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Effect of Ethanol Leaf Extract of *Aspilia Africana* on Activities of Some Serum Enzymes of Male Rats

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Annotation: The effect of ethanol leaf extracts of *Aspilia africana* (hemorrhage plant) on some serum enzyme activities on male albino Wistar rats were determined. A total of 48 male Wistar albino rats were used in this research, these were shared into 4 groups of 12 each. Group 1 was placed as the control, group 2 were given 500mg/kgBW of the extract, group 3 were given l000mg/kgBW of the extract and group 4 were given 2000mg/kgBW of the extract. The administration of the extract was done in batches (14 and 28days). Following the administration for the 14 days, batch 1 (which was gotten by selecting 6 rats randomly from each group) were sacrificed and the administration were continued with the remaining rats at the same doses for 28 days. Statistical analysis of serum enzyme activities for 14days showed that there was no significant difference in activities of ALT, AST and ALP of the treatment groups compared to the control. In 28 days, there were no significant difference in the activities of both alanine amino transferase and alkaline phosphatase among the groups, Aspartate aminotransferase concentration

in the different experimental groups of the 28 days differed significantly from the control at $p<0.05$, showing potential toxicity to the heart tissue. Conclusively, that the ethanol leaf extract of *Aspilia africana* is safe and can be useful in the management hepatic conditions and other ailments due to it low toxicity.

Keywords: Ethanol Leaf Extract, Aspilia Africana, Serum Enzymes and Male Rats.

1.0 INTRODUCTION

Man has been widely depending on medicinal plants for health care right from the beginning of age. Medicinal plants have been considered important even in international market. In African and Asian countries, over 60% of the population depend completely on plants as a source of medicine due to the ease of accessibility, low cost and availability (2, 12). Consequently, WHO in 2002 recommended the use of herbal medicine and countries in the world, especially African and Asian, are now using traditional medicines as a major component of their health care programs (2).

Aspilia africana has long history of traditional use. It is one of the many indigenous plants used by tradomedical practitioners in Nigeria to cure certain illnesses. It is known by different names across the various ethnic groups in Nigeria; the Ibos call it organgila, Tazalian by the Hausas, Yungung by the Yorubas and Edemedong by the Efiks (3). It has several uses in tradomedical sciences, for example, it is used for the treatment of various ailments (3). Adeniyi and colleque, (2000) & Okoli ***et al.,*** (2007a) reported some therapeutic benefits of this plant. The Antimicrobial and antiinflammatory activities have also been associated to this plant (6).

Alkaloids, saponins, glycosides and tannins are immeasurable in the plant (1). Diterpenes; kaurenoic and grandiflorenic acids have been reported (8). It is used in the management of rheumatic problems (6, 10). In Kenya, it is used to kill intestinal worms; in Uganda, for gonorrhea treatment (10). The methanol extract of the leaf is reported to cure malaria and respiratory problems (5). A concussion of the leaves is used to cure eye problem and as a lotion for the face to relieve frabrile headache. They are also used to cure ringworm and dysentery.

Although *Aspilia africana* is used traditionally to manage a plethora of ailments, for instants in the treatment of wound, cure of malaria and respiratory problems, etc., there is limited report on its toxicity. *Aspilia africana* can induce adverse effects if incorrectly used. This study investigated the effect of *Aspilia africana* on some serum enzymes of male rats.

2.0 MATERIALS AND METHODS

2.1 Collection, Identification and plant materials preparation.

2.1.1 Collection / identification.

Aspilia africana leaves were collected from the Cross River University of Technology (CRUTECH) and identified by a Botanist in University of Calabar, Calabar, Nigeria.

