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Toxicological assessment of *abeere* seed (*Huntaria umbellata* K. Schum)

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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 liquid extract of *abeere* seed or the alcoholic extract (w/v) and examined for 14 days. The effect of the seed extracts on the haematological parameters, selected enzymes, liver function and body weights of the affected rabbits were analysed. There was a shift in the leukocyte 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 liquid extract of *abeere* was observed. There was no significant difference ($p > 0.05$) in the histology of major organs and body weights of test and control rabbits.

INTRODUCTION

Abeere is the Yoruba name for *Huntaria umbellata* seed. The plant grows well in West Africa and it belongs to the family Apocynaceae.[1] Many genera in the Apocynaceae family have been well studied, especially their chemical composition and economic importance.[2] But not much is known about the in vivo toxicity of *Huntaria umbellata*.

Huntaria umbellata is a medicinal plant of long-standing use in the treatment of various ailments in Nigeria and Ghana[1] especially the leaves, roots and bark.[3] There is relatively low demand for its medicinal application because of existing uncertainty about its value and, possibly, the fear of a higher concentration of alkaloids and other toxic materials in the roots and bark than in other parts of the plant.[3] Hence, there is a need for a laboratory analysis of its toxicological properties. The present study was therefore initiated to determine the systemic impact of *Huntaria umbellata* seed in mammals.

KEY WORDS: *Leucocyte, enzyme, seeds, toxicity, rabbit*

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MATERIAL AND METHOD

Huntaria umbellata seeds were collected from fresh fruit pods obtained from markets in Benin City. The seeds were washed, dried and the coat removed. Ten grammes as then macerated in a sterile grinder. The macerate was transferred into a 250ml pyrex flask containing 90ml of either sterile distilled water or 70% ethyl alcohol and allowed to soak for four hours, for the extraction of the juice. After extracting the juice the homogenate was

filtered using Whatman filter paper (Number 1), the filtrates were labelled as either water or alcoholic extracts for subsequent use.

Thirty age-matched healthy adult male rabbits (New Zealand White) were divided into six equal groups (alcohol and water control groups and four treatment groups) for two dosage levels of 0.5ml and 1.0ml extracts. Two treatment groups were given intraperitoneal injection of 0.5ml and 1.0ml liquid extract of *abeere,* while the remaining two treatment groups were given 0.5ml and 1.0ml alcoholic extract of *abeere.* The control groups received 1.0ml sterile distilled water and 1.0ml ethyl alcohol (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, venous blood was collected from rabbits in the groups through the marginal ear veins into heparinized and non-heparinized plastic tubes for haematological and biochemical investigations.

The body weights of the rabbits were taken during treatment and up to 14 days after, with a top-loading weighing balance.

Fourteen days after the last treatment, the rabbits were sacrificed by anaesthetising them with ether, and after laparotomy and evisceration, the heart, liver, kidney and testes were removed, weighed and placed in 10% formalin for processing for histopathology.

Haematopathology

Blood samples were analysed for packed cell volume (PCV), haemoglobin level (Hb), total white blood cells count (TWBC) and differential white blood cells count (DWBC) using accepted methods.[4]

Biochemistry

Sera obtained from clotted blood samples from rabbits were analysed for alkaline phosphatase (ALP).[5] Aspartate transaminase (AST) and alanine transaminase (ALT) were determined following the conventional methods.[6,7] The total and conjugated bilirubin was determined using standard methods.[5]

Histology

Serial sections of the formalin fixed organs were cut (5µm thick), fixed on microscope slides, de-waxed and stained with haematoxylin and eosin (H & E) following the methods outlined.[8] The sections were mounted in Canada balsam and examined under light microscopy for studying the presence or absence of architectural defects.

Statistics

The data were analysed using one-way analysis of variance (ANOVA), and F-test and t-test were used 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.[9]

RESULTS

Table 1 shows the haematological impact of *Huntaria umbellata* seed extracts on rabbit blood. The mean total white blood cell counts for the groups of rabbit were $4.8x10^3$ cells/ml for the group treated with 1.0ml alcoholic extract, $5.1x10^3$ cells/ml for the group treated with 1.0ml water extract, and $5.1x10^3$ cells/ml for the control group. The mean percentage of lymphocytes was 50.31 for rabbits treated with alcoholic extract, 52.81 for rabbits treated with water extract and 45.64 for the control rabbits. The mean percentages of monocytes were 0.25 for alcoholic extract, 3.52 for water extract and 2.34 for the control.

