TOXICOLOGICAL ASSESSMENT OF 'ABEERE' SEED *HUNTERIA UMBELLATA* K. SCHUM (APOCYANCEAE)

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ABSTRACT

Thirty age – matched healthy adult male New Zealand white rabbits (2.0 ± 0.5kg BW) were randomly divided into six groups (four treatment and two control groups). The treatment groups were given intraperitoneal injection of either 0.5ml or 1.0ml of water extract of 'abeere' seed or the alcoholic extract (w/v) respectively and examined for 14 days. The effect of the seed extracts on the heamatological parameters, selected enzymes, liver function and body weights of the affected rabbits were analyzed. There was a shift in the leucocyte population towards lymphocytes in the rabbits treated with extracts of 'abeere' seed and a reduction in neutrophylls. An enhancement in the activities of alkaline phosphatase, aspartate transaminase and alanine transaminase in rabbits exposed to 0.5ml of water extract of 'abeere' was observed. There was no significant difference ($P>0.05$) in the histology of major organs and weights of test and control rabbits.
Keywords: *Hunteria umbellata*, seeds, Toxicity studies, Rabbit

INTRODUCTION

Abere is a Yoruba name for Hunteria umbellata seed. The plant grows well in West Africa (SOFOWURA, 1982) and it belongs to the family Apocynaceae. Many genera in the Apocynaceae family have been well studied, especially their chemical composition and economic importance (SOUNDBERG & PORUTIN, 1979). But not much is known about the 'in vivo' toxicity of Hunteria umbellata.

Hunteria umbellata is a medicinal plant of a long-standing use in the treatment of various aliments in Nigeria and Ghana (SOFOWURA, 1982), especially the leaves, roots and bark (ADEGOKE and ALO, 1986). The seed of *Hunteria umbellata* is relatively of less demand for medicinal application because of existing uncertainty about its value and possibly fear of a higher concentration of alkaloids and other toxic materials than the other parts of the plant (ADEGOKE and ALO, 1986). This situation creates the need for toxicological evaluation of the seed of *Hunteria umbellata*. The present study was therefore initiated to determine the systemic impact of *Hunteia umbellata* seed in mammals.

MATERIAL AND METHOD

Hunteria umbellata seeds were harvested from fresh fruit pods obtained from markets in Benin City. The seeds were washed, dried and the coat removed. Ten grammes were macerated in a sterile grinder. The macerate was transferred into 250ml Pyrex flask containing 90ml of either sterile distilled water or ethyl alcohol and allowed to soak for 4 h, for the extraction of the seed. At the end of extraction, the homogenate was filtered through Whatman filter (Number 1), the filtrates were labelled water or alcoholic extracts for subsequent use.

Thirty age matched healthy adult male rabbits, New Zealand white were divided into 6 equal groups (Alcohol and water control groups and 4 treatment groups) for 2 dosage level of 0.5ml and 1.0 of extracts. Two treatment groups were given intraperitoneal injection of 0.5ml and 1.0ml of water extract of 'Abeere' respectively while the remaining two treatment groups were given 0.5ml and 1.0ml of alcoholic extract of abeere respectively. The control groups received 1.0ml sterile distilled water and 1.0ml ethyl alcohol respectively (the vehicles in which the abeere extracts were suspended). The various materials were administered at three days interval for 12 days. Two days after the last treatment, veinous blood was collected from rabbits in the groups through the marginal ear venous into heparinized and non-heparinized plastic tubes for haematologial and biochemical investigations.

The body weights of rabbits were taken during treatment and up to 14 days after with a top-loading weighing balance (5 goat Brand, China).

Fourteen days after the last treatment, the rabbits were sacrificed by anaesthetizing them with ether and

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after laporatomy and evisceration, the heart, the liver, the kidney and testes were removed, weighed and placed in 10% formalin for processing for histopathology.

HEAMATOPATHOLOGY

Blood samples were analyzed for packed cell volume (PCV), haemoglobin level (Hb), total white blood cells count (TWBC) and differential white blood cells count (DWBC) following the methods outlined by DACIE and LEWIS (1975).

BIOCHEMISTRY

Sera obtained from clotted blood samples from rabbits were analyzed for alkaline phosphatase (ALP) (VERLEY, 1967). Aspartate transaminase (AST) and alanine transaminase (ALT) were determined following the methods outlined by ANON (1984a, 1985b).

The total and conjugated bilirubin was determined following the method described by VARLEY (1967).

