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Effect of Aqueous *Nauclea pobeguinii* Leaf Extract on Rats Induced with Hepatic Injury

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Abstract: Forty male albino rats (Wistar strain) weighing between 150 and 170 g were used to study the antioxidant property of *Nauclea pobeguinii* extract. The rats were divided into four groups, each group consisting of 10 animals. The antioxidant activity of the extract was evaluated using CCl₄-induced lipid peroxidation model. Group one was kept on normal diet and served as control, the second group received the extract alone three times daily for 10 days by oral route, the third received only CCl₄ in olive oil by subcutaneous injection, while the fourth group received the extract at the same dose and duration as group two before exposure to CCl₄. Eighteen hours after CCl₄ administration, the animals were sacrificed, blood was collected and serum separated for analysis. Biochemical analysis of serum indicate increased activities of L-alanine aminotransferase (L-ALT), L-aspartate aminotransferase (L-AST) and alkaline phosphatase (ALP) in CCl₄ administered rats which is an indication of liver damage occasioned by lipid peroxidation. Prior treatment of CCl₄ exposed rats with the plant extract lowered the serum activities of these enzymes to levels that were comparable to control. The study indicates that aqueous extract of *N. pobeguinii* possess antioxidant property since it improves recovery or reduces the toxic effects of CCl₄ in liver cells of male rats.

Key words: *Nauclea pobeguinii*, protection, antioxidant property, carbon tetrachloride, liver, rats

INTRODUCTION

The liver is vulnerable to a wide variety of metabolic, toxic, microbial, circulatory and neoplastic insults. The level of impairment and the state of injury to the hepatocyte can be ascertained by various markers. The most commonly used markers of hepatocyte injury are L-aspartate aminotransferase and L-alanine aminotransferase. Although, if hepatic disease is primarily of an obstructive nature (cholestatic), alkaline phosphatase will be significant enzyme marker. It has been shown that hepatic injury or disease results in the leakage of these enzyme markers into the circulation thereby altering their concentration (Uliéná *et al.*, 2003). However, the pathogenesis of induced hepatic injury is not quite clear, but there is no doubt that Reactive Oxygen Species (ROS) and free radicals play important roles in biochemical changes taking place in the liver. Biochemical membranes are particularly prone to the effect of ROS. The peroxidation of unsaturated fatty acids in biological membranes lead to a decrease of membrane fluidity and a disruption of membrane integrity and function which may cause serious biochemical changes.

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Carbon tetrachloride (CCl_4) is a hepatotoxin. Trichloromethyl radicals are generated from it *in vivo*. The radicals stimulate a sequence of reactions that culminate in the initiation of the peroxidation of membrane lipids (Reinke *et al.*, 1988) and hence liver damage. Trichloromethyl radical is believed to be the immediate product of the reductive dechlorination of CCl_4 , catalysed by certain cytochrome P_{450} isoenzymes (Sipes *et al.*, 1977) particularly the ethanol inducible isoform of the cytochrome (Reinke *et al.*, 1988). Several endogenous protective mechanisms have been evolved to limit ROS and the damage caused by them (Uliéná *et al.*, 2003), but this may not provide complete protection from oxidative stress. Hence, there is increased need of natural and artificial agents possessing antioxidant properties.

Nauclea pobeguini, a family of Rubiaceae, consist of indole alkaloids having such significant biological activities as antiproliferative. This plant can also be thought to possess other components, yet to be identified, which has antioxidant activity. The aqueous extract of the plant is used by the local community in Abraka, Nigeria as a remedy for jaundice. It is usually prepared by boiling the leaves in water. CCl_4 -induced lipid peroxidation process provides a model with which *N. pobeguini* can be assessed for antioxidant property. The ability of extracts of *N. pobeguini* to exhibit antioxidant action was therefore investigated by administering it to rats prior to CCl_4 treatment. The effect was compared with that in extract-free, CCl_4 -treated rats.

MATERIALS AND METHODS

Materials

Forty male albino rats, Wistar strain (150-170 g) bred in the animal house of the College of Medicine, University of Lagos, Nigeria were used for the study after permission from appropriate authorities. They were divided into four experimental groups with ten animals per group. The animals were left to acclimatize to laboratory conditions for two weeks, before the commencement of the study.

L-alanine/L-aspartate aminotransferase kits were products of Quimica Clinica Applicada (QCA), Spain. Para-nitrophenol phosphate and para-nitrophenol were purchased from BDH chemicals (Poole, England). Tartaric and citric acids were obtained from Merck chemicals (Darmstadt). Grower's mash was obtained from Livestock Feeds Plc., Nigeria. The fresh leaves of *N. pobeguini* were obtained from a farm in Abraka, Nigeria and authenticated by the department of Botany, Delta State University, Abraka, Nigeria.

