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RELATIONSHIP BETWEEN THE CONCENTRATION OF TOTAL PROTEINS AND SPECIFIC ACTIVITY OF CATALASE IN INDOMETHACIN-ULCERATED RATS PRE-TREATED WITH *ANACARDIUM OCCIDENTALE* METHANOL LEAF EXTRACT

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ABSTRACT

Increased needs for proteins and anti-oxidants are required to promote wound healing and hence, this research was originated to investigate the relationship between the concentration of total proteins and specific activity of catalase in indomethacin-ulcerated rats pre-treated with *A. occidentale* methanol leaf extract. The effects of the methanol leaf extract of *A. occidentale* on the concentration of total proteins and specific activity of catalase were determined and assayed respectively using standard methods. The extract at the three doses [100, 200 and 400 mg/kg body weight (b.w)] caused significant ($p < 0.05$) and dose-related increases in the concentration of total proteins and specific activity of catalase in the rats of the test groups compared to those of the rats in the ulcer-untreated group (group 2). The 400 mg/kg b.w of the extract exerted the greatest effects in a manner similar to those of the standard anti-ulcer drug, famotidine at the dose of 50 mg/kg b.w. The remarkable amelioration of the amount of total proteins and specific activity of catalase in the ulcerated rats by the methanol leaf extract of *A. occidentale* encourages the local utilisation of the leaves of the plant in the treatment of gastric ulcer.

KEYWORDS: Proteins, Anacardium occidentale, Catalase and Gastric ulcer.

INTRODUCTION

Medicinal agents have always arisen from nature for thousands of years and a great deal of modern drugs has been isolated from natural sources. Many of them are based on their use in traditional medicine. It has been noted that the original sources of many important pharmaceuticals in current use have been plants used by the indigenous people.[1],[2] The plant, *A. occidentale* (Linn) (Fig. 1) belongs to the Anacardiaceae family and is indigenous to the tropical regions such as northeast Brazil. Its fruit popularly known as cashew consists of two parts: the fruit itself (the nut) and the accessory fruit (or flower stalk) also known as the cashew.[3] The cashew apple contains tannins, vitamin C, sugars, carotenoids, organic acids, proteins, fibres and water.[4] There are several reports on the pharmacological properties of cashew tree byproducts such as anti-inflammatory and anti-diabetic effects as well as acetylcholinesterase-inhibitory action. Substances derived from the fruit of cashew have also been shown to be tyrosinase-inhibitive. Also, the ethanol extract especially from the stalk of *A. occidentale* could both be used as a food additive as well as an ingredient in pharmaceutical preparations to replace the synthetic anti-oxidants.[5]

Peptic ulcer is an excoriated area of the gastric or duodenal mucosa caused by the action of gastric juice.[6] Peptic ulcer occurs in that part of the gastrointestinal tract which is exposed to gastric acid and pepsin (i.e., the stomach and duodenum). Common cause of ulcer is the intake of nonsteroidal anti-inflammatory drugs (NSAIDs) for examples: indomethacin, diclofenac and aspirin (especially in high doses). Their anti-inflammatory and analgesic actions are based mainly on their inhibitory effects on cyclooxygenase, thus blocking prostaglandin synthesis (from arachidonic acid). The critical element is suppression of the constitutive form of cyclooxygenase-1 (COX-1) in the mucosa and by consequence, decreased production of the cytoprotective prostaglandins (prostaglandins E_2 and I_2). NSAIDs damage the mucosa locally by nonionic diffusion into the mucosal cells.[7] In recent years, a widespread search has been launched to identify new anti-ulcer drugs from natural sources. The aim of the present investigation was to evaluate the effects of the methanol extract of the leaves of *A. occidentale* on the concentration of total proteins and specific activity of catalase in ethanol-ulcerated rats.

MATERIALS AND METHODS

Plant

Fresh leaves of *A. occidentale* were plucked from their tree at University of Nigeria, Nsukka. The leaves were identified by Mr. Alfred Ozioko of Bioresource Development and Conservation Programme (BDCP) Research Centre, Nsukka where the voucher specimen was deposited in the herbarium.

Preparation of the extract

The fresh leaves of *A. occidentale* were washed with distilled water. The leaves were spread on a clean mat in a well-ventilated room with regular turning to enhance even drying and avoid decaying and allowed to shade-dry for 3 weeks. A known weight (500 g) of the pulverised leaves was macerated in 5 volumes (w/v) of methanol and left for 24 hours. The mixture was thereafter, filtered using Whatman No 1 filter paper and the filtrate concentrated in a rotary evaporator and weighed.

