Effect of Extracts of *Hymenocardia acida* Tul (Hymenocardiaceae) on Rats

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Abstract: The aqueous and methanol leaf extracts of *Hymenocardia acida* Tul. (Hymenocardiaceae) were evaluated for their effect on superoxide dismutase (SOD) catalase (CAT); and Ca^{2+} concentrations in rats. The extracts were orally administered at a dose of 200mg^{-kg} daily for 7 days. Results showed elevated activity of SOD ($p<0.05$) while CAT activity was depressed ($p<0.05$) in the treated rats. Effects on enzymes' activities suggest that the extracts induced SOD activity while inhibiting CAT activity, indicating an altered oxidative status in the animals. Ca^{2+} concentrations were higher ($p<0.05$) in the treated rats which suggests an effect on Ca^{2+} metabolism. Correlation between activities of SOD/CAT and Ca^{2+} concentration were r = 0.055 and r = -0.787 for SOD; r = 0.533 and r = -0.908 for catalase. Study reveals that pharmacological action credited to *H. acida* is due to effect on enzymatic antioxidants and Ca^{2+} metabolism. [Journal of American Science 2010;6(2);143-146] (ISSN: 1545-1003).

Keywords: *H. acida*, superoxide dismutase, catalase, calcium.

1. Introduction

Oxidative stress and its related biological damage have been proposed to be involved in the development and maintenance of rheumatoid arthritis in animal and human models, cardiovascular diseases, diabetes and viral infections (Darlington and Stone, 2001 ; Repetto and Llesuy, 2002). Consequently, the use of medicinal plants exhibiting antioxidative activity in the treatment and management of diseases has been on the increase in recent times (Pourmorad *et al*., 2006). In living organisms the first line of defence against free radicals is the oxidative stress enzyme superoxide dismutase (SOD). Superoxide dismutase (EC 1.15.1.1) catalyses the dismutation of superoxide anions converting them to hydrogen peroxides. The toxic hydrogen peroxide in turn, is converted to molecular oxygen and water by catalase (EC 1.11.1.6) or glutathione peroxidase (EC 1.11.1.9) (Aksoy *et al*., 2004).

Furthermore, many medicinal plants also contain different proportions of micro nutrients (Okwu and Josiah 2006). One of such micro nutrient is Ca^{2+} which is the most common mineral in the body. The physiological functions of Ca^{2+} which include its roles as major structural element (particularly in bone formation), in cell signalling, and as cofactor for enzymes are vital to an organism's survival (Gross, 2005). Ca^{2+} in many organisms including humans may become deficient with advancing age and this negative calcium balance appear to be associated with several conditions including osteoporosis (Fujita *et al*., 2000). Thus, studies are focusing on the role of micro nutrients in the pathogenesis and management of different diseases (Okochi and Okpuzor, 2005).

Hymenocardia acida Tul (Hymenocardiaceae), widespread in tropical Africa is a small tree of about 6 m high. Ethnomedical investigation of *H. acida* reveals a variety of medicinal uses in tropical African countries. In Senegal and Ivory Coast, an infusion or decoction of its leaves is used for the treatment of chest complaints, small pox, in baths and draughts as a febrifuge, and is taken as snuff for headaches or applied topically for rheumatic pains and toothaches (Sofidiya *et al*, 2009). The bark and leaves are used together in various ways in Nigeria for abdominal and menstrual pains and as poultices on abscesses and tumours. The powdered leaves of the plant are used for the treatment arthritis (Burkhill, 1994). Our previous study on *H. acida* showed that it possesses free radical scavenging activity and strong reducing power *in vitro* (Sofidiya *et al*., 2006). Antioxidative screening of this plant revealed the presence of flavonols, flavonoids, phenols, proanthocyanidins, steroids and triterpenoids (Sofidiya *et al*., 2009). However, despite the extensive use of *H. acida* in the treatment of many diseases in traditional medicine, the *in vivo* basis for its pharmacological activity has not been evaluated.

This is the objective of this work which is to investigate the antioxidative property of the leaves *in vivo*, in order to ascertain the mode of the pharmacological action of the plant. Furthermore, in view of the role of Ca^{2+} in biological processes, the study aims at determining the relationship between the antioxidative property of the plant and Ca^{2+}

concentration in treated animal.