Preparation of Aqueous Extract of *Nauclea pobeguini*

The aqueous extract was prepared by boiling 100 g of the leaves in 1000 mL of water for 15 min with subsequent standing to allow for cooling down to room temperature. After separation of undissolved residue, the solution was used for the study.

Treatment of Animals

Rats in groups 2 and 4 were given 5 mL of extract kg^{-1} body weight orally by incubations. At the same time animals in groups 1 and 3 received an equal volume of de-ionized water kg^{-1} body weight by the same route. This treatment was carried out three times daily for ten days during which the rats were allowed free access to food and water. Following the last treatment, rats in group 3 and 4 received a mixture of CCl_4 in olive oil (1:1) subcutaneously at a dose of 6 mL kg^{-1} . The CCl_4 -free control rats (group 1 and 2) were given 6 mL corn oil kg^{-1} body weight subcutaneously. Treatment of the animals was in accordance with the principles of laboratory animal care (NIH, 1985).

Preparation of Serum

Eighteen hours after CCl₄ treatment, each rat was anaesthetized in a chloroform saturated chamber. The thoracic and abdominal regions were opened to expose the heart. Blood was obtained through heart puncture by means of a 5 mL hypodermic syringe and needle and placed in ice-cold 10 mL centrifuge tube. It was allowed to clot and then centrifuged at 3000 g for 5 min. The serum sample was collected and left standing on ice until required.

Biochemical Assays

Serum aspartate and alanine aminotransferases activities were assessed using Quimmica Applicada kits based on the methods of Reitman and Frankel (1957). The activities of L-ALT and L-AST are expressed as units mL⁻¹. Alkaline phosphatase activity was determined by the method of Annino and Giese (1976). The enzyme activity is expressed in units L⁻¹ in which one unit represent one micromole p-nitrophenol produced per minute.

Statistics

The results are expressed as mean±SEM and the mean values of the groups were compared using ANOVA and least square difference. The significance level was set at p<0.05.

RESULTS

It was observed that the size of the liver was increased in CCl₄-intoxicated rats, but it was not significantly different from the control in rats both treated with extract alone and those pretreated with extract before CCl₄ administration. The body weight gain of rats did not differ significantly between the groups. Thus the study indicates that aqueous extracts of *N. pobeguinii* restored to normal the CCl₄-induced increase in liver/body weight ratio of rats (Table 1).

Table 2 present the effect of *N. pobeguinii* extract on liver function indices of CCl₄ treated rats. The activities of serum aminotransferases (L-ALT and L-AST) of rats administered CCl₄ (group 3) and extract alone (group 2) were significantly increased relative to control. Prior administration of the extract to CCl₄ treated rats (group 4) decreased the activities of the serum aminotransferases to levels that were similar to the control. Similarly the serum alkaline phosphatase activity was significantly increased in CCl₄ treated extract-free rats as compared to control and extract treated rats. However

Table 1: Effect of *N. pobeguinii* extract on liver/body weight ratio and body weight gain of CCl₄ treated rats

Parameters	Treatments			
	Group 1 -CCl ₄ -Ext.	Group 2 -CCl ₄ +Ext.	Group 3 +CCl ₄ -Ext.	Group 4 +CCl ₄ +Ext.
Liver/body weight ratio (10 ⁻²)	3.40±0.58 ^a	3.30±0.30 ^a	6.70±0.40 ^b	3.48±0.50 ^a
Body weight gain (g rat ⁻¹ day ⁻¹)	4.80±0.13 ^a	4.40±0.23 ^a	4.30±0.60 ^a	4.50±0.50 ^a

Values are mean±SEM, Values on the same row with different superscripts differ significantly (p<0.05) from each other

Table 2: Effect of *N. pobeguunii* extract on serum L-aspartate aminotransferase (L-AST), L-alanine aminotransferases (L-ALT) and alkaline phosphatase (ALP) activities of CCl₄ treated rats

Parameters	Treatments			
	Group 1 -CCl ₄ -Ext.	Group 2 -CCl ₄ +Ext.	Group 3 +CCl ₄ -Ext.	Group 4 +CCl ₄ +Ext.
AST (units mL ⁻¹)	126.60±3.78 ^a	127.60±2.30 ^a	165.70±2.10 ^b	129.40±3.50 ^a
ALT (units mL ⁻¹)	78.80±1.33 ^a	75.40±1.03 ^a	115.80±2.60 ^b	81.50±3.80 ^a
ALP (units L ⁻¹)	58.40±2.46 ^a	52.60±3.10 ^a	83.00±2.63 ^b	60.70±5.20 ^a

Values are mean±SEM, Values on the same row with different superscripts differ significantly (p<0.05) from each other

pretreatment of CCl₄ administered rats with the extract significantly decreased the activity of alkaline phosphatase relative to CCl₄ treated extract-free rats. Therefore the study indicates that the extract protects rats from CCl₄-induced liver damage.