HISTOLOGY

Serial sections of the formalin fixed organs were cut (5μm thick), fixed on microscope slides, dewaxed and stained with heamatoxylin and eosin (H&E) following the methods outlined by IBEH (1998). The sections were mounted in Canada balsam and examined under light microscopy for studying presence or absence of architectural defects.

STATISTICS

Data obtained were analyzed by one-way analysis of variance (ANOVA) using F – test and T-test to determine the significance of differences in group result and Duncan's multiple range test to locate points of significant differences following the methods outlined by BAILEY (1981).

RESULTS

Table 1 shows the heamatological impact of *Hunteria umbellata* seed extracted on rabbit blood. The mean total white blood cell counts for the rabbit were 4.8 x 10^3 cells for alcoholic extract treated group, 5.1 x 10^3 cells/ml for water extract treated group and 5.1 x 10^3 cells/ml for control rabbit. The mean percentage of lymphocytes was 50.31 for alcoholic extract – treated rabbit, 52.81 for water extract – treated rabbit and 45.64 for control rabbit. The mean percentages of monocytes were 0.25 for alcoholic extract treated rabbit, 3.52 for water extract – treated rabbits and 2.34 for control rabbits.

Table 2 shows the effect of *Hunteria umbellata* seed extracts on enzymes and liver functions of rabbit. The water extract (0.5ml) treatment group of rabbits yielded the highest serum levels of alkaline phosphatase (71.34), aspartate transaminase (70.34) alanine transaminase (57.2 ± 0.7). There was no significant difference ($P>0.05$) in the serum bilirubin of rabbits in all the groups.

Table 3 shows the effect of *Hunteria umbellata* seed extract on the body weight and other physical parameters of rabbit. There was no significant difference in the body weight ($P>0.05$), fur and eye appearance and texture of feaces in all the groups of rabbit. Exposure to extracts of *Hunteria umbellata* seed extracts and their control showed no significant difference in the organ structure ($P>0.05$) in all the groups of rabbit.

DISCUSSION

The impact of extracts of abeere seed (*Hunteria umbellata* seed) on rabbits was determined in this study using short-term investigation protocol. The results in Table 1 suggest that exposure to abeere seed extracts caused changes in some hematological parameters of rabbit. There was significant shift to lymphocytes in the population of white blood cells, which suggests presence of lymphocytosis in the abeere treated rabbits. This result may be due to the immune response of the rabbit to the extract, which led to the mobilization of immune-competent cells. The implication of this finding is that the extracts of abeere were immunogenic, with water extract at a dosage of 1.0ml providing a more effective stimulus than the alcohol extract. This view is supported by the significant increase in monocytes in the 1.0ml water extract-treated group of rabbit, which may be an indication of an increased capacity to produce antibodies by the affected rabbits. This opinion is not at variance with the report of FIDENBERG *et al.,* (1976) concerning the functions of immune-competent cells.

The effect of abeere seed extracts on selected enzymes showed an enhancement in the activities of alkaline phosphates, aspartate transaminase and alanine transaminase in the 0.5ml water extract-treated group of rabbits. This may be positive development if it relates to enhancement of metabolic activities in the effected group of rabbits. This may be the case since there was no significant difference in the liver functions of rabbits in all the groups as suggested by the bilirubin estimation results which otherwise would have pointed to tissue or organ damage as being responsible for the increase in the activities of the three enzymes in the 0.5ml water extract-treated rabbits. Further, a corresponding increase in effect would be expected to occur with an increase in dosage if normal toxicity has been expressed, which was not the case. These views are not at variance with the report of VARLEY (1967) and IBEH (1992) with respect to the functions of enzymes.

Exposure to extracts of abeere seed did not change significantly the body weights of affected rabbit (Table 3), which suggests no adverse effect on metabolic activities of the rabbits treated with the seed extract. Similarly, there was no significant difference in the organ weights and tissue histology of rabbits in both treatment

and control groups (P>0.05). The implications of these results are that the water and alcoholic extracts of abeere at the dosage levels employed in this investigation did not exhibit marked toxicity in the animals and therefore could be regarded as safe doses (approximately 50mg – 100mg/2kg body weight).

The extracts of abeere seed used in this study would contain among other things water and alcohol soluble alkaloids (ADEKOGE and ALAO, 1988), the existence of which may be responsible for the less choice of the seed for medicinal application than the roots, leaves and bark of *Hunteria umbellata*. The results from the present study suggest that the seed may not be any different from the roots, leaves or bark of the plant in terms of toxicity. However, further studies are needed to properly evaluate the toxicity of *Hunteria umbellata* products using long-term study protocol.

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Table 1: Effect of *Hunteria umbellata* seed on the Heamotologic Parameters of Rabbit.

