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Effects of the methanol extract of the leaves of *Azadirachta indica* on the concentration of total proteins and specific activity of catalase in ethanol-ulcerated rats

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ABSTRACT

Background and Aim: Increased needs for proteins and anti-oxidants are required to promote wound healing and thus, this study was originated to evaluate the effects of the methanol extract of the leaves of *Azadirachta indica* on the concentration of total proteins and specific activity of catalase in ethanol-ulcerated rats. **Methods :** The effects of the methanol extract of the leaves of *A. indica* on the concentration of total proteins and specific activity of catalase were determined and assayed respectively using standard methods. **Results:** 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, cimetidine at the dose of 100 mg/kg b.w. **Conclusion:** The remarkable amelioration of the amount of total proteins and specific activity of catalase in the ulcerated rats by the methanol extract of the leaves of *A. indica* encourages the local utilisation of the leaves of the plant in the treatment of gastric ulcer.

KEYWORDS: Proteins, *Azadirachta indica*, Catalase and Gastric ulcer

1. INTRODUCTION

Nature has been a source of medicinal agents for thousands of years and an impressive number 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 indigenous people[1]. *Azadirachta indica* (Linn) (Fig. 1) also known as neem, belongs to Meliaceae family and is one of the two species of the genus, *Azadirachta*. Although, it is native to Bangladesh, it is also available in other tropical and sub-tropical regions such as India, Burma, Sri Lanka, Malaysia and Pakistan[2]. Neem is an evergreen and fast-growing tree reaching an average height of 15-20 metres and 35-40 metres in rare cases. Numbers of biologically active compounds have been isolated from the leaf, bark and seeds of *A. indica*. A bitter principle, nimbidin found in the bark of the plant was found to be anti-pyretic, non-irritant and effective in treating skin diseases such as eczema, arsenical dermatitis, burn ulcers, furunculosis, herpes labialis, scabies and seborrhaeic dermatitis[3]. It is also useful against warts and dandruff. Nimbidin and sodium nimbidinate from neem bark were reported to show spermicidal actions. Its bark extracts had shown very promising diuretic, anti-microbial, cytotoxic, analgesic and anti-inflammatory properties[4].

Fig. 1: *Azadirachta indica* (Linn)

Peptic ulcer is an excoriated area of the gastric or duodenal mucosa caused by the action of gastric juice[5]. 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

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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[6]. 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. indica* on the concentration of total proteins and specific activity of catalase in ethanol-ulcerated rats.

2. MATERIALS AND METHODS

2.1 Plant

Fresh leaves of *A. indica* were plucked from their tree at the Botanical Garden of the 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.

2.2 Preparation of the Extract

The fresh leaves of *A. indica* 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.

2.3 Animals

Adult male albino Wistar rats of between 3 and 4 months old with average weight of 120 ± 20 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.

2.4 Chemicals

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

2.5 Determination of the Concentration of Total Proteins

The concentration of total proteins was determined using the method described by Lowry *et al*[7].

2.6 Assay of the Specific Activity of Catalase

The specific activity of catalase was assayed according to the method described by Aebi[8].

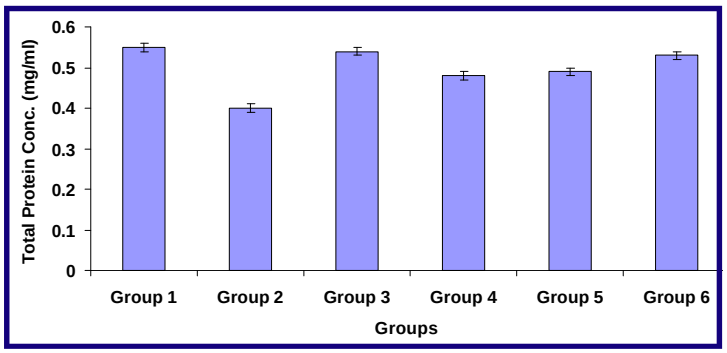
2.7 Statistical Analysis

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.

