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Research Paper

Alkaline Phosphatase Activity (Alp) In Albino Rats Treated With Aqueous Extract of Fresh Leaves of *Nauclea Latifolia*

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ABSTRACT:- Most medicinal plants have been shown to elicit toxic effects even within their therapeutic doses. This research was carried out to investigate the effect of aqueous extract of fresh leaves of *Nauclea latifolia* on activity of alkaline phosphatase (ALP) in albino rats. Twenty adult male albino rats, used, were grouped into four groups (A, B, C and D) of five rats per group. Aqueous extract of fresh leaves of *Nauclea latifolia* was administered to groups A, B and C with the following doses 100,200 and 300mg/kg respectively. While group D served as control. The administration lasted for seven consecutive days. The body weight and physical activities of the rats decreased in groups when compared to the control. The total protein concentration of the groups treated with the extract did not vary significantly ($p>0.05$) from the values obtained in the animals in the control group. There was a significant increase ($p<0.05$) in the ALP activity of the treated groups when compared to the control. These effects were found to be dose-dependent. The results obtained from the research suggest that the adverse effect of *Nauclea latifolia* may include hepatoxicity, since there was elevation in the level of ALP in the treated groups.

Keywords:- *Nauclea latifolia*, alkaline phosphatase, hepatoxicity and physical activities.

I. INTRODUCTION

Alkaline phosphatase is an enzyme that appears in many places in the body. Though this enzyme has many known functions, which range from diagnosing medical problems to manipulating DNA in laboratories, there are also many functions that have yet to be discovered and are being researched today. Researchers work with this enzyme every day, and hope to find even more ways that it may be useful to the scientific community (Arbonnier 2000).

Alkaline phosphatase is an enzyme that is found mostly in the liver and in the bones. Alkaline phosphatase is the enzyme that causes the chemical reactions that remove phosphates from a number of different molecules. These enzymes work best in an environment with a pli that is alkaline, and in removing acidic phosphates from molecules, they help to create that environment (Shigemori et *al.,* 2002).

The alkaline phosphatase (ALP) known as metalloenzyme [phosphaVe-monoester phosphohydrolase (alkaline optimum); EC 3.1.3.1] exists as severa^ tissue-specific isozymes encoded by separate genes. The enzyme, which expressed in many species (plants, bacteria and animals), catalyzes the hydrolysis of phosphomonoesters, R-O-PO_3, with little regard to the identity of the 'R' group. The catalytic mechanism involves the formation of a serine phosphate at the active site which reacts with water at alkaline pH to release inorganic phosphate from the enzyme (Shigernori *et al.,* 2002). In the presence of high concentrations of an organic alcohol, a transphosphorylation reaction results when the organic alcohol releases the enzyme-bound inorganic phosphate and becomes phosphorylated (Shigemori *et al.,* 2002).

In vertebrates, the enzyme is an ectoenzyme, which is attached to the outer face of the plasma membrane through a phosphatidyl inositol-glycophospholipid (GPI) anchor covalently attached to the C-terminus of the enzyme. Humans have four ALP genes corresponding to intestinal, placental, placental-like and liver/bone/kidney (tissue nonspecific; TNAP) gene products (Arbonnier 2000) ALP has many different functions in the many organisms and tissues where it occurs.

Nauclea latifolia Smith (Rubiaceae) is a shrub or small spreading tree that is a widely distributed plant that grows in the north Nigeria and other African countries (Arbonnier 2000). It is found in the forest and fringe tropical forests. *Nauclea latifolia* roots decoctionj.s one of such herbal preparations that have been used traditionally for treating different disease conditions. Medicinal uses vary from one traditional setting to another, its traditional uses include: fever, pain, dental caries, septic mouth, malaria, dysentery, diarrhea, and diseases of the central nervous system such as epilepsy (Arbonnier, 2000, Amos *et al.,* 2005; Ngo Bum *et al.,* 2009; Abbah

et al., 2009). The root of *Nauclea latifolia* is the preferred part of the plant used in Cameroonian traditional medicine for treating pain and fever. This part is usually harvested, sun dried and pulverized to obtain powder. About 100 g of the powdered material is macerated in 500 ml of water and boiled. The decoction obtained is administered orally at the dose rage of 80-160 mg/kg (Arbonnier 2000).