2. Materials and methods

Plant material

The leaves of *H. acida* were collected in January 2004 at Olokemeji reserves in Ogun State, Nigeria and were authenticated at the Forestry Research Institute of Nigeria (FRIN), Ibadan. Voucher specimen (FHI 38672) was prepared and deposited at the herbaria of both the Institute and at the Pharmacognosy Department, University of Lagos

Extraction

The leaves were air dried at room temperature for 21 days, pulverized and a portion (850 g) of the powdered material was extracted with 7.5 L of water on a shaker at room temperature overnight. The extract was filtered through Whatman No.1 filter paper and the filtrate concentrated in a lyophilizer. The residue after aqueous extraction was further extracted with methanol (3 L) and filtered. The filtrate was evaporated to dryness under reduced pressure. The extracts were stored at 4^oC until required.

Preparation of extracts solutions

The extracts solutions were prepared by dissolving 0.4 g of the extract in 10 ml of 5% Tween 20, to give an effective concentration of 40 mg mL^{-1}. The formula: Dosage mg^{-1} kg)/1000 x Wt of animal (g)/concentration (mg mL^{-1}) was used to calculate the volume of the extract solution to be administered to each animal. The extracts solutions were prepared fresh daily before administration.

Animal source

Eighteen Wistar rats used for the experiment were purchased from the rat colony at Department of Veterinary Parasitology and Entomology University of Nigeria, Nsukka. The Ethical committee of the University of Lagos approved the use of these animals for experimental purposes. They were housed in standard rat cages and were fed with commercial livestock feed and water *ad libitum*.

Animal treatment

The rats weighing between 200-250 g were sorted into 3 groups of 6 rats each (2 tests and one control). The extracts solutions [1 mL (200 mg^{-1} kg)] were administered orally to the test groups for 7 days. The control group received 1ml of 5% Tween 20 only. The animals were fasted overnight, sacrificed and blood was collected. The blood was centrifuged at 1500 rpm for 5 min; and serum was separated for enzymes assay and Ca^{2+} levels determination.

Enzyme assays

Chemicals

Hydrogen peroxide (H_2O_2), Epinephrine ($C_9H_{13}O_3N$) and Tween 20 were purchased from Sigma-Aldrich, Germany. Kit for calcium determination was from BDH (Poole, UK). All other chemicals used, including solvents, were of analytical grade.

Determination of SOD activity

The method of Sun and Zigma (1978) was adopted. The reaction mixture (3 ml) contained 2.95 ml 0.05 M sodium carbonate buffer pH 10.2, 0.02 ml of serum and 0.03 ml of epinephrine in 0.005 N HCL was used to initiate the reaction. The reference cuvette contained 2.95 ml buffer, 0.03 ml of substrate (epinephrine) and 0.02 ml of water. Enzyme activity was calculated by measuring the change in absorbance at 480 nm for 5 min.

Determination of Catalase activity

Serum catalase activity was determined according to the method of Beers and Sizer as described by Usoh *et al.,* (2005) by measuring the decrease in absorbance at 240nm due to the decomposition of H_2O_2 in a UV recording spectrophotometer. The reaction mixture (3 ml) contained 0.1 ml of serum in phosphate buffer (50 mM, pH 7.0) and 2.9 ml of 30 mM H_2O_2 in phosphate buffer pH 7.0. An extinction coefficient for H_2O_2 at 240 nm of 40.0 $M^{-1}cm^{-1}$ (Aebi 1984) was used for the calculation. The specific activity of catalase was expressed as moles of H_2O_2 reduced per minute per mg protein.

Determination of Ca^{2+} concentration

Calcium concentration in $mmolL^{-1}$ was estimated by the method of Biggs and Moorehead as described by Yakubu *et al.* (2007)

Statistical Analysis

Data were reported ±SEM of 6 measurements and were subjected to one-analysis of variance and significance of the difference between groups was by student's *t*-test ($p<0.05$). Test of correlation between groups was by Pearson correlation test.

3. Results

SOD activity

Results, summarised in Table 1, show a significant increase ($p<0.05$) in serum activity of SOD in the rats treated with aqueous and methanol extracts of *H. acida* as compared to the untreated rats. This increase was more significant ($p<0.05$) in the methanolic extract than the aqueous extract.

Catalase activity

Catalase activity was significantly lower ($p<0.05$) in rats treated with both extracts (Table 1). However, this depressed activity was more significant in the methanol extract than the aqueous extract.

A linear positive correlation ($r = 0.9751$) existed between SOD and catalase activities in the control rats. There was positive correlation ($r = 0.533$) between SOD and catalase activities in the methanol extract-treated rats. However, in the aqueous extract, SOD and catalase activities had negative correlation ($r = -0.9083$).