Direct change in organ weight or organ/body weight ratio has been used as an index of CCl₄ toxicity (Uemitsu *et al.*, 1986; Uemitsu and Nakayoshi, 1984). The latter method was used in this study since it has been shown to be a more sensitive indicator of CCl₄ toxicity than absolute liver weight (Uemitsu *et al.*, 1986). Therefore the observed increase in liver/body weight ratio of rats administered CCl₄ (Table 1) is consistent with these reports and that of others (Vilstrup, 1983; Okazaki *et al.*, 1985). Increase in organ weight after exposure to a toxicant may be due to a tumor, fluid or triglyceride accumulation. Triglyceride accumulation in liver (fatty liver) is a common response to CCl₄ toxicity (Timbrell, 1991; Junnila *et al.*, 2000) and this may account for the observed increase in the liver/body weight ratio of the CCl₄-treated rats. In this study, the hepatoprotective activity of *N. pobeguini* extract in CCl₄-induced toxicity was evaluated using changes in liver/bodyweight ratio and body weight gain of rats since they are well known indices of CCl₄ toxicity. The reversal of the CCl₄-induced increase in liver/body weight ratio of rats by the extract is an indication of its protective effect.

Serum aminotransferases (ALT and AST) and serum alkaline phosphatase activities were also used to measure both CCl₄-induced hepatotoxicity and protection of *N. pobeguini* extract against the same effect of CCl₄ in rats. In agreement with previous reports (Reinke *et al.*, 1988), our results show that CCl₄ caused an elevation in the serum levels of ALT, AST and ALP which is indicative of damage to the liver. Treatment of rats with aqueous extract of *N. pobeguini* caused less hepatotoxicity than with CCl₄ alone (Table 2) as evidenced by the decreased serum content of the above enzymes relative to the CCl₄-treated extract-free group. Various mechanisms have been proposed for CCl₄-induced liver damage (Brattin *et al.*, 1985). One view is that a trichloromethyl radical (CCl₃) is produced from CCl₄ by reductive dechlorination. The trichloromethyl radical in turn abstracts a hydrogen atom from a fatty acid to form chloroform and a lipid radical. The lipid radical may then react with molecular oxygen to initiate lipid peroxidation which is thought to ultimately cause the cytotoxic response (Sipes *et al.*, 1977; Recknagel, 1983; Brattin *et al.*, 1985; Reinke *et al.*, 1988). In the present study, we did not attempt to address any mechanistic concept but merely adopted CCl₄-induced liver injury as a model for assessing *N. pobeguini* for antioxidant activity. Since the mechanism of action of CCl₄ involves oxidation, we are of the view that if *N. pobeguini* possesses antioxidant action it would prevent lipid peroxidation and therefore membrane damage. Present results clearly demonstrate that *N. pobeguini* is an excellent preventive agent for CCl₄-induced liver damage in rats and this finding appears to suggest that the aqueous extract of *N. pobeguini* possesses antioxidant activity.

Many natural and artificial agents possessing anti-oxidative properties have been proposed to prevent and treat hepatopathies induced by oxidative stress (Lieber, 1997; Èervincová and Drahota, 1998). There is increasing evidence for the hepatoprotective role of hydroxyl- and polyhydroxy-organic compounds particularly from vegetables, fruits and some herbs (Bass, 1999). Although the biochemical mechanism of the antioxidant effect of *N. pobeguini* extract against CCl₄-induced hepatotoxicity was not examined in the present study, it is possible that the bioactive principles in the extract may have acted directly or indirectly in protecting the liver against damage. Directly it may be breaking the sequence of events between the reductive dechlorination of CCl₄ and the subsequent abstraction of hydrogen from unsaturated fatty acids in the membrane and peroxide formation. Indirectly, it may inhibit the activities of cytochrome P₄₅₀ isoenzymes (Sipes *et al.*, 1977; Reinke *et al.*, 1988) required for trichloromethyl radical formation or it may be an effective scavenger of the reactive metabolite, the trichloromethyl radical. Besides the increase in serum L-ALT and L-AST of rats exposed to the extract alone (Table 2) may arise following increased synthesis of these enzymes

in the liver. Activation of liver aminotransferases may promote regeneration of damaged hepatocytes and this could contribute to the restoration of the CCl₄-induced liver damage by prior treatment of rats with the extract.

CONCLUSION

In conclusion the present study indicates that aqueous extract of *N. pobeguinii* has anti-oxidant activity since it improves recovery or reduces the toxic effects of CCl₄ in liver cells of male rats.

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