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HAEMATOLOGICAL EFFECTS OF THE HYDRO-METHANOLIC SEED EXTRACT OF THE *AZANZA GARCKEANA* ON WISTAR RATS

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ABSTRACT
The hydromethanolic extract of the seed of *Azanza-garckeana* (Az-*garckeana*) fruit was evaluated for its effects on some basic haematological parameters. 25 male were divided into 5 groups of 5 rats each: Group 1 received 1ml of distilled water and served as negative control; Groups 2, 3 and 4 received respectively 500, 1500 and 3000mg/kg bw of the extract; Group received 0.2ml/kg bw of Bioferon and served as positive control. After treatments and analysis of blood samples, there was significant ($p<0.05$) changes in haematological parameters: Hb, PCV, mean corpuscular volume, mean corpuscular haemoglobin and neutrophil amongst the various rat groups involved in the study ($p<0.05$); with group 3 rats administered the medium dose of the extract having consistently and significantly lower values of these indices compared to both negative control (Group 1) and positive control (Group 5) rats ($p<0.05$). In conclusion, the results indicated a general reduction in the values of some red and white blood cell indices.

KEYWORDS: *Medicinal plant, Azanza garckeana, haematological profile.*

INTRODUCTION
Azanza garckeana (Az-*garckeana*) commonly known as "snot apple" or "Goron tula" (in Hausa language) is a plant widely used in Nigeria for its medicinal (traditional) and nutritional value and economic potentials. Although the therapeutic potentials of a particular plant could arise from several or any given portion including the leaves, roots, bark, fruit, seeds and flowers.[1] Various portions of the Az-garckeana plant have also been reported to possess therapeutic attributes.[2,3,4]

However the most explored and reported part of the Az-garckeana plant are its fruit pulp. For instance, the fruit pulp of Az-garckeana has been described to possess aphrodiasiac, fertility enhancing immune stimulating and antioxidant properties.[5,6] Additionally a few of the potential benefits attributable to the fruit pulp include anti-inflammatory antidiabetic analgesic, antimicrobial, nutritional and properties.[2,3,7,8,9,10] Despite these studies, information on the biological effects of the seed portion of the plant are scanty, particularly its potential effects on the formed elements of the blood.[11,12,13]

In evaluating the effects of medicinal agents, an assessment of their potential effects on haematological indices using biological models is invaluable.[14] This is because the status of haematological indices is reliable in evaluating the beneficial or toxic effects induced by therapeutic agents under screening.[15,16]

Therefore, the present study aims at an evaluation of the potential effects of the extract on haematological parameters using male Wistar rats as experimental models. This is with the view of further exploring the potential beneficial effects on the haematological system of a now widely and popularly consumed fruit among Nigerians.

MATERIALS AND METHODS
Plant materials collection and preparation of extract
Plant materials collection and preparation of extract from fresh fruits of Az-garckeana were harvested from Tula town in Kaltungo local council area of Gombe State, Nigeria was used for the present study. A sample of the plant was deposited as a voucher at the University of Port Harcourt Herbarium. The plant and fruits were identified and authenticated by Dr M. Suleiman of the

department of Pharmacognosy and Phytotherapy, faculty of Pharmacy, University of Port Harcourt, Nigeria and assigned with a herbarium number, UPHM0596.

The pulps of the fruits of *the A. garckeana* pant were carefully removed and the seeds were air dried for two weeks after which they were pulverized into fine powder using a motorized blender. The powdered sample obtained was then soaked in hydro-methanol solvent (20:80 v/v). The solution obtained was periodically stirred to ensure a uniform mixture. After 72 hours, the solution was filtered with Whatman filter paper (size 4) and then concentrated using a rotary evaporator followed by water bathing at 50˚C. The recovered gelatinous-like extract was then refrigerated at about -4˚C before use.

Study Animals and Determination of Lethal dose

Twenty five (25) male Wistar rats weighing between 160 and 200gm were obtained and kept in the animal house, faculty of basic medical sciences, university of port Harcourt, Nigeria. They were maintained under 12-hour light/dark cycle at room temperature. All the animals were treated according to guidelines for the care and use of experimental animals.[17] The LD_{50} of the extract was determined using the Lorke's Method.[18]

Drugs: all drugs, reagents and chemicals used in the present study were analytical grade and obtained from authorized dealers (mainly Sigma Aldrich).