Animals

Adult male albino Wistar rats of between 3 and 4 months old with average weight of 120 ± 25 g were obtained from the Animal house of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka. The rats were acclimatised to a standard environmental condition for one week with a 12 hour light and dark cycle and maintained on a regular feed and water *ad libitum*. The Principles of Laboratory Animal Care were adhered to.

Chemicals

The chemicals used in this study were of analytical grade and included: methanol (BDH Chemicals Ltd., Poole, England), indomethacin [standard ulcerating agent (Sigma-Aldrich, Inc., St. Louis, USA)], famotidine [standard anti-ulcer drug (Sigma-Aldrich, Inc., St. Louis, USA)] and distilled water.

Determination of the concentration of total proteins

The concentration of total proteins was determined using the method described by Lowry *et al*.[8]

Assay of the specific activity of catalase

The specific activity of catalase was assayed according to the method described by Aebi.[9]

Statistical analysis

The data obtained were subjected to one-way Analysis of Variance (ANOVA). The results are expressed as means ± standard errors of the means (SEM). Significant differences are observed at $p < 0.05$. The analysis was done using the computer software known as Statistical Products and Service Solutions (SPSS), Version 18.

RESULTS AND DISCUSSION

Results

Effect of the methanol extract of the leaves of A. occidentale on the concentration of total proteins

Fig. 2 shows that the total protein concentration (0.52 ± 0.01 mg/ml) of the rats in the normal control group (group 1) was significantly ($p < 0.05$) higher than that of the rats (0.37 ± 0.01 mg/ml) in the ulcer-untreated group (group 2). The 100, 200 and 400 mg/kg body weight of the extract significantly ($p < 0.05$) and dose-dependently increased the total protein concentrations of the rats in groups 4 (0.43 ± 0.01 mg/ml), 5 (0.46 ± 0.01 mg/ml) and 6 (0.50 ± 0.01 mg/ml) when compared to the value obtained for the rats in group 2 (0.37 ± 0.01 mg/ml). The effect of the extract at the dose of 400 mg/kg body weight was comparable to that of the standard anti-ulcer drug [famotidine (50 mg/kg body weight)] as there was no significant ($p > 0.05$) difference between the total protein concentration of the rats in group 6 (0.50 ± 0.01 mg/ml) and that of the rats in group 3 (0.51 ± 0.01 mg/ml).

Effect of the methanol extract of the leaves of A. occidentale on the specific activity of catalase

As shown in Fig. 3, the specific activity of catalase [$(10.40 \pm 0.40) \times 10^{-3}$ ΔA_{240}/min/mg proteins] of the rats in the normal control group (group 1) was significantly ($p < 0.05$) higher than that of the rats [$(4.30 \pm 0.70) \times 10^{-3}$ ΔA_{240}/min/mg proteins] in the ulcer-untreated group (group 2). The 100, 200 and 400 mg/kg body weight of the extract significantly ($p < 0.05$) and dose-relatedly increased the specific activities of catalase of the rats in groups 4 [$(7.90 \pm 0.60) \times 10^{-3}$ ΔA_{240}/min/mg proteins], 5 [$(9.60 \pm 0.50) \times 10^{-3}$ ΔA_{240}/min/mg proteins] and 6 [$(11.20 \pm 0.50) \times 10^{-3}$ ΔA_{240}/min/mg proteins] when compared to the value obtained for the rats in group 2 [$(4.30 \pm 0.70) \times 10^{-3}$ ΔA_{240}/min/mg proteins]. The effects of the extract at the doses of 200 and 400 mg/kg body weight were comparable to that of the standard anti-ulcer drug, famotidine at the dose of 50 mg/kg body weight as there were no significant ($p > 0.05$) differences between the specific activities of catalase of the rats in groups 3 [$(11.00 \pm 0.70) \times$

10^{-3} ΔA_{240}/min/mg proteins], 5 [(9.60 ± 0.50) x 10^{-3} ΔA_{240}/min/mg proteins] and 6 [(11.20 ± 0.50) x 10^{-3} ΔA_{240}/min/mg proteins] and that of the rats in group 1 [(10.40 ± 0.40) x 10^{-3} ΔA_{240}/min/mg proteins].