2.1.2 Preparation / Treatment of Plant Materials.

The dead matter was sorted out and eliminated. The air-drying took 2 weeks and were then blended using a blender manually. 150g of the leaves was soaked in 700ml of ethanol and kept for about 48 hours for proper extraction of the plant active component. The filtration process was done using Whatman no. 1 filter paper to obtain the soluble portion of the pant. This was evaporated in water bath to dryness and the extracts refrigerated at 2-5°C until when used. Required amount of extract

was made by diluting with distilled water into the appropriate concentrations according to the weight of the animals

2.2 Experimental Design

Forty eight (48) male Wistar rats weighing 150-180g were used for this study. They were placed in four groups of twelve rats. Group I- control group, II, III and IV extract treated groups (500mg/kg, 1000mg/kg and 2000mg/kg, extract). Extract administration to the animals was done using syringe and feeding tubes. Each group was housed in wooden cages with wire mesh top and fed rat chow (Guineaa feeds products) from the High Quality Livestock feeds, Calabar, Nigeria. They were given tap water freely. Measurement of weight changes were taken every week all through the administration period

Table 1.0: Experimental design

Groups	Animals	Administered Treatment
I	12	Controls.
11	12	500mg/kg extract
III	12	1000mg/kg extract
IV	12	2000mg/kg extract

2.3 Collection of Sample for Analysis

The dissection was done after 24 hours of fasting after last feeding. Whole blood was collected via cardiac puncture using sterile syringes and needles. The blood was emptied into plain tubes and allowed to clot for about 2 hours. The clotted blood was thereafter centrifuged using bench top centrifuge (MSE) at 3000pm for 10 minutes to recover serum from clotted cells. Serum was separated with sterile dropper and stored frozen until used for biochemical analysis. Serum liver enzymes- AST, ALT and ALP analysis was done according to the method described by (9) with Randox test kits.

2.4 Biochemical assays

The activity and concentration of these enzymes was done as below

2.4.1 Estimation of serum AST activities

Alpha-oxoglutarate + L-aspartate $\xrightarrow{AST}$ L-glutamate+ oxaloacetate

Oxaloacetate, with concentration in proportion to aspartate consumed by enzyme and hence its activity is measured by monitoring the concentration of oxaloacetate hydrazone formed with 2,4 - dinitrophenyl hydrazine.

Reagent provided

- Buffer reagent phosphate buffer, 100mm, ph. 7.4, L-aspart. I 00mM.

  oxoglutarate, 2mM
- Reagent 2 2,4-dinitro phenyl hydrazine, ImM

Reagent preparation: contents are ready for use.

Procedure: As per schedule below, pipette into test tubes.

	Reagent blank	sample
Sample -	-	0.2ml
Solution 1 (buffer)	1ml	1ml
Distilled water	0.2ml	-

Mixed and incubated for exactly 30minutes at 37°C

Solution 2 1ml 1ml

Mixed and was allowed to stand for exactly 20 minutes at 20 - 25°C

Sodium hydroxide 10 ml 10 ml

Mixed and absorbance of sample was read against the reagent blank at 546 nm after 5minutes.

Calculation:

Absorbance	u/1	Absorbance	u/1
0.020	7	0.100	36
0.030	10	0.110	41
0.040	13	0.120	47
0.050	16	0.130	52
0.060	19	0.140	59
0.070	23	0.150	67
0.080	27	0.160	76
0.090	31	0.170	89

2.4.2 Estimation of serum ALT activities

The activity of alanine aminotransferase (ALT) was estimated in the serum sample by the reaction of a-oxoglutarate with L- alanine to give L- glutamate and pyruvate as by product.

Alpha-oxoglutarate + L-alanine $\longrightarrow$ ALT L-glutamate + pyruvate

Pyruvate whose concentration depends on the amount of L-alanine tramsaminated and hence the activity of ALT is measured by monitoring the concentration of pyruvate hydrazone formed with 2,4-dinitrophenylhydrazine at 546nM.

Reagent provided:

Buffer reagent: phosphate buffer, 100mM, ph - 7-4, L-alanine, 200mol/L, alpha-oxoglutarate, 2mmol/l

Reagent 2: 24-dinitrophenyl hydrazine, 1 mM.

