



Effects of Aqueous and Ethanol Corm Extracts of *Zygotritonia crocea* on Haematological Indices and Serum Electrolyte Levels in Wistar Rats

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SUMMARY

Objectives: To study the effects of both aqueous and ethanol corm extracts of *Zygotritonia crocea* on haematological indices and serum electrolyte levels in wistar rats.

Methods: Forty-two adult female rats were grouped into six (A1, A2, A3, B1, B2 and B3) of seven rats per group. Groups A1 and B1 served as control for aqueous and ethanol extracts respectively. A2 and A3 were treated with 100 and 200 mg/kg body weight aqueous corm extract respectively for twenty-one days while B2 and B3 were treated with 100 and 200 mg/kg body weight ethanol corm extract respectively for twenty-one days.

Results: Both extracts significantly increase ($p < 0.05$) the mean Hb, PCV, RBC, and MCV values. MCH and Neutrophils values were also increased. Platelets value was increased in ethanol extract treated rats but decreased in aqueous treated rats. Both extracts increased mean WBC value in rats treated with 200 mg/kg body weight extract while percentage lymphocytes was reduced. Ethanol extract significantly reduced ($p < 0.05$) K^+ level while it was increased with 200 mg/kg weight aqueous extract. The extracts have non-significant ($p > 0.05$) effect on Na^+ , Cl^- and HCO_3^- levels.

Conclusion: The results therefore show that the extracts of the plant, *Z. crocea* are capable of stimulating erythropoiesis while it may create some level of electrolytes imbalance.

INTRODUCTION

In most parts of Nigeria, traditional medicine has been noted to be vital in preventing and curing various diseases, thereby playing an important role in the health services of the areas especially among the low socio-economic class. Herbs are normally administered orally or can be applied onto the skin surface as ointment. Various herbalists and researchers have led credence to the facts that many plants are actually medicinal in nature and therefore, can be used as antimicrobial and antifungal, [1-2] antidiabetic and antihyperlipidemic [3-5] while some have been reported to have haematological effects. [6-8]

Zygotritonia crocea is a member of Iridaceae family, known in the Yoruba part of Nigeria as "Baka" and the corm as "Isu baka". [9-10] The plants in this family are perennial herbs with underground storage organs of rhizomes, bulbs, or corms. [10] They have adventitious roots and sword-shaped leaves that overlap each other transversely at the base. [11] *Zygotritonia crocea* is prominent tradomedically for the treatment of intestinal disorder particularly those associated with typhoid, diarrhoea, dysentery and also as components of traditional anti-tuberculosis recipes. [12] There are few published works so far on the plant and the only one known is a report on the antimicrobial potential of the plant, that it exhibited a significant anti-fungal activity using the corms

methanolic extract of the plant. [13]

Zygotritonia crocea contains alkaloids [13] which is of great importance pharmacologically. This study is designed to evaluate the possible effect of both aqueous and ethanol corm extracts of the medicinal plant on some haematological and electrolytes parameters.

MATERIALS AND METHODS

Animal

Forty-two female albino rats of the wistar strain weighing between 160-215g were bought from the Animal House of the College of Veterinary Medicine (COLVET), University of Agriculture (UNAAB), Abeokuta, Ogun State, Nigeria. The animals were kept in standard cages to acclimatize for 14 days under standard temperature and relative humidity of $27 \pm 1^\circ C$ and $54 \pm 2\%$ respectively. They were fed freely on normal feed pellets obtained from Ola Feed Mills, Asero, Abeokuta, Ogun State, Nigeria and water *ad libitum*.

Plant material

Fresh corms of *Zygotritonia crocea* were collected from the local herbs dealers in the month of May, 2008 at Obantoko Market, Abeokuta, Ogun State, Nigeria and were authenticated at the Forest Research Institute of Nigeria

(FRIN), Ibadan, Nigeria where an herbarium specimen was deposited.

Preparation of aqueous and ethanol extracts

Two separate 200g (A and B) of fresh corm were peeled, sliced, washed and oven dried at 60°C. 50 g each of dried samples A and B was grounded to powder. 250ml boiled distilled water was added to sample A and stirred while sample B was macerated in 250ml ethanol. They were allowed to stand for 3 days. The decanted filtrates of sample A and sample B were oven dried at 60°C and 40°C respectively. Sample extracts were reconstituted in normal saline to give two final concentrations of 100 mg/ml and 200 mg/ml each.

Animal grouping and extracts administration

The 42 rats were randomly grouped into six groups (A1, A2, A3 and B1, B2, B3) of 7 rats per group. Groups A1 and B1 (controls) received 0.3 ml normal saline orally. Groups A2 and A3 received 100 and 200mg/kg body weight aqueous extract respectively per day for 21 days. Groups B2 and B3 received 100 and 200 mg/kg body weight ethanol extract respectively per day for 21 days.