3. RESULTS

3.1 Effect of the Methanol Extract of the Leaves of *A. indica* on the Concentration of Total Proteins

Fig. 2 shows that the total protein concentration (0.55 ± 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 (0.40 ± 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 (0.48 ± 0.01 mg/ml), 5 (0.49 ± 0.01 mg/ml) and 6 (0.53 ± 0.01 mg/ml) when compared to the value obtained for the rats in group 2 (0.53 ± 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 [cimetidine (100 mg/kg body weight)] as there was no significant ($p > 0.05$) difference between the total protein concentration of the rats in group 6 (0.53 ± 0.01 mg/ml) and that of the rats in group 3 (0.54 ± 0.01 mg/ml).



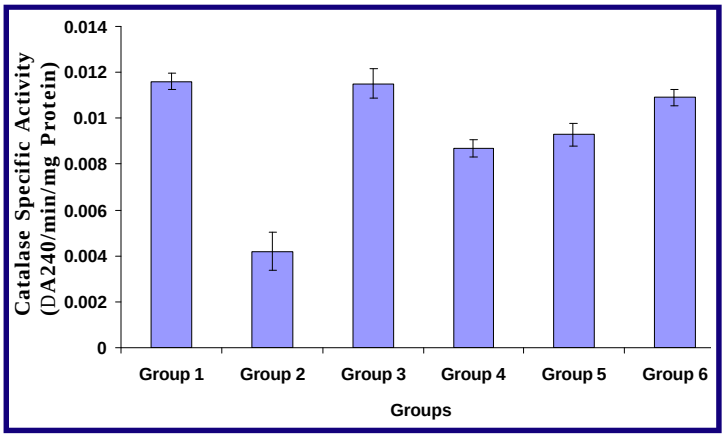
Group 1: 5 ml/kg body weight (b.w) of distilled water only (Normal control)
Group 2: 5 ml/kg b.w of distilled water + 5 ml/kg b.w of absolute ethanol (Positive control)
Group 3: 100 mg/kg b.w of cimetidine + 5 ml/kg b.w of absolute ethanol
Group 4: 100 mg/kg b.w of the extract + 5 ml/kg b.w of absolute ethanol
Group 5: 200 mg/kg b.w of the extract + 5 ml/kg b.w of absolute ethanol
Group 6: 400 mg/kg b.w of the extract + 5 ml/kg b.w of absolute ethanol

Fig. 2: Effects of the graded doses of the methanol extract of the leaves of *A. indica* on total protein concentration

3.2 Effect of the Methanol Extract of the Leaves of *A. indica* on the Specific Activity of Catalase

As shown in Fig. 3, the specific activity of catalase [(11.60 ± 0.40) x 10^{-3} Δ A_{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

[(4.20 ± 0.80) x 10^{-3} Δ A_{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 activity of catalase of the rats in groups 4 [(8.70 ± 0.40) x 10^{-3} ΔA_{240}/min/mg proteins], 5 [(9.30 ± 0.50) x $10^{-3}\Delta A_{240}$/min/mg proteins] and 6 [(10.90 ± 0.40) x 10^{-3} ΔA_{240}/min/mg proteins] when compared to the value obtained for the rats in group 2 [(4.20 ± 0.80) x 10^{-3} ΔA_{240}/min/mg proteins]. The effect of the extract at the dose of 400 mg/kg body weight was comparable to that of the standard anti-ulcer drug, cimetidine at the dose of 100 mg/kg body weight as there was no significant ($p > 0.05$) difference between the specific activity of catalase of the rats in group 6 [(10.90 ± 0.40) x 10^{-3} ΔA_{240}/min/mg proteins] and that of the rats in group 3 [(11.50 ± 0.60) x 10^{-3} ΔA_{240}/min/mg proteins].