Reagent preparation: Contents are ready for use.

Procedure: As per scheduled below, pipette into test tubes.

	Reagent blank	sample
Sample	-	0.2ml
Solution 1	1 ml	1ml
Distilled water	0.2ml	-
Mix, incubate for	exactly 30minutes at 37°C	
Solution 2	1ml	

Mix, allow standing for exactly 20 minutes at 20 - 25°C

Sodium hydroxide 10 ml 10 ml

Mixed and absorbance of sample was read against the reagent blank at 540nm after 5 minutes Calculation: obtain ALT activity from the conversion table below.

Absorbance	u/1	Absorbance	u/1
0.025	4	0.275	48
0.050	8	0.300	52

0.075	12	0.325	57
0.100	17	0.350	62
0.125	21	0.375	67
0.150	25	0.400	72
0.175	29	0.425	77
0.200	34	0.450	83
0.225	39	0.475	88
0.250	43	0.500	94

2.4.3 Estimation of serum ALP activities

ALP activity in the serum was estimated by kit method as described by (11) based on the measurement of the rate of hydrolysis of phosphate esters. Colorless P-Nitrophenol, which absorbs strongly at 405nm. The rate of increased absorbance at 405nM is proportional to the enzyme activity.

Reaction equation

P-Nitro-Phenyl phosphate + H20 $\longrightarrow$ P- Nitrr ALP Phosphate

Procedure:

The working reagent and the photometer were brought to reaction temperature (30°C). 1ml of the working reagent and 0.2ml of the sample was pipette into a cuvette and mixed. Initial absorbance was recorded after one minute (1) intervals thereafter for three (3) minutes. The difference between consecutive absorbance and the average change in absorbance per minute was calculated as:

Catalytic concentration (p/1) =

$$\frac{DA7min \times 1000 \times TV}{E \times LP \times SV}$$

Where 1000 = conversion of pml to pi DA/Min change in absorbance per minute.

TV = total reaction volume

E = Millimolar absorbtivity of P-Nitro phenol (18.75)

LP light path (1.0cm)

SV Sample volume (0.01ml)

p/1 DA/min x 1000 x 1.025

18.75 x I x 0.025 DA/min x 2187

2.5 Data Computation

This was done using students t-test following Anova to detect differences in the concentration of enzyme between groups. Test with a probability value of <0.05 was taken as significant.

3.0 RESULTS AND DISCUSSION

3.1 Results

3.1.1 Effect of *Aspilia africana* on Some Serum Enzymes of Male Rats

Serum enzymes include; amino transferase (alanine NH_2 transferase ALT and aspartate NH_2 transferase AST) and ALP their activities were carried out and the results is presented in figure (1-3). The result of (14days) indicate no difference in activities of ALT, AST and ALP among the groups. In figure (4-6) and table 5 of the appendix (28days), there were no significant difference in both Ala amino transferase and ALP activity among the groups. However, aspartate aminotransferase concentration in the different experimental groups of the 28days, differed

significantly from group 1 at $p<0.05$.

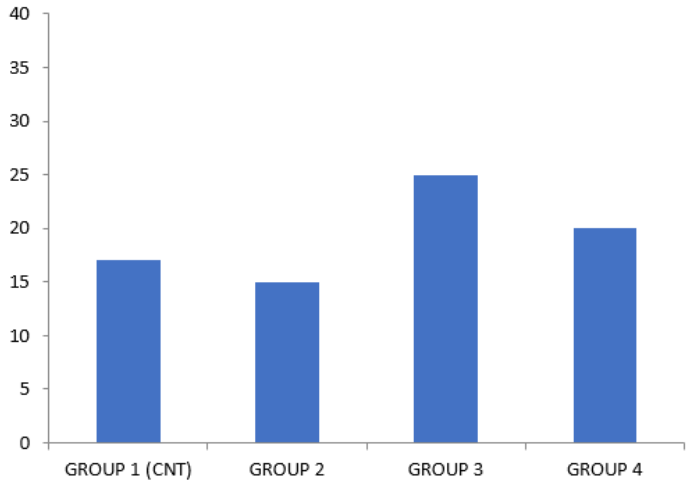

Figure 1.0: Comparing alanine aminotransferase concentrations in the different experiment groups of batch 1.