Sample collection

At the end of 21 days period of extracts administration, the rats were sacrificed under slight ether anaesthesia. Blood samples were collected from each rat through heart puncture into clean lithium heparinized bottles for haematological analysis and determination of electrolyte levels.

Determination of haematological parameters and electrolyte levels

Haemoglobin (Hb) concentration was determined by cyano-methaemoglobin method,[14] Packed Cell Volume (PCV) was determined using Haematocrit method as described by Dacie and Lewis (1991).[15] The Red Blood Cell (RBC), total White Blood Cell (WBC), White Blood Cell differentials and the platelet counts were estimated using the improved Neubauer chamber.[14-15] About 3ml of the blood collected was also used for the determination of electrolyte levels.

Statistical analysis

Results are reported as mean \pm standard deviation (SD). Statistical analysis was carried out using 2-way analysis of variance (ANOVA). Statistical significance was considered at $p < 0.05$.

RESULTS

The effects of treatment of aqueous and ethanol corm extracts of *Z. crocea* at 100 and 200 mg/kg body weight on the haematological parameters of female albino rats for 21 days are shown in Tables 1 and 2 respectively. There was a significant ($p < 0.05$) increase in the mean values of Hb of rats treated with 100 and 200 mg/kg body weight aqueous extract (10.15 \pm 0.61g/dL and 12.57 \pm 3.18g/dL) respectively compared with control (6.71 \pm 2.61g/dL) (Table 1) and rats treated with 100 and 200 mg/kg body weight ethanol extract (9.70 \pm 3.03g/dL and 10.63 \pm 3.50 g/dL) respectively compared with control (6.71 \pm 2.25 g/dL) (Table 2).

The percentage (%) of PCV was significantly increased ($p < 0.05$) in rats treated with 100 and 200 mg/kg

body weight aqueous extract (30.50 \pm 1.73% and 28.67 \pm 0.91%) respectively compared with control (20.19 \pm 2.15%) (Table 1) and rats treated with ethanol extract 100 and 200 mg/kg body weight (29.14 \pm 4.03% and 33.01 \pm 5.12%) respectively compared with control (20.14 \pm 3.05 %) (Table 2).

There was a significant ($p < 0.05$) increase in the mean total RBC of rats treated with aqueous extract 100 and 200mg/kg body weight (3.55 \pm 0.17 x 10¹²/L and 3.36 \pm 0.96 x 10¹²/L) respectively compared with control (2.51 \pm 0.78 x 10¹²/L) (Table 1) and rats treated with ethanol extract 100 and 200 mg/kg body weight (3.41 \pm 0.90 x 10¹²/L and 3.80 \pm 1.03 x 10¹²/L) respectively compared with control (2.51 \pm 0.78 x 10¹²/L) (Table 2).

There was a significant ($p < 0.05$) increase observed in the MCV of rats treated with aqueous extract 100 and 200mg/kg body weight (85.77 \pm 1.94 fl and 84.33 \pm 4.33 fl) respectively compared with control (78.56 \pm 3.25 fl) (Table 1) and rats treated with ethanol extract 100 and 200 mg/kg body weight (83.67 \pm 5.57 fl and 85.75 \pm 5.02 fl) compared with control (78.56 \pm 4.25 fl) (Table 2).

The MCH increased non-significantly ($p > 0.05$) in rats treated with aqueous extract 100 and 200 mg/kg body weight (28.57 \pm 0.29 pg and 28.13 \pm 1.35 pg) respectively compared with control (26.17 \pm 2.09 pg) (Table 1) and rats treated with ethanol extract 100 and 200 mg/kg body weight (27.84 \pm 1.62 pg and 28.50 \pm 1.73 pg) respectively compared with control (26.17 \pm 2.09 pg) (Table 2).

The values of the MCHC in rats treated with aqueous extract 100 and 200 mg/kg body weight (33.25 \pm 0.11 g/dL and 33.37 \pm 0.11 g/dL) respectively is the same with that of the control (33.54 \pm 0.57 g/dL) (Table 1). The MCHC was non-significantly increased ($p > 0.5$) in the rats treated with ethanol extract 100 mg/kg body weight (34.60 \pm 2.61g/dL) and non-significantly decreased ($p > 0.05$) in rats treated with ethanol extract 200 mg/kg body weight (32.22 \pm 0.08 g/dL) compared with the control (33.54 \pm 0.57 g/dL) (Table 2).

There was a non-significant ($p > 0.05$) decrease in WBC value of rats treated with 100 mg/kg body weight aqueous extract (1.61 \pm 0.76 x 10⁶/L) while it increased significantly ($p < 0.05$) in rats treated with 200 mg/kg body weight aqueous extract (2.97 \pm 1.65 x 10⁶/L) compared with the control (1.75 \pm 0.78 x 10⁶/L) (Table 1). The WBC values in the rats treated with the ethanol extract was observed to significantly decreased ($p < 0.05$) in rats treated with 100 mg/kg body weight (1.16 \pm 0.66 x 10⁶/L) but significantly increased ($p < 0.05$) in rats treated with 200 mg/kg body weight (2.73 \pm 0.58 x 10⁶/L) compared with the control (1.76 \pm 0.78 x 10⁶/L) (Table 2).