Fig. 1: ***Anacardium occidentale*** **(Linn).**

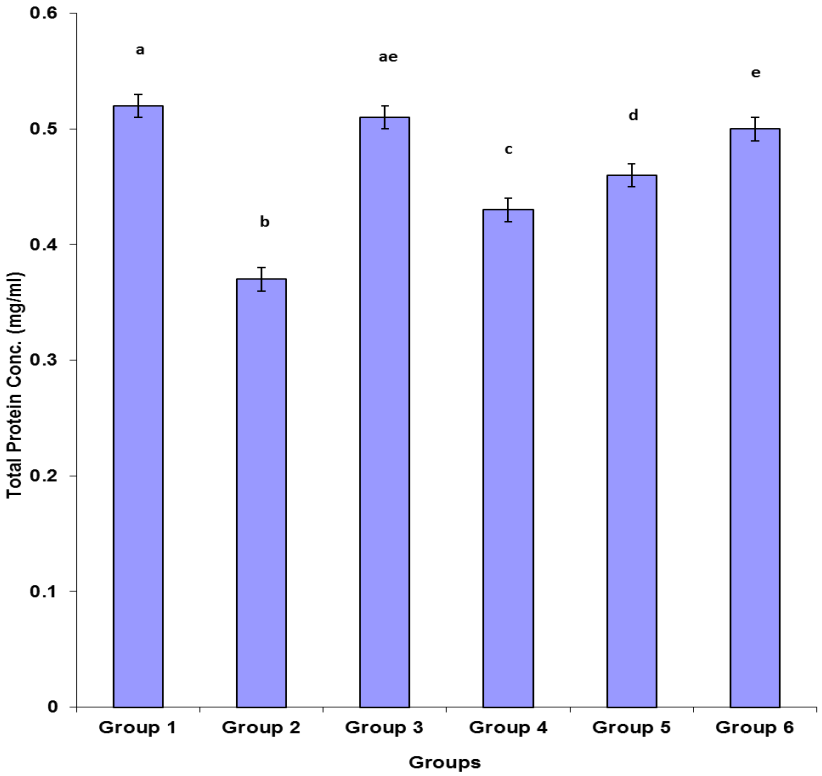

Fig. 2: Effects of the graded doses of the methanol extract of the leaves of A. occidentale on total protein concentration [Values for groups with different letters are significantly (p < 0.05) different]

Group 1: 5 ml/kg b.w of distilled water only (Normal control).

Group 2: 5 ml/kg b.w of distilled water + 100 mg/kg b.w of indomethacin (Positive control).

Group 3: 50 mg/kg b.w of famotidine + 100 mg/kg b.w of indomethacin (Standard control).

Group 4: 100 mg/kg b.w of the extract + 100 mg/kg b.w of indomethacin.

Group 5: 200 mg/kg b.w of the extract + 100 mg/kg b.w of indomethacin.

Group 6: 400 mg/kg b.w of the extract + 100 mg/kg b.w of indomethacin.