mean values ± S.E.M, n = 6

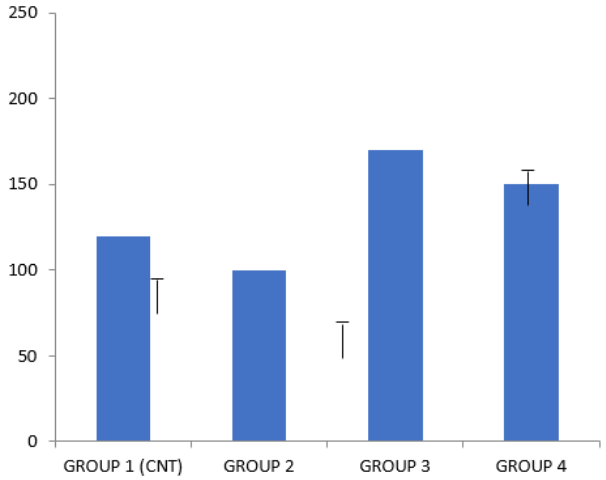

Figure 2.0: Comparison of aspartate aminotransferase concentrations in among groups of batch 1.

mean values ± SEM, n = 6

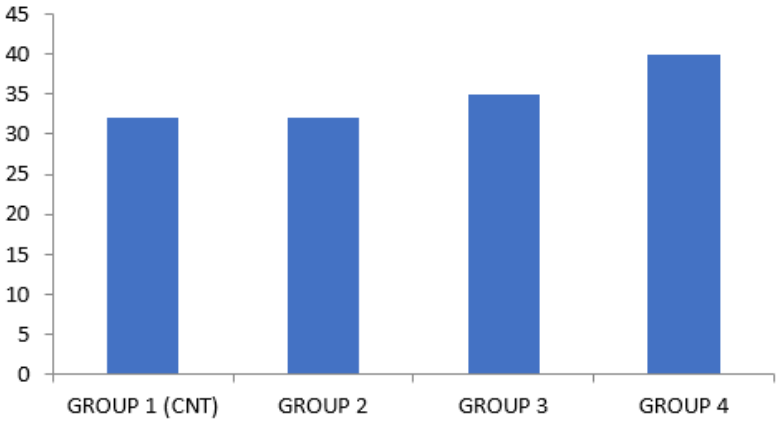

Figure 3.0: Comparing alkaline phosphate concentrations in the experimental groups in batch 1.

Values mean ± SEM, n = 6

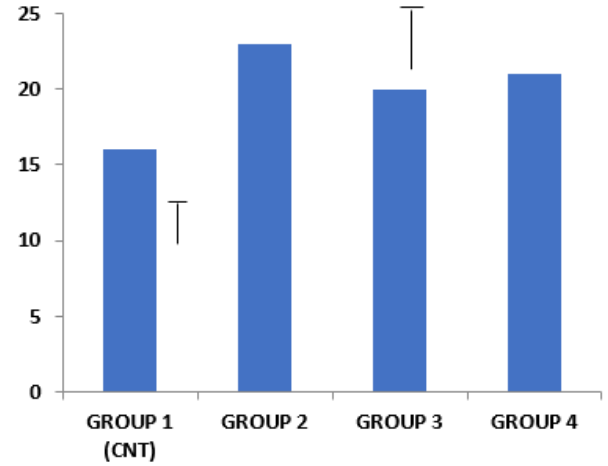

Figure 4.0: Comparing alanine aminotransferase concentrations of experimental groups of batch 2.