Table 2 shows the effect of *Huntaria umbellata* seed extracts on enzymes and liver functions of the rabbits. Rabbits treated with water extract (0.5ml) yielded the highest serum levels of alkaline phosphatase (71.34), aspartate transaminase (70.34) and 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 1 **Effect of *Huntaria umbellata* seed on the haematological parameters of rabbit**

Parameter tested	Treatment groups					
	TAE (0.5ml)	TAE (1.0ml)	TWE (0.5ml)	TWE (1.0ml)	Control	
					Water (1.0ml)	Alcohol (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
Haemoglobin (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)	$4.9x10^3$±1.34*	$4.8x10^3$±1.14*	$5.2x10^3$±1.52	$5.1x10^3$±1.12	$5.4x10^3$±1.81	$5.1x10^3$±1.11
Neutrophylls (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±0.12	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 alcoholic extract; TWE = treated with water extract.

Table 2 **Effect of *Huntaria umbellata* seed on the biochemistry of rabbit serum**

Parameter tested	Treatment groups					
	TAE (0.5ml)	TAE (1.0ml)	TWE (0.5ml)	TWE (1.0ml)	Control	
					Water (1.0ml)	Alcohol (1.0ml)
Alkaline phosphatase (IU/l)	56.24±0.24	57.31±0.45	71.34±0.71*	65.72±0.23	58.58±0.35	55.37±.079
Aspartate transaminase (IU/l)	64.72±0.26	60.43±0.21	70.34±0.24*	51.34±0.83	60.17±0.26	61.35±0.14
Alanine transaminase (IU/l)	43.31±0.33	46.32±0.51	57.64±0.14*	44.33±0.18	47.42±0.12	48.54±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±0.02	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 alcoholic extract; TWE = Treated with water extract.

Table 3 **Effect of *Huntaria umbellata* seed extracts on body weights/physical parameters of rabbit**

Parameter tested	Treatment groups					
	TAE (0.5ml)	TAE (1.0ml)	TWE (0.5ml)	TWE (1.0ml)	Control	
					Water (1.0ml)	Alcohol (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	F1
Eye	Sp	Sp	Sp	Sp	Sp	Sp
Faeces	N	N	N	N	N	N

Sp = sparkling; Fl = full lustre; N = normal; TAE = treated with alcoholic extract; TWE = treated with water extract.

Table 3 shows the effect of *Huntaria umbellata* seed extracts on the body weight and other physical parameters of the rabbits. There was no significant difference in the body weight ($p > 0.05$), fur and eye appearance and texture of faeces in all the groups of rabbit.

The histological findings in rabbits exposed to extracts of *Huntaria 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 effect of *abeere* seed extracts (*Huntaria umbellata* seed) on rabbits was determined in this study using a short-term investigation protocol. The results in Table 1 suggest that exposure to *abeere* seed extracts caused changes in some haematological parameters of the rabbits. There was a significant shift to lymphocytes in the population of white blood cells, which suggests the presence of lymphocytosis in rabbits treated with *abeere.* This may be due to the immune response of the rabbit to the extract, which led to the mobilisation of immuno-competent cells. The implication of this finding is that the extracts of *abeere* were immunogenic, with water-based extract at a dosage of 1.0ml providing a more effective stimulus than the alcohol-based extract. This is evidenced by the significant increase in monocytes in the group of rabbits treated with 1.0ml water extract, which may be an indication of an increased capacity to produce antibodies by the affected rabbits. This finding is in agreement with a previous report[10] concerning the functions of immuno-competent cells.

The effect of *abeere* seed extracts on selected enzymes showed an enhancement in the activities of alkaline phosphatse, aspartate transaminase and alanine transaminase in the group of rabbits treated with 0.5ml water extract. This may be a positive development if it relates to enhancing metabolic activities in the affected group of rabbits. It is possibly the case as the bilirubin estimation results did not reveal any significant difference in the liver functions of rabbits in all the groups. It would otherwise have pointed to tissue or organ damage as being responsible for the increase in the activities of the three enzymes in rabbits treated with 0.5ml water extract. Furthermore, 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 an earlier report[5,11] on the functions of enzymes.

Exposure to extracts of *abeere* seed did not change significantly the body weights of affected rabbits (Table 3), which suggests no adverse effect on metabolic activities of 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 50-100mg/2kg body weight). The results obtained would not have been different if feeding time had exceeded the 12 days used, as the feeding period still falls within acceptable range of time.

The extracts of *abeere* seed used in this study would contain, among other things, water and alcohol soluble alkaloids,[3] the existence of which may be responsible for the less choice of the seed for medicinal application than the roots, leaves and bark. Results from this study suggest that the seed may not be 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 *Huntaria umbellata* products using long-term study protocol.

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