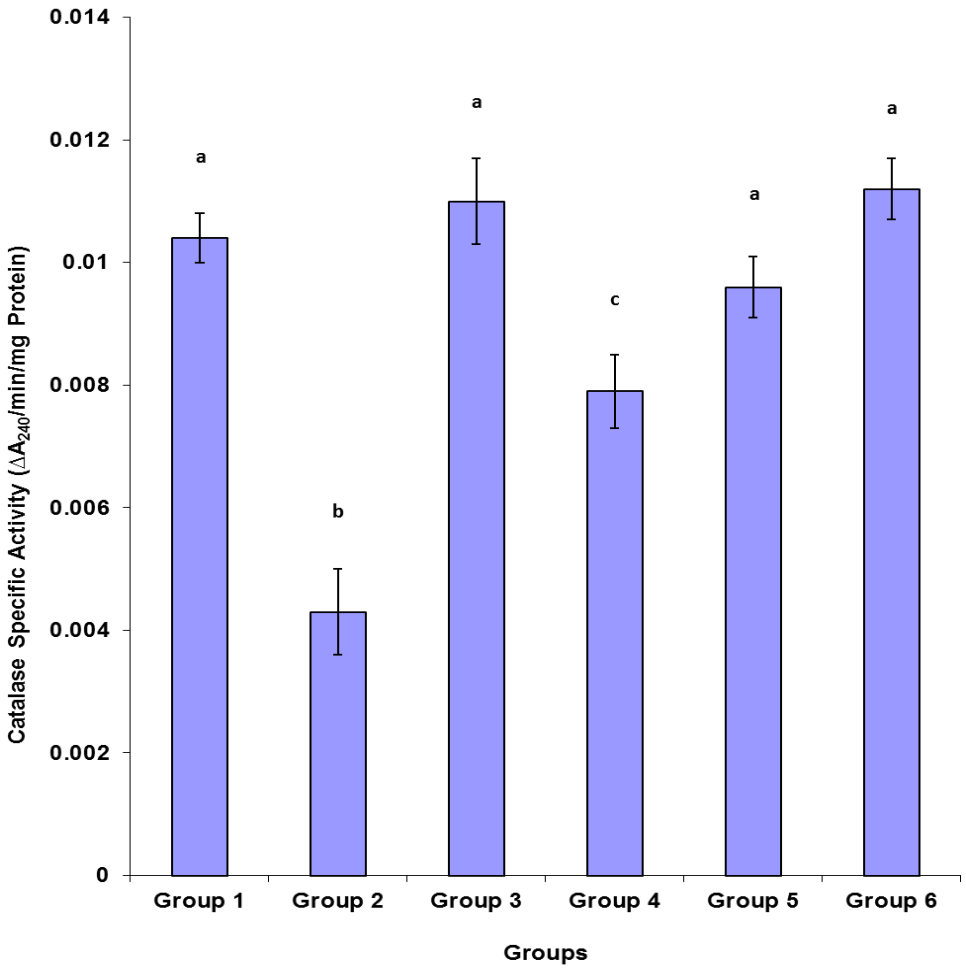

Fig. 3: Effects of the graded doses of the methanol extract of the leaves of A. occidentale on catalase specific activity [Values for groups with different letters are significantly ($p < 0.05$) different]

Group 1: 5 ml/kg b.w of distilled water only (Normal control).

Group 2: 5 ml/kg b.w of distilled water + 100 mg/kg b.w of indomethacin (Positive control).

Group 3: 50 mg/kg b.w of famotidine + 100 mg/kg b.w of indomethacin (Standard control).

Group 4: 100 mg/kg b.w of the extract + 100 mg/kg b.w of indomethacin.

Group 5: 200 mg/kg b.w of the extract + 100 mg/kg b.w of indomethacin.

Group 6: 400 mg/kg b.w of the extract + 100 mg/kg b.w of indomethacin.

DISCUSSION

In this study, the relationship between the concentration of total proteins and specific activity of catalase in indomethacin-ulcerated rats pre-treated with *A. occidentale* methanol leaf

extract were evaluated with a view to elucidating the probable mechanism(s) of action(s) of the leaves of *A. occidentale* on gastric ulcer.

The amount of total proteins in the rats of the ulcer-untreated group (group 2) was remarkably depleted. The depletion might have been a consequence of peroxidation of protein moieties in the ulcerated rats as a result of free radical generation. That the graded doses of the methanol extract of the leaves of *A. occidentale* restored the concentrations of total proteins of the rats in the treated groups to near normalcy, demonstrates in part, an ulcer-ameliorative effect of the leaves of *A. occidentale*. The dose-dependent and pronounced rises in the concentrations of total proteins of the rats in the treated groups might be due to the presence of proteins or agents that exert stimulatory effect on protein synthesis as well as anti-oxidant phytochemicals in the leaves of the plant as documented earlier[10] which might have annulled the indomethacin-caused gastric ulcer.

The decrease in the specific activity of catalase in the rats of the ulcer-untreated group might be due to the ulcerating agent (indomethacin)-initiated generation of free radicals which culminated in ulceration. Indomethacin may be associated with a disturbance in the balance between gastric mucosal protective and aggressive factors. Gastric mucosa is exposed to aggressive factors as gastric acid, pepsin and stimulants among others while gastroprotective factors maintain the integrity of the gastric mucous layer, microcirculatory system, HCO_3^-, prostaglandins, epidermal growth factor synthesis, and epithelial cell restitution. It has been proposed that neutrophil and oxygen radical-dependent microvascular injuries may be important processes that lead to mucosal damage in response to NSAID administration.[11] Treatment of rats with the graded doses of the methanol extract of the leaves of *A. occidentale* along with indomethacin appreciably raised the specific activity of catalase in the rats in a dose-dependent fashion. Catalase is among the endogenous defences which are primarily involved in maintaining the integrity and physiology of tissues. Hydrogen peroxide (H_2O_2) is catalytically converted by catalase into ground-state oxygen and hydroxyl radicals whose accumulation can play a critical role in the pathophysiology of ulcer. The ability of the methanol extract of the leaves of *A. occidentale* to augment the specific activity of catalase against the indomethacin-induced toxicity is indicative of its potential in the prevention or amelioration of gastric lesions induced by free-radical reactions.

CONCLUSION

In conclusion, there are indications as presented in this study that the methanol extract of the leaves of *A. occidentale* in part, protects against indomethacin-induced gastric ulcer in rats by elevating the amount of total proteins and specific activity of catalase.

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