Values are mean ± SEM, n = 6

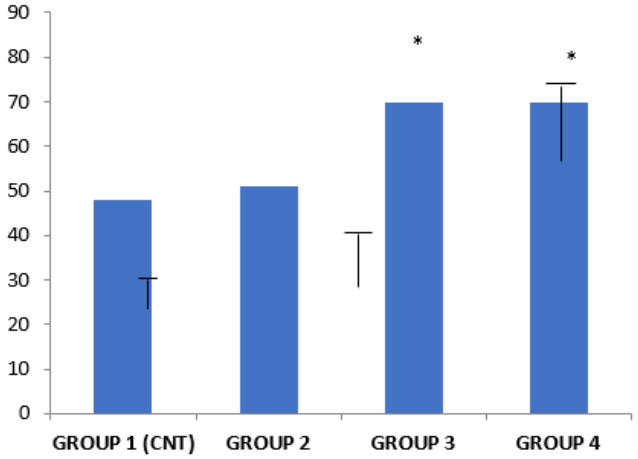

Figure 5.0: Comparing aspartate aminotransferase concentrations of experimental groups of batch 2.

Values are Mean ± SEM, n = 6

***Significant from control at p < 0.05**

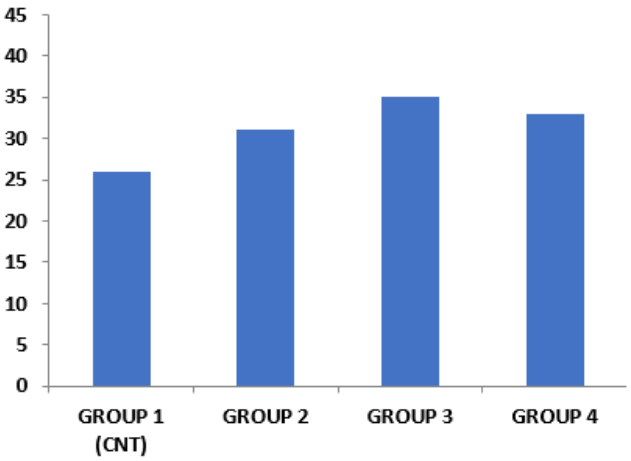

Figure 6.0: Comparing alkaline phosphate concentrations in the experimented groups of batch 2.

Mean Values ± SEM, n = 6

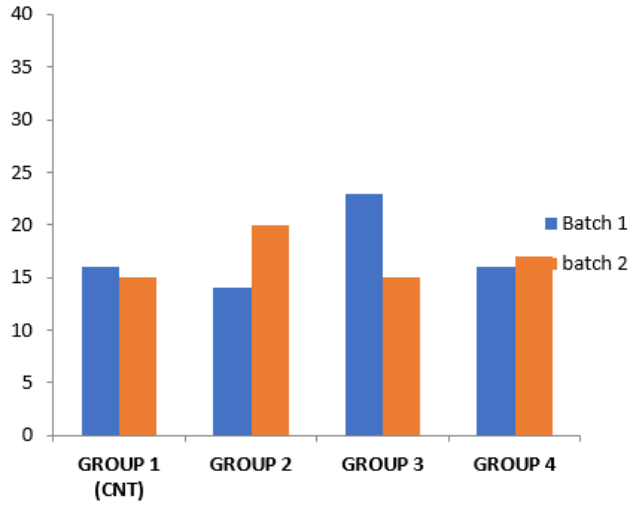

Figure 7.0: Batches 1 and 2 Comparison of alanine aminotransferase concentrations.