Study Protocol

After 14 days of acclimatization, the Wistar rats were randomly assigned into 5 groups of 5 rats each and were subsequently treated as follows for 30 days during which all the animals were allowed free access to tap water and rat feed *ad libitum.*

Group 1: Negative control; received only 1ml of distilled water daily.

Group 2: Low dose of Az-garckeana group; received 1ml of 500mg/kg bw of the hydro- methanol extract of the seed of Az-garckeana.

Group 3: Medium dose of Az-garckeana group; received 1ml of 1500mg/kg bw of the hydro-methanol seed extract of the seed of Az-garckeana.

Group 4: High dose of Az-garckeana group; received 1ml of 3000mg/kg bw of the hydro-methanol seed extract of the seed of Az-garckeana.

Group 5: Positive control; received 0.23ml/kg bw of bioferon.

Determination of Haematological parameters

At the end of the study period, 5ml of blood was collected from the experimental animals via direct cardiac puncture. The blood was immediately transferred into appropriate sample bottles and properly labelled. Determination of haematological parameters was done using automated haematological analyzer (URIT-2900Vet Plus - Auto Hematology Analyzer). The following haematological parameters were subsequently determined: red blood cell count, haemoglobin concentration, the corpuscular indices and platelet count; also determined were total white and differential white blood cell counts. Total lymphocyte count was calculated as a product of the total white blood cell count and percentage lymphocyte count.

Ethical approval

The ethical approval was obtained from our insttuitional research ethics committee vide reference number: UPH/R&D/REC/EXEC/085.

Statistical Analysis

Data obtained was subjected to statistical analysis using SPSS version 21.0. Statistical significance was determined using one-way analysis of variance (ANOVA) followed by Post-Hoc multiple comparison test. A P-value less than 0.05 was considered significant. Results are presented in Tables 1, 2 and 3.

RESULTS

Table 1: Acute toxicity study of hydromethanolic extract of the seed of *Az-garckeana* administered orally in male wistar rats.

Doses (mg/kg)	Number of rats per group	Deaths within 24 Hours
250	4	0
500	4	0
1000	4	0
2000	4	0
4000	4	1

Table 1 shows the acute toxicity study of the extract administered orally in male wistar rats The LD_{50} of the hydro-methanolic extract of the seed of Az-garckeana was found to >4000mg/kg.

Table 2 below shows effects of hydromethanol extract of the seed of *Az-garckeana* on red cell count, haemoglobin concentration, packed cell volume, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration and platelet count.

Table 2: Effect of hydromethanol seed extract of *Azanza garckkeana* (HSEA) administration on some red blood cells indices and platelet count.

Parameters	Group 1: Control	Group 2: 500 mg/kg bw HSEA	Group 3: 1500mg/kgbw HSEA	Group 4: 3000mg/kg HSEA	Group 5: Bioferon	Significant differences
Red blood cell count (RBC) ($X10^{12}$/L)	6.32 ± 0.29	6.32 ± 0.24	5.92 ± 0.37	6.65 ± 0.57	7.07 ± 0.23 [c]	p>0.05
Haemoglobin concentration (Hb) (g/dL)	12.47 ± 0.89	13.10 ± 0.59	10.50 ± 0.92 [b]	12.65± 1.19	13.40 ± 0.34 [c]	p<0.05
Packed Cell Volume (PCV) (%)	38.50 ± 2.98	40.75 ± 2.17	28.75 ± 5.28 [b]	39.50 ± 3.92 [c]	41.00 ± 1.08 [c]	p<0.05
Mean Corpuscular Volume (MCV) (fL)	61.17 ± 2.56	65.12 ± 3.10	55.70 ± 2.41 [b]	59.60 ± 1.64	58.62 ± 1.34	p<0.05
Mean Corpuscular Haemoglobin (MCH) (pg/cell)	19.57 ± 0.82	20.57 ± 0.44	16.77 ± 0.79 [a, b]	18.92 ± 0.45 [c]	18.90 ± 0.30 [c]	p<0.05
Mean Corpuscular Haemoglobin Concentration (MCHC) (g/dL)	32.12 ± 0.31	32.52 ± 1.69	32.72 ± 0.42	31.85 ± 0.33	32.37 ± 0.31	p>0.05
Platelet count ($X10^{9}$/L)	514.50 ± 61.22	498.75 ± 90.38	493.00 ± 90.93	489.75 ± 77.61	637.75 ± 45.01	p>0.05

Values represent mean ± SEM, n=5; [a] Significant at p<0.05 when compared to control; [b] Significant at p<0.05 when compared to group 2; [c] Significant at p<0.05 when compared to group 3; [d] Significant at p<0.05 when compared to group 4.

