl_4	72.0 ± 3.5 ++	119.0 ± 0.7 +	38.0 ± 0.7 ++
N.S. + Paracetamol	95.0 ± 3.7	120.4 ± 4.3	47.7 ± 3.2
N.S. + Carbon tetrachloride	120.5 ± .6	138.0 ± 9.2	69.0 ± 2.4

,** Significantly different from paracetamol control group ($p<0.05$, $p<0.01$) respectively
*+, ++ significantly different from carbon tetrachloride control group ($p<0.05$, $p<0.01$ respectively.
Ext = Extract, Silb. = Silibinin, N.S. = Normal saline

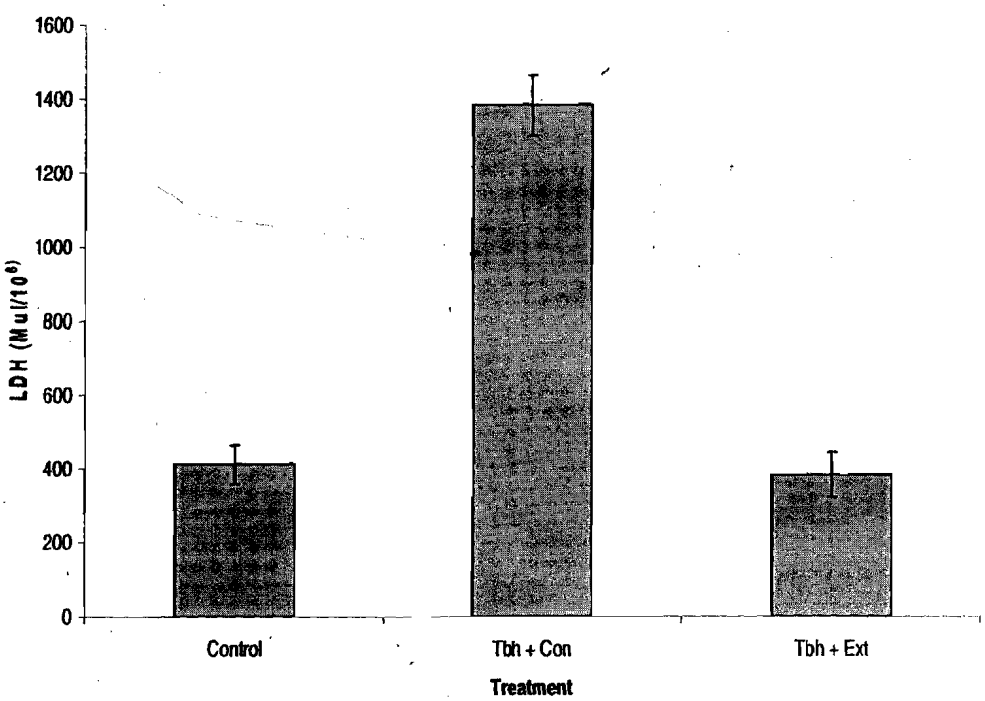

Figure 2: The effects of the methanolic extract of N. latifolia root bark on lactate dehydrogenase (LDH) enzyme