The aqueous extract of leaves of the plant has been used as a remedy for diabetes in northern Nigeria (Gidado *et al.,* 2005). The plant also has been reported to have antihypertensive and laxative activities (Akpanabiantu *et. al.,* 2005). Previous works have shown that the aqueous extract of the bark of *Nauclea latifolia* (freeze-dried extract) attenuated writhing episodes induced by acetic acid and increased the threshold for pain perception in the hot plate test in mice. The extract remarkably decreased both the acute and delayed phases of formaline-induced pain in rats and also caused a significant reduction in both yeast-induced pyrexia and egg albumin-induced edema in rats (Abbah *et al.,* 2009). In the course of pharmacological studies, anticonvulsant, anxiolytic and sedative properties oi *Nauclea latifolia* roots decoction (Ngo Bum *et al.,* 2009) have already been reported from this laboratory. Phytochemical investigations of the bark and wood of *Nauclea latifolia* have reported the presence of naucleamides A-E, new monoterpene indole alkaloids from *Nauclea latifolia* (Shigemori *et al.,* 2002). Unfortunately, none of these compounds have been tested for their pharmacological activities.

Biochemical evaluation of the plant (*Nauclea Latifolia*) indicates that the plant has high anti-diabetic property, high anti-hypertensive properly,anti-palpitation,anti-convulsant,anti-depressant and anti-abortifient.In African traditional medicine,Naucle.a Latifolia (Egbesi Plant)has neuropharmacological effects and it is also being used as herbal recipe for its cardiovascular activities.

Aim/Objectives

The aim of this research work is to evaluate the role of/alkAline phosphatase activity in albino rat treated with *Nauclea latiolia* leaves extract.

Medicinal plants have been found to elicit various toxicity. This research work evaluate the heptotoxicity of agnous extract of fresh leaves of *Nauclea lotiolia* by measuring it effect on alkaline phosphatase levels in treated albino rats.

II. MATERIALS AND METHODS

Methods

Collection of Albino Rats

Twenty (20) albino rats obtained from the disease free stock of the animal house, department of Biochemistry College of medical science, university of Nigeria, Nsukka.

Collection plant leaves

Nauclea latifolia leaves was obtained from a seller in Mgbabor village Abakaliki and it was identified by a botanist (Dr. Ibiam. F.) of the department of Applied Biology in Ebonyi state university, Abakaliki. Ebonyi State.

Preparation of Plant Extract

Fresh leaves of *Nauclea latifolia* were washed and crushed with mortar and pestle until it turned into paste. Then 300g of this paste was soaked in 1000ml of ethanol and left for 48 hours. A Musclin cloth was then used to filter out the liquid path of the extract paste to get the ethanol extract of *Naulea latifolia* leaves. The ethanol was allowed to evaporate to obtain the extract of *Naulea latifolia* leaves.

Animal grouping: The animals were kept in four cages, five animals per cage and were labeled accordingly.

Measurement of the Weight of the Animals

The weight of the animals was taken daily using chemical balance. The results obtained were used to monitor the weight changes and determine the volume of the sample to be administered to each of the animal.

Animal treatment/administration

The animals were fed with growers match and water on daily basis for seven consecutive days for acclimatization. The samples were administered to the animals using 2ml syringe. The animals in groups A, B, and C, were given 100, 200, and 300mg/kg body weights respectively while the animals in group D (the control) were given distilled water for seven consecutive days.

Collection of samples from animals

After seven days of treatment with the plant extract, the animals were starved for 24hrs and their blood samples were collected into a sterile bottle using sterile blade.

Determination of protein concentration
Lowry method of protein assay 1951 as described by Bradford (2001) was used in protein assay.

Principle: Under alkaline conditions divalent copper ions forms a complex with peptide monovalent ion. Monovalent copper ion and the radical groups of tyrosine, tryptophan and cysteine react with phenol reagent to produce an unstable product that becomes reduced to molybdenum or tungsten blue.

Procedure: this experiment was carried out on the serum and was performed using the Bovine serum albumin as the standard 0.4ml sample was added to 0.4ml of two times Lowry concentrate and mixed together, the mixture was incubated at room temperature for 10minutes. 0.2ml of 0.2N Folin reagents was added, stirred immediately and incubated for additional 30munites at room temperature. The absorbance was read at 750nm and was subjected to a standard curve. It is important to mix the 0.2N Folin reagent rapidly to avoid the decomposition of the reagent.

Statistical analysis
Result was expressed as mean ± standard deviation. The data were subjected to one way analysis of ANOVA.

III. RESULTS

Physical Observations

On the first day, the animals were very normal and were in active condition before the extract was administered. After the administration, a remarkable decrease in activity was noticed. Subsequent days gave rise to further decrease in activity especially in Groups that had higher doses like C and B respectively. Also there was a decrease in water and food intake as the days progressed.

Changes in body weight of the animals

The average weight of animals in groups A,B,C and D after seven (7) days of the Administration is shown below the animals in Group A, B and C showed an insignificant decrease (P> 0.05) in weight, while the animals in Group D, which where not treated, showed an insignificant increase (P<0.05) in weight.

