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Toxicological Studies on the leaf extract of *Azadirachta indica* in the heart of rat (*Rattus novegicus)*

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ABSTRACT

The effect of *Azadirachta indica* (leaf extract) on some enzyme activities in the heart was investigated. These enzymes include Aspartate transaminase, Alanine transaminase and Alkaline phosphatase. The result obtained showed a decrease in the activities of aspartate transaminase and alkaline phosphatase in the heart ($P<0.05$) after the administration of first dose. The reduction in aspartate transaminase activities continued to the termination of experiment (seventh day). Generally, the activities of all the enzymes studied reduced on the seventh day. Reduction of alkaline phosphatase activity in the heart is with a corresponding increase in the serum on the third day of administration. This reflects tissue damage which might have been done to the plasma membrane with corresponding efflux of the cytoplasmic content of the enzyme into extracellular space. Though, recovery of aspartate transaminase and alkaline phosphatase on the third day after administration of the second dose, indicates denovo synthesis of enzyme molecules. The result obtained shows that continual usage of the leaf extract of *Azadirachta indica* may lead to cell destruction and initiation of heart diseases.

Key words: *Azadirachta indica*, alanine transaminase, aspartate transaminase, alkaline phosphatase, tissue damage.

INTRODUCTION

Azadirachta indica A. Juss (Neem) has been widely used as an antimalaria in Nigeria and the West Coast of Africa as anti-inflammatory agent and insect repellent with antifeeding properties (Egunjobi, 1976; Bhanwra, 2000)

Neem is now widely distributed in many countries of the world by cultivation. It was also demonstrated that no plant material showed greater activity against a broad spectrum of insect pest species than the neem tree (Ekong, 1967). Several compounds were isolated from the seeds of neem one of these, azadirachtin, was found to both repel and disrupt the growth and reproduction of many destructive insect species. The stem and leaf extracts have been shown by Okpanyi, 1977; Bhanwra 2000; Subapriya, 2005) to have antipyretic, anti-inflammatory and analgesic effects. The antibacterial, anti-viral, and anti-inflammatory actions of this plant justify its use in the treatment of fevers, malaria and skin diseases. Due to the therapeutic and pesticidal properties of *Azadirachta indica* the extracts of its various parts has been the subject of extensive investigations leading to the isolation of interesting constituents. The constituents of *Azadirachta indica* includes a wide range of terpenoids bioactive limonoids and flavonoids amidst other chemical constituents. The objective of this study is to assess the effect of *Azadirachta indica* (neem) on the heart using rat as animal model.

MATERIAL AND METHODS

Plant material

Fresh leaves of *Azardirachta indica* were obtained from the local plant in Ado-Ekiti, Nigeria and identified in the Department of Plant Science

and Forestry Herbarium, University of Ado-Ekiti, Nigeria.

Animal grouping

Twenty rats of both sexes (3 months old) weighing between 100-200g were obtained from the Animal breeding Unit of Anatomy and Physiology department, University of Ibadan, Ibadan, Nigeria. The animals were divided equally into four groups of five animals each. Rats in group one served as control. They were fed with pellets and water *ad libitum.*

Administration of extract

Fresh leaves of Neem were sun dried and made into powdery form. Aqueous extract of the leaves was prepared by soaking 6g of the powdered leaves in 100ml of distilled water ($6\%^{w}/_{v}$). The resulting suspension was left overnight at room temperature (37°C) Thereafter, the suspension was filtered. The filtrate was administered orally to the rats at a dose of 100mg/kg body weight. Rats in group two were given only one dose, those in group three were administered two doses at 24h intervals, while those in group four were administered three doses at 24h intervals and were kept alive for four days. The control group (Group one) was given distilled water, instead of the extract. All the experimental rats were closely examined for signs of restlessness, excitement, intoxication and other behavioural changes.

Collection of tissue

The rats were bled while under anaesthesia, into a clean dry beaker and serum was prepared as described by (Akanji and Nlumanze, 1987) after the completion of each dose regimes. They were sacrificed and dissected while still under anaesthesia by cervical dislocation. Heart was removed into ice-cold 0.25M sucrose solution, washed free of blood and weighed. It was later cut into tiny pieces with a sterile blade into ice-cold 0.25M Sucrose solution. The homogenates were kept frozen overnight (-20°C) prior to enzyme assay. This was to ensure the maximum release of those enzyme systems located in the cell organelles (Ngaha *et al*, 1989).