Group 1: 5 ml/kg body weight (b.w) of distilled water only (Normal control)
Group 2: 5 ml/kg b.w of distilled water + 5 ml/kg b.w of absolute ethanol (Positive control)
Group 3: 100 mg/kg b.w of cimetidine + 5 ml/kg b.w of absolute ethanol
Group 4: 100 mg/kg b.w of the extract + 5 ml/kg b.w of absolute ethanol
Group 5: 200 mg/kg b.w of the extract + 5 ml/kg b.w of absolute ethanol
Group 6: 400 mg/kg b.w of the extract + 5 ml/kg b.w of absolute ethanol

Fig. 3: Effects of the graded doses of the methanol extract of the leaves of *A. indica* on catalase specific activity

4. DISCUSSION

In this study, the effects of the methanol extract of the leaves of *A. indica* on the concentration of total proteins and specific activity of catalase in ethanol-ulcerated rats were evaluated with a view to elucidating the probable mechanism(s) of action(s) of the leaves of *A. indica* 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. indica* 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. indica*. 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[9]) which might have annulled the ethanol-caused gastric ulcer.

The decrease in the specific activity of catalase in the rats of the ulcer-untreated group might be due to ulcerogen (ethanol)-initiated generation of free radicals which culminates in ulceration. The effects of ethanol on gastric mucosa are complicated and multifaceted. Ethanol 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. Ethanol injures the vascular endothelial cells of the gastric mucosa and induces microcirculatory disturbance and hypoxia, resulting in the overproduction of oxygen radicals[10]. Treatment of rats with the graded doses of the methanol extract of the leaves of *A. indica* along with ethanol 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. indica* to augment the specific activity of catalase against the ethanol-induced toxicity is indicative of its potential in the prevention or amelioration of gastric lesions induced by free-radical reactions.

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

REFERENCES

1. Balick JM, Cox PA. Plants, People and Culture: The Science of Ethnobotany. Scientific American Library, New York 1996; p. 228.
2. Schumacher M, Cerella C, Reuter S, Dicato M, Diederich M. Anti-inflammatory, pro-apoptotic and anti-proliferative effects of a methanolic neem (*Azadirachta indica*) leaf extract are mediated via modulation of the nuclear factor-kB pathway. Genes and Nutrition. 2011;6(2):149-160.
3. Khan NM, Khan FA, Mu TH, Khan ZU, Khan M, Ahmad S, Behram T. Potentiality of neem (*Azadirachta indica*) powder in rheology modification of oil-in-water emulsion. Journal of Food Process Engineering. 2015;38:190-196.
4. Emran TB, Nasir Uddin MM, Rahman A, Uddin Z, Islam M. Phytochemical, anti-microbial, cytotoxic, analgesic and anti-

inflammatory properties of *Azadirachta indica*: A therapeutic study. Journal of Bioanalysis and Biomedicine. 2015;12:1-7.

5. Guyton AC, Hall JE. Textbook of Medical Physiology. 11th ed, Elsevier publication, Philadelphia;2006:795-821.
6. Wandale NN, Pagore RR, Biyani KR. Gastroprotective and anti-secretory effects of polyherbal formulation on gastric lesion induced in rats. International Journal of Research in Pharmaceutical and Biomedical Sciences. 2012;3(3):993-1003.
7. Lowry OH, Rosebrough NJ, Farr AL, Randal RJ. Protein measurement with folin phenol reagent. Journal of Biological Chemistry. 1951;193:265-275.
8. Aebi H. Catalase In vitro: Methods of Enzymatic Analysis. Academic Press, New York; 1984:671-684.
9. Prashanth GK, Krishnaiah GM. Chemical composition of the leaves of *Azadirachta indica* Linn (Neem). International Journal of Advancement in Engineering Technology, Management and Applied Sciences. 2014;1(5)21-31.
10. Suzuki H, Nishizawa T, Tsugawa H, S, Hibi T. Roles of oxidative stress in stomach disorders. Journal of Clinical Biochemistry and Nutrition. 2012;50(1):35-39.

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