Table 1: Changes in average body weight (g) of animals during 7 days of treatment Treated with *Nauclea latifolia*

Days Of Administration	AVERAGE WEIGHT OF RATS (g)			
	GROUP A	GROUP B	GROUP C	GROUP D
1.	142.2±7.43	94.88±4.85	89.50±5.63	90.01±4.65
2.	141.60±7.35	82.46±3.45	88.28±5.62	93.25±4.69
3.	141.10±7.32	80.42±3.43	88.02±5.58	96.00±5.45
4.	140.42±7.42	70.50±2.95	87.32±4.49	96.24±5.89
5.	140.00±6.90	67.30±3.90	78.82±3.93	97.30±6.37
6.	138.62±6.57	65.28±3.75	78.03±3.63	99.48±7.35
7.	138.10±6.36	64.20±3.73	77.05±3.60	100.20±7.63

Values are mean ± standard Deviation, n=5.

The table above the average weight of albino rats treated with *Nauclea latifolia* for seven days. The mean body weight of the animals decreased while the control group increased

AVERAGE TOTAL PROTEIN CONCENTRATION, ENZYME ACTIVITY AND SPECIFIC ENZYME ACTIVITY IN THE ANIMALS

Table 2: enzyme and specific enzyme activity in the animals during the 7days of administration

Animal group	Total protein concentration (mg/ml)	Enzyme activity (u/l)	Specific enzyme activity (L/mg)
A	0.50 ± 0.06^{a}	183.57 ± 7.07^{b}	352.08 ± 7.76^{b}
B	0.55 ± 0.09^{a}	237.00 ± 7.07^{c}	340.38 ± 8.82^{b}
C	0.44 ± 0.08^{a}	273.29 ± 8.73^{c}	527.27 ± 0.65^{c}
D	0.67 ± 0.05^{a}	108.68 ± 6.89^{a}	131.27 ± 5.36^{a}

Value are mean ± standard Deviation, n = 5. The values with different superscript differs significantly. (p<0.05).

The average total protein concentrations of the treated groups (A-D) were insignificantly lower ($p>0.05$) than that of the control group (D) the alkaline phosphatase activity of the control group was significantly lower ($p<0.05$) then those obtained in the group to which the extract was administered.

IV. DISCUSSION

Serum alkaline phosphatase activity was investigated with the view of establishing hepatotoxicity in treatment with *Nauclea latifolia,* plant extract, which is a medicinal plant extract.

A decrease in physical activity was noticed in the groups that were treated with the extract, while the animals in the control were more physically active. The decrease in physical activity is not well understood, however the introduction of the sample and the subsequent accumulation of high dose in the liver, and other related organs, may have led to intrahepatotoxic condition and other necessary conditions. This condition is capable of impairing energy related metabolism of the liver resulting in depreciation of energy in such animals and reducing the basal metabolic rate of such animals with characteristic calmness. As was observed by Agbafor (2004) in determining the effect of *Baphia nitida* on acetyl cholinesterase in albino rats. The average body weight of the treated animals (Group A, B, and C) decreased insignificantly ($p>0.05$), while that of group D showed a non significant increase ($p>0.05$).Although the precise biochemical mechanism of this effect cannot be concluded of this stage of the research, it may be attributed to the reported decrease in food and water intake work done by Bray (1998) on effect of *Irvingia gabonesis* seed extract on rat showed a similar result.

Determination of the protein concentration (level) in the serum of groups A, B and C revealed an insignificant difference ($p>0.05$) in the treated animals, compared with the control (Ground D).

This plant extract may not be playing a significant role in protein synthesis and degradation. This is in line with work done by Agbafor (2004) in determining the effect of *Baphia nitida* on acetytclolinestrase in albino rates.

The activity of serum alkaline phosphatase showed a significant increase ($p<0.05$) in the treated animals when compared with the control group. It has also been reported that *Nauclea latifolia,* though a medicinal plant, when used in a large doses, can lead to liver damage with consequent increase in serum alkaline activity (Laureuce and Beumett, 1997). The elevation in serum alkaline phosphatase activity was observed to be higher in group C than in group B and A, this could mean that the potency of the plant extract is dose dependent.

V. CONCLUSION

The observations made in this work suggest that *Nauclea latifolia* may induce metabolic responses, which may include hepatocellular injury. Thus care must be taken in the use of treatment of diseases.

However, the results generated from this research are not enough to confirm all claims since only one (alkaline phosphatase), out of the four serum hepatomarkers indicative of intrahepatic damage was accessed.

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