Values are Mean ± SEM, n = 6

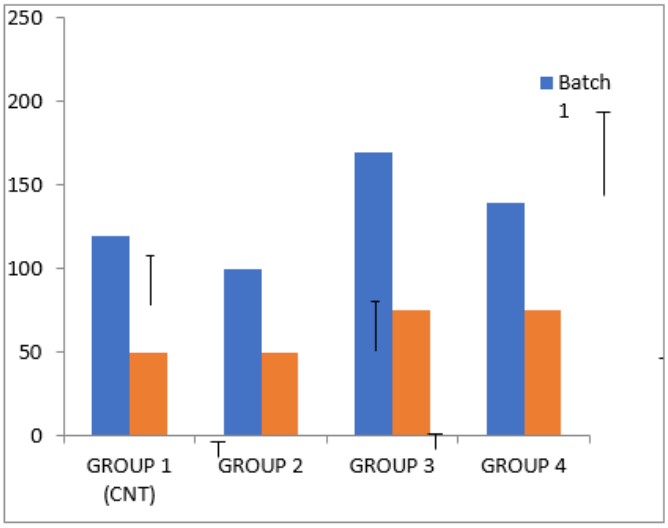

Figure 8.0: Batches 1 and 2 comparison of aspartate aminotransferase concentrations. Values are mean ± SEM, n = 6

***significant from batch 1 at p <0.05**

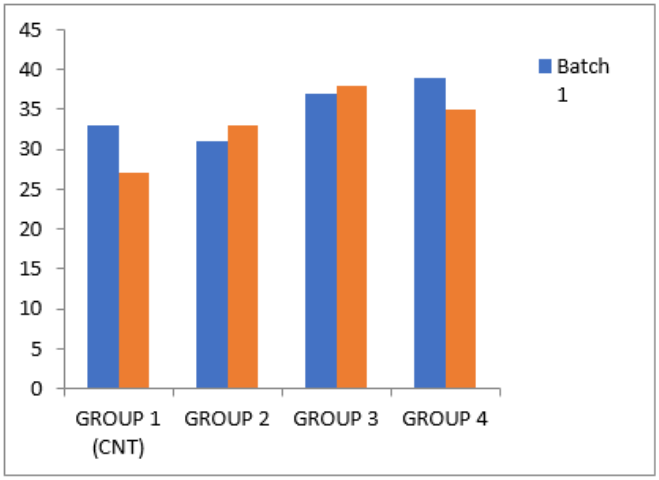

Figure 9.0: Comparison of alkaline phosphatase concentrations between batches 1 and 2 in different groups.

Mean Values ± SEM, n = 6

3.2 Discussion

Herbal preparations given without any standard dosage has raised concerns on their toxic properties. Toxicity studies are used to assess potential health risk in humans using animals. These may be observed in the activity of substances such as enzymes and products of metabolism, normal functioning and histomorphology of the organs. This study assessed the toxicological effects of *Aspilia africana* in acute and sub chronic administration on serum enzyme activities, since it is widely used as a therapeutic.

Different enzymes are often found in significant quantity in the serum not extracellular fluid derived. This is due to tissue damage and disrupted cell membranes that leak enzymes from the tissue and become increased in the serum (4). Hence, enzyme measurement in the serum provides a valuable tool in clinical diagnosis. Decrease in the activities of serum ALP, AST and ALT in the 14days administration as presented in figure (1-3) and in table 4 of the appendix may suggest inactivation of the enzyme molecules. In the 28 days administration in figure (4-6), there were reduction in the activities of both ALP and ALT, but the concentration of AST among the different experimental groups and compare significantly with control ($p<0.05$).

3.3 Conclusion

From the result of this study, *Aspilia Africana* was nontoxic to serum enzyme. Hence, it is concluded that the ethanol leaf extract of ***Aspilia africana*** is safe and can be useful in the management hepatic conditions and other ailments.

3.4 Recommendations

Based on the findings of this study, further advance research can be recommended in this area such as conducting more phytochemical study of this plant to know its content and investigating the underlying mechanisms by which this plant exert its effects.

3.5 Disclaimer (Artificial Intelligence)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (Chat GPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

3.6 Competing Interests

Authors have declared that no competing interests exist.

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