Tbh = Tert-butyl hydroperoxide, Con = Control, Ext = Extract

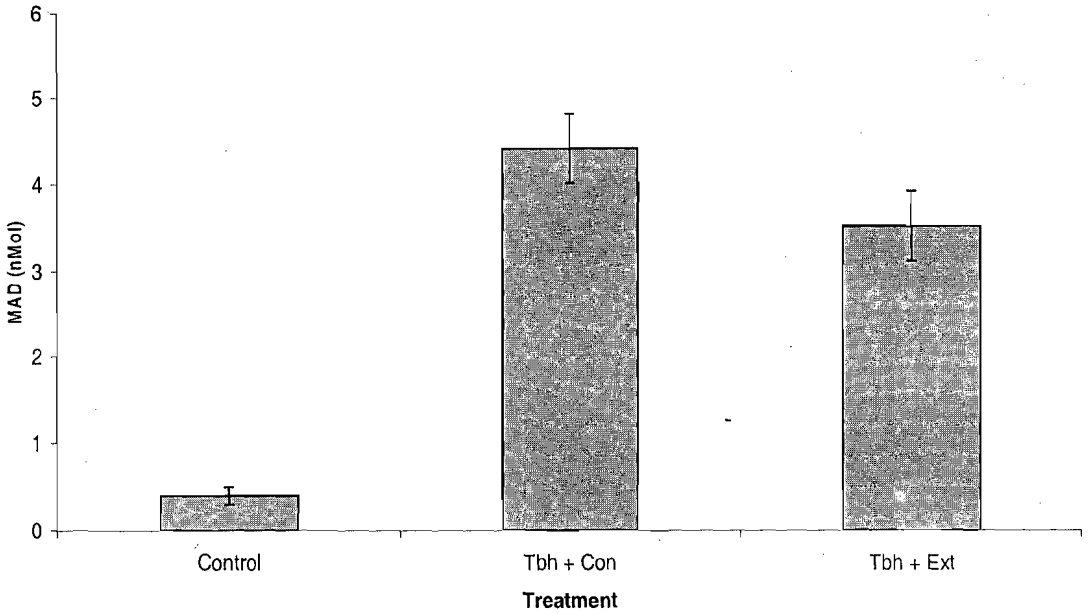

Figure 3: The effects of the methanolic extract of *N. latifolia* on lipid peroxidation

Tbh = Tert-butyl hydroperoxide, Con = Control, Ext = Extract

TBH-induced elevation of malondialdehyde (MAD) and lactate dehydrogenase(LDH) in isolated rat hepatocytes

Lipid peroxidation, expressed in terms of malondialdehyde formation, significantly increased thirty minutes after the addition of tBH. Incubation of the hepatocytes with the methanolic extract (1mg/ml) significantly ($p<0.05$) reduced the tBH-induced LDH leakage but had no remarkable effect on MAD formation (Figures 2 and 3).

DISCUSSION

This work primarily investigated the antihepeatotoxic activities of the methanolic extract of *Nauclea latifolia* root bark in paracetamol and CCl_4-induced liver injury. The essence of this is to validate or invalidate its use in Nigerian traditional medicine for the treatment of liver ailments. The results of the acute toxicity test showed that the extract was toxic to the CNS at a high dosage levels. This did not manifest at doses below 400 mg/kg. The ability of the methanolic extract to reduce the prolongation of the pentobarbital sleeping time in rats in both models of liver-induced injury is suggestive of the antihepatotoxic potential of the extract. This observation was not as a result of the effect of the extract on CNS since at the dose of 100 mg/kg, it did not influence the duration of barbiturate-induced sleep in normal animals.

The overall mechanism of action of paracetamol induced liver injury is known. The drug is transformed to its toxic metabolite N-acetyl-p-benzoquinonime, through the action of cytochrome P-450 (Mitchel *et al.*, 1973, Jollow *et al.*, 1973). This metabolite reacts with reduced glutathione (GSH) to yield non-toxic 3 GS yl-paracetamol (Prescott and Critchely, 1989). Depletion of GSH causes the remaining quinone to bind to cellular macromolecules leading to cell death (Potter *et al.*, 1974). It has been established that the hepatotoxicity of CCl_4 is dependent on its metabolism to CCl_3 radical by NADPH-cytochrome P-450 enzyme system of the liver cell endoplasmic reticulum and that the subsequent binding of CCl_3 radical to cellular macromolecules and the peroxidation of the phospholipids of the endoplasmic reticulum are the main sequences of the liver injury. The hepatotoxicity of these agents was evidenced by high increases in serum activities of

The antihepatotoxic actions of the extract was manifested by the significant

reduction of the increased activity of serum transaminases in the liver of rats intoxicated with paracetamol and CCl_4 following pretreatment with the methanolic extract. A phytochemical investigation of the root bark of this plant showed that it contains flavonoids and tannins. Some flavonoids have been reported to inhibit drug metabolism suggesting the possibility of the active component in the methanolic extract of *N. latifolia* exhibiting its antihepatotoxic effect by inhibiting the biotransformation of paracetamol and CCl_4 to their respective toxic radicals. However, the extract appeared to elevate the level of alkaline phospahtase in the serum, suggesting that it may be inducing the blockage of the bile duct rather damaging the liver cells. The leakage of LDH induced by tBH in isolated hepatocytes was significantly reduced by the extract indicating that the liver protective component in the extract may be exercising its action by preventing liver cell necrosis.

However, the extract showed no significant influence on the increased formation of MAD-induced by tert-butyl hydroperoxide in the isolated rat hepatocytes which suggests that the active component in the extract does not act by preventing the CCl_4-induced lipid peroxidation.

In conclusion, the methanolic extract of *N. latifolia* showed some protective effects on the experimental model of paracetamol and CCl_4-induced hepatic injury. The prophylactic effect is fairly comparable to that of silibinin, suggesting that the extract could be a potential source of antihepatotoxic agents. The results of the present study showed a pharmacological basis for the folklore medicinal application of the root bark of *N latifolia in* Nigeria.

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