Significant variations were only found in the values of the following haematological parameters: haemoglobin concentration, packed cell volume, mean corpuscular volume and mean corpuscular haemoglobin amongst the various rat groups involved in the present study (p<0.05). noteworthy, the values of each parameter amongst group3 rats administered the medium dose of the extract being consistently and significantly of the lowest value compared to both the negative control (group 1) rats and the positive control (group 5) rats (p<0.05). furthermore, the values of all four haematological parameters were found to be marginaly higher amongst group 2 rats administered with low doses of the hydromethanol seed extract of Az-garckeana as compared to both group 3 and group 4 rats administered medium and high doses of the hydromethanolic seed extract of Az-garckeana respectively.

Non-significant differences were observed in the mean values of red blood cell count amongst the different rat groups treated with hydromethanol extract of the seed of Az-garckeana (groups 2, 3 and 4) compared to negative control (group 1) rats (p>0.05). However the value of the red blood cell count for the bioferon treated (group 5) rats was found to be significantly higher than those of all the rat groups treated with Az-garckeana and amongst the negative control (group 1) rats (p<0.05). Although, the highest of value red blood cell count was found amongst group 4 rats administered the highest dose of the extract. The values of both the mean corpuscular haemoglobin concentration (MCHC) and platelet count showed a similar non-significant variation amongst the various rat groups under investigation (p>0.05).

Table 3: Effect of hydromethanol seed extract of *AzG* (HSEA) on total and differential white blood cell and total lymphocyte counts.

Parameters	Group 1: Control	Group 2: 500 mg/kg bw HSEA	Group 3: 1500mg/kgbw HSEA	Group 4: 3000mg/kg HSEA	Group 5: Bioferon	Significant differences
White blood cell Count (WBC) ($X10^{9}$/L)	14.82 ± 2.51	17.70 ± 2.67	12.07 ± 1.57	15.15 ± 2.83	14.90 ± 2.14	p>0.05
Neutrophils (%)	8.75 ± 0.75	6.25 ± 1.43	5.25 ± 0.94 [a]	6.75 ± 0.85	7.75 ± 1.03	P<0.05

Lymphocyte (%)	84.25 ± 4.59	86.75 ± 4.55	88.50 ± 2.06	88.00 ± 2.55	87.25 ± 3.70	p>0.05
Oesinophils (%)	1.50 ± 0.29	1.00 ± 0.00	1.00 ± 0.00	1.25 ± 0.25	1.25 ± 0.25	p>0.05
Monocytes (%)	4.75 ± 1.37	4.75 ± 1.25	4.5 ± 1.19	4.75 ± 1.11	5.00 ± 1.58	p>0.05
Total Lymphocyte Count (µL) (TLC)	12.76 ± 2.68	14.99 ± 1.44	10.71 ± 1.50	13.18 ± 2.14	13.08 ±2.07	p>0.05

Values represent mean ± SEM, n=5; [a] Significant at p<0.05 when compared to control; [b] Significant at p<0.05 when compared to group 2; [c] Significant at p<0.05 when compared to group 3; [d] Significant at p<0.05 when compared to group 4.

Table 3 above shows the effect of hydromethanol extract of the seed Az-garckeana on total white blood cell count, differential white blood cell count and total lymphocyte count. Significant variations were only observed for differential neutrophil counts amongst the various rat groups involved in the present study.

A pattern essentially similar to that seen for the red blood cell indices was also observed for white blood cell indices. For instance group 3 rats who were administered medium doses of the hydromethanol extract of the seed Az-garckeana were observed to have consistently lower values of total white blood cell count, percentage neutrophils, percentage eosinophils, percentage monocytes and subsequently total lymphocyte counts compared to all the other rat groups. However, significant differences were only observed for percentage neutrophils counts, group 3 rats were found to have significantly lower values of percentage neutrophil compared to group 2 rats administered with low doses of the hydromethanol extract of the seed Az-garckeana (p<0,05).