Determination of enzyme activities

Alkaline phosphatase (ALP), alanine transa-minase (ALT) and aspartate transaminase (AST) activities were determined using the appropriate buffer systems Spectrophotometric method of (Kings, 1960) was used to measure alanine and aspartate transaminase. Alanine transaminase was measured by monitoring the concentration of pyruvate hydrazone formed into 2,4-dintrophenyl hydrazine at 546nm. Aspartate transaminase was measured by monitoring the concentration of oxaloacetate hydrazone formed with 2,4…dinitrophenyl hydrazine at 546nm. Alkaline phosphatase activity was determined by measuring the p-nitrophenol liberated form p-nitrophenyl phosphate at 400nm (Wright *et al*, 1972).

RESULTS AND DISCUSSION

Enzyme activities are expressed as specific activity m/l± standard deviation (SD).

Statistical significance was tested using student's t-test, compared with control value ($P<0.05$)

Table 1 shows the changes in the activities of aspartate transaminase (AST), alanine transaminase (ALT) alkaline phosphatase (ALP) in the heart of rat following administration of aqueous extract from neem leaf when compared with control.

Table 1: Effect of neem leaf extract on some enzyme activities (µ/l) in rat heart

Days after injection	Group	GOTAST	GPTALT	ALP
0	1 (Control)	177.80 ± 2.63	1114.30. ± 2.21	5964.0± 1.43
1	2	111.10 ± 1.20	1142.90 ± 1.64	2840.0 ± 2.30
3	3	195.50 ± 0.64	1114.30 ± 2.34	6134.0± 3.10
7	4	53.30 ± 2.50	991.40 ± 1.56	4544.0 ± 0.56

Aspartate transaminase and alkaline phosphatase activities reduced in the heart (P<0.05) after the administration of the first dose when compared with control animals. The activities of these enzymes (AST and ALP) in the heart recovered on the third day Alanine transaminase on the other hand increased appreciably after the administration of the first dose on the first day. The activity of ALT was found to decrease in the heart from the third day after the animals have received second dose of the extract. The reduction continued to the seventh day. The significant reduction of alkaline phosphatase activity in the heart on the third day (Table 1) may be attributed to the destruction of the plasma membrane and hence efflux of cytoplasmic content of the enzyme into extracellular space. This report is confirmed by corresponding increase in ALP activity noticed in the serum on the same day after the administration of the second dose. (Table 2)

Table 2: Effect of neem leaf extract on some blood serum enzyme activities (m/l) in rat heart.

Days after injection	Group	AST	ALT	ALP
0	1 (Control)	31.70 ± 2.67	151.40. ± 085	511.20± 2.14
1	2	17.80 ± 3.10	28.60 ± 1.62	1192.80± 3.25
3	3	71.10 ± 1.47	62.90 ± 0.25	1278.00± 3.10
7	4	155.50 ± 2.34	457.10 ± 3.14	1505.20± 1.73

Statistical significance was tested by student's t-test (P<0.05)

Coodly, 1970 reported that enzyme activities present in the serum (or plasma) represent an "overflow" from their tissues of origin. The reduction of aspartate transaminase activity (AST) does not show a significant effect on the serum rather they may be inactivated at the cellular level or as a result of the enzyme being inhibited by the extract. It is possible that low serum values may be due to released enzymes not getting into the serum due to inhibition of enzyme molecules insitu (Akanji and Nlumanze, 1987).

The recovery of the activities of the enzymes (AST and ALP) on the third day after administration of the second dose (Table 1) indicates denovosynthesis of the enzyme molecules.

High serum levels of the enzymes (AST, ALT and ALP) on the seventh day is an indication of a diseased state. This is confirmed by the significant reduction of the enzyme activities in the heart (Table 1). This report revealed the effect of the high dosage of the extract on the heart, which implies that the heart is exposed to insult with chemical compounds which result in cell damage (i.e plasma membrane) hence efflux of the enzyme into extracellular environment.

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