PARAMETER TESTED			*TREATMENT GROUPS*		*CONTROL*	
	TAE (0.5ml)	**TAE (1.0ml)**	**TWE (0.5ml)**	**TWE (1.0ml)**	**Wt. (1.0ml)**	**Alc. (1.0ml)**
Parked Cell Volume (%)	24.61 ± 0.01	29.3 ± 0.02	24.7 ± 0.03	28.4 ± 0.01	21.71 ± 0.03	28.31 ± 0.04
Heamoglobin (g/dl)	8.2 ± 0.04	9.42 ± 0.04	8.12 ± 0.03	7.24 ± 0.02	9.02 ± 0.07	8.13 ± 0.01
Total white blood cell count (cell/ml)	4.9×10^3 ± 1.34*	4.8×10^3 ± 1.14*	5.2×10^3 ± 1.52	5.1×10^3 ± 1.12	5.4×10^3 ± 1.81	5.1×10^3 ± 1.11
Neutophylls (N%)	47.52 ± 0.13	46.14 ± 0.33	44.62 ± 0.69	42.72 ± 0.47	52.38 ± 0.15	51.62 ± 0.70
Lymphocytes (L,%)	50.17 ± 0.04*	50.31 ± 0.12*	54.61 ± 0.41*	52.81 ± 0.26*	44.37 ± 0.84	45.64 ± 0.90
Eosinophyll (E, %)	0.24 ± 0.07	0.14 ± 0.05	0.13 ± 0.14	0.26 ± 0.21	1.55 ± 0.11	1.50 ± 0.37
Monocytes (M,%)	0.23 ± 0.12	0.25 ± 0.09	0.22 ± 0.25	3.52 ± 0.05*	2.51 ± 0.13	2.34 ± 0.04

Values are mean ± S.E, N = 5

* = Location of Significant difference using Duncan's Multiple Range Test.

TAE = Treated with alcohol extract; TWE = Treated with water extract; Wt = Water, Alc = Alcohol

Table 2: Effect of *Hunteria umbellata* seed on some Enzymes and Bilirubin in the blood of Rabbit

PARAMETER TESTED			***TREATMENT GROUPS***		***CONTROL***	
	TAE (0.5ml)	**TAE (1.0ml)**	**TWE (0.5ml)**	**TWE (1.0ml)**	**Wt. (1.0ml)**	**Alc. (1.0ml)**
Alkaline Phosphatase (I.U/1)	56.24 ± 0.24	57.3 ± 0.45	71.34 ± 0.71*	65.72 ± 0.23	58.58 ± 0.35	55.37 ± 0.79
Aspartate Transaminase (I.U/1)	64.72 ± 0.26	60.43 ± 0.21	70.4 ± 0.24*	51.34 ± 0.83	60.17 ± 0.26	61.35 ± 0.14
Alanine Transaminase (I.U/1)	43.31 ± 0.33	46.32 ± 0.51	57.64 ± 0.14*	44.33 ± 0.18	47.42 ± 0.12	48.51 ± 0.61
Total Bilirubin (mg/dl)	1.54 ± 0.01	1.12 ± 0.04	1.43 ± 0.02	1.48 ± 0.07	1.04 ± 0.02	1.17 ± 0.01
Conjugated Bilirubin (mg/dl)	1.08 ± 0.03	0.82 ± 0.02	0.94 ± 0.04	0.91 ± 0.04	0.63 ± 0.02	0.92 ± 0.01

Values are mean ± S.E, N = 5

* = Location of Significant difference using Duncan's Multiple Range Test.

TAE = Treated with alcohol extract; TWE = Treated with water extract; Wt = Water, Alc = Alcohol

Table 3: Effects of *Hunteria umbellata* seed extracts on Body weights/Physical Parameters of Rabbit.

PARAMETER TESTED			***TREATMENT GROUPS***		***CONTROL***	
	TAE (0.5ml)	**TAE (1.0ml)**	**TWE (0.5ml)**	**TWE (1.0ml)**	**Wt. (1.0ml)**	**Alc. (1.0ml)**
Mean Body weight (kg)	1.58 ± 0.07	1.62 ± 0.25	1.56 ± 0.12	1.61 ± 0.31	1.57 ± 0.14	1.47 ± 0.71
Physical appearance	FL	FL	FL	FL	FL	FL
Eye	Sp	Sp	Sp	Sp	Sp	Sp
Feaces	N	N	N	N	N	N

Key: Sp + Sparkling, FL = Full Lustre, N = Normal

TAE = Treated with alcohol extract; TWE = Treated with water extract; Wt = Water, Alc = Alcohol