Table 1. SOD/Catalase Activities and Calcium concentrations in *H. Acida*-Treated Rats

Group	SOD (units/g Protein $x10^{-3}$)	Catalase (moles of H_2O_2 degraded/min $x10^{-2}$)	Calcium (mmol/L)
Control	2.35 ±0.16	28.0±0.03	1.91±0.23
MeOH	3.79±0.21*	0.2±0.0001*	2.75±0.31
H_2O	4.03±0.42*	4.0±0.03*	3.33±0.06*

*Significant at $p<0.05$

Ca^{2+} concentration

Serum calcium concentration was higher ($p<0.05$) in the *H. acida* aqueous extract-treated rats, while there was no significant difference between Ca^{2+}concentration in methanol extract-treated and the untreated rats (Table 1).

There was a relationship between Ca^{2+} and the enzymes (Table 2). The untreated rats showed positive correlation between Ca^{2+} concentration, SOD and catalase activities. In methanol extract, there was minimal correlation ($r = 0.055$) between Ca^{2+} and SOD activity, while there was a positive correlation ($r = 0.533$) between Ca^{2+} and catalase activity. However, in the aqueous extract, there was a strong negative correlation between Ca^{2+} levels and the enzymes' activities.

Table 2. Correlation between SOD/Catalase Activities and Calcium Concentration in Untreated and *H. acida*-treated Rats

		Extracts	
Parameters	Untreated (r value)	*H. acida* methanol (r value)	*H.acida* water (r value)
SOD/Ca^{2+}	0.673	0.055	-0.787
Catalase/Ca^{2+}	0.957	0.533	-0.9083

4. Discussion

Plants are generally believed to be rich in a wide variety of secondary metabolites such as alkaloids, flavonoids, terpenoids and saponins. Of these metabolites, plant antioxidants such as the numerous phenolic compounds have received increased attention as useful nutraceutical in management of diseases (Wan and Diaz-Sanchez, 2007). For this reason, research has focused on evaluating the antioxidant properties of plants used in ethnomedicine, in order to relate these properties to their mode of action.

The different effects demonstrated by *H. acida* leaf extract on SOD, catalase and Ca^{2+} concentration appear to be directly related to the types and proportions of compounds extractable by the two solvents – H_2O and methanol. Our studies showed an elevated SOD activity which was might be as a result of the induction of the synthesis of the enzyme. Earlier, the *in vitro* studies of *H. acida* aqueous and methanol extract reported a significant proportion of phenolic compounds, which accounted for its antioxidative activity (Sofidiya *et al*., 2006). Thus, we believe that the induction of SOD is probably due the presence of the phenolic compounds in the extract. This agrees with Yeh and Yen (2006), who reported that four different phenolic acids induced antioxidant enzymes SOD, catalase and gluthathione peroxidase.

Moreover, the depressed activity of catalase in our study suggests an inhibitory effect of H_2O_2 which accumulated due to elevated activity of SOD. H_2O_2 even though a natural substrate of catalase, acts as an inhibitor of the enzyme at elevated levels (Aksoy *et al*., 2004).

The relationship between SOD and catalase is buttressed in aqueous extract-treated rats which demonstrated that about 90% of the decline in the activity of catalase was due to the activity of SOD. This is supported by studies that in the presence of H_2O_2, SOD acts as a pro-oxidant (Bast *et al.*, 1991). This created oxidative stress in the animals which agrees with Maduka and Okoye (2002), who reported an elevated activity of SOD and inhibition of catalase activity in the red cells and the liver by stem bark of *Sacoglotis gabonensis* Baill Urb. (Humiriaceae), a Nigerian beverage additive. In view of the important role oxidative stress plays in resolution of inflammation in mammals, our results suggest the mode of the pharmacological action of *H. acida.*

A significant correlation was observed between SOD/catalase and Ca^{2+} concentration which suggest that a relationship exist between them. This was apparent in the aqueous extract-treated rats which exhibited higher Ca^{2+} concentration. This group showed that 78 and 90% of the activity of SOD and catalase respectively was due to the increase in Ca^{2+} concentration. These results are in agreement with previous findings that there is a relationship between

Ca^{2+} and antioxidative enzyme (Brown *et al*., 2003). Oxidative stress created in the rats is believed to affect calcium channels in mammals (Hool and Corry, 2007). Furthermore, H_2O_2 is reported to interact with cell signalling pathway by way of modification of key thiol groups on proteins that possess regulatory function (Hool and Corry, 2007).

This study reports that *H. acida* extract exerts its pharmacological activity in rats, by interaction with antioxidant enzymes, reactive oxygen species and extra cellular calcium. This supports the use of this plant for the treatment of rheumatoid arthritis in traditional medicine practice in Nigeria.

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14/09/2009