DISCUSSION

Plants can have medicinal qualities derived from its different parts and it is such that within a given plant the varied portions could contain distinct active ingredients thus making it possible for a plant to have toxic parts and yet harmless or therapeutic parts.[4] Considering such possibility of variability in therapeutic potency of medicinal plants, the therapeutic profiling of such plants should holistically evaluate the varied portions of the plant.[19] Thus, in the present study an uncommon portion of the A. garckeana plant, the seed, has been evaluated of its possible influence on the haematological system of a mammalian model and main findings are so discussed in the subsequent paragraphs.

The outcome of the present study indicated a non-significant effect of the different doses of the HSEAG on the RBC, Hb and PCV with respect to its untreated groups. Although there were generally no depressive effects of the HSEAG on the said parameters, but the bioferon (a synthetic drug with haematinic effect due to the iron-containing complex).[20,21] Treated group indicated improved levels of the RBC, Hb, PCV and MCH values. Remarkably, it was seen that the group treated with 1500mg/kg bw HSEAG has marked decreases in the aforementioned parameters when compared to those of the bioferon treated group. The above outcome of the present study is consistent with the earlier report of Ahmed et al.[22], that stated how the A. garckeana extract did not exhibit significantly different levels of PCV, WBC, MCH, or kidney function indices from the control group. While this may imply that the fruit of HSEAG may be safe for the erythrocyte population of the haematological system of the study model, a higher dose (like beyond the 1500mg/kg dose) may exert possible undesirable effects on the system. This outcome is different from the findings on Kolawole et al.[23] and Charles et al.[24], who separately worked on effects of extract Citrullus lanatus (watwermelon) and fleurya aestuans respectively and submitted clear cut haematinic effects of their plants. It is therefore suggestive to state here that the 500, 1500 and 3000mg/kg of HSEAG may not elicit haematinic effect and caution must be taken when treating with higher doses as it may be counterproductive.

The finding of the present study on the effect of the different doses of HSEAG revealed a marginally depressive potency on the platelet population of the haematological system of the study models. This was magnified by the platelet level elevated in the bioferon treated group. The above outcome is variant with the earlier submissions of Iyojo et al.[25], and Ibrahim et al.[26], who separately worked on the pulp and leave extract of Az-garckeana respectively and reported marked elevations in leucocyte and platelet population in similar mammalian models. And these outcomes wee likened to the antioxidant properties of the different portions of the plant. Juxtaposing this notion to the finding of the present study, it is deducible that such attribute may be lacking in the seed portion of the plant and higher level of other active ingredients may be responsible for this platelet depressive tendency. It also implies that on further confirmation of this attribute by the seed portion in the present study, it may be a portent candidate for managing thrombocytosis conditions.

Again, the present study found only marginal changes in the levels of total WBC and WBC differentials. But remarkable was the depressive tendency of the 1500mg/kg of the HSAE on the aforementioned parameters. Similar to the outcome on platelet level of the present study, these effects on WBC and its differentials are opposite the findings of previous investigations using other parts of the plant.[25,26] Another note of caution from the above finding of the present study is that total WBC, neutrophil, monocyte and total lymphocyte levels were much more reduced in the 1500mg/kg HSEAG treated group. It thus, validates the

previous assertion of the present study that higher doses of the HSEAG may not enhance haematological profile but could rather exert depressive tendency as seen with these parameters.

CONCLUSION

In conclusion, the present study has found that at lower doses (500mg/kg), the hydromethanol seed extract of Az-garckeana (HSEAG) may not exert any toxic effect on the haematologic profile of the study model, but at higher dose (particularly 1500mg/kg and possibly above) may depress the levels of most of the haematological parameters (including RBC, Hb, PCV, MCH, platelets, total WBC, neutrophil, monocytes and total lymphocytes). While this may be different from the result of previous studies but indicates that the higher doses of HSEAG may be potent candidates for managing disorders that elevate the aforementioned haematological parameters. Consequently, further studies may be needed to specifically isolate the active compounds and elucidate their possible mechanisms that may be responsible for such attributes as seen recorded in the present study.

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