The neutrophils values in rats treated with aqueous extract was significantly ($p < 0.05$) higher in rats treated with both 100 and 200 mg/kg body weight (16.25 \pm 1.20 % and 21.67 \pm 2.10 %) than the untreated control (13.57 \pm 1.60 %) (Table 1) while in the rats treated with ethanol extract, neutrophils was non-significantly ($p > .05$) higher in 100 mg/kg body weight (14.29 \pm 0.47%) treated rats but significantly ($p < 0.05$) higher in 200 mg/kg body weight (21.50 \pm 1.18 %) treated rats than the control (13.57 \pm 1.23 %) (Table 2).

The lymphocytes values in rats treated with aqueous extract was significantly ($p < 0.05$) lower in both 100 and 200mg/kg body weight rats (83.75 \pm 4.79 % and

Table 1: Changes in the haematological parameters of rats treated with aqueous corm extract of *Zygotritonia crocea* for 21 days

Haematological Parameters	Control	100mg/kg body weight	200mg/kg body weight
Hb (g/dL)	6.71±2.61	10.15±0.61 ^a	12.57±3.18 ^a
PCV (%)	20.19±2.15	30.50±1.73 ^a	28.67±0.91 ^a
RBC (x 10 ¹² /L)	2.51±0.78	3.55±0.17 ^a	3.36±0.96 ^a
MCV (fl)	78.56±3.25	85.77±1.94 ^a	84.33±4.33 ^a
MCH (pg)	26.17±2.09	28.57±0.29 ^c	28.13±1.35 ^c
MCHC (g/dL)	33.54±0.57	33.25±0.11	33.37±0.11
WBC (x 10 ⁶ /L)	1.75±0.78	1.61±0.76	2.97±1.65 ^a
Neutrophils (%)	13.57±1.56	16.25±1.20 ^a	21.67±2.10 ^a
Lymphocytes (%)	86.42±5.56	83.75±4.79 ^b	78.33±3.50 ^b
Platelets (x 10 ⁹ /L)	231.50±7.39	231.50±7.38	219.10±8.52 ^b

Values are mean ± SD (n=7). Values with ^{a, b} and ^c represent those that increased significantly, decreased significantly and increased but non-significantly respectively at p<0.05 compared to the control rats.

Table 2: Changes in the haematological parameters of rats treated with ethanolic corm extract of *Zygotritonia crocea* for 21 days

Haematological Parameters	Control	100mg/kg body weight	200mg/kg body weight
Hb (g/dL)	6.71±2.25	9.70±3.02 ^a	10.63±3.50 ^a
PCV (%)	20.14±3.05	29.14±4.03 ^a	33.01±5.12 ^a
RBC (x 10 ¹² /L)	2.51±0.78	3.41±0.90 ^a	3.80±1.03 ^a
MCV (fl)	78.56±4.25	83.67±5.57 ^a	85.75±5.02 ^a
MCH (pg)	26.17±2.09	27.84±1.62 ^c	28.50±1.73 ^c
MCHC (g/dL)	33.54±0.57	34.60±2.61 ^c	32.22±0.08 ^d
WBC (x 10 ⁶ /L)	1.76±0.78	1.16±0.66 ^b	2.73±0.58 ^a
Neutrophils (%)	13.57±1.23	14.29±0.47 ^c	21.50±1.18 ^a
Lymphocytes (%)	86.43±5.50	85.71±6.73 ^d	78.00±7.07 ^b
Platelets (x 10 ⁹ /L)	163.50±11.11	221.93±9.67 ^a	247.50±11.13 ^a

Values are mean ± SD (n=7). Values with ^{a, b} and ^{c, d} represent those that increased significantly, decreased significantly, increased but non-significantly and decreased but non-significantly respectively at p<0.05 compared to the control rats

Table 3: Changes in the electrolyte levels of rats treated with aqueous corm extract *Zygotritonia crocea* for 21 days

Electrolyte levels	Control	100mg/kg body weight	200mg/kg body weight
Na ⁺ (mmol/L)	139.01±2.79	140.05±1.29	142.00±2.65
K ⁺ (mmol/L)	5.24±0.98	5.22±0.29	6.27±0.83 ^a
Cl ⁻ (mmol/L)	99.42±3.40	99.75±3.30	104.00±1.00 ^a
HCO ₃ ⁻ (mmol/L)	23.14±2.67	23.00±1.72	25.67±1.53

Values are mean ± SD (n=7). Values with ^a represent those that increased significantly at p<0.05 compared with the control rats.

78.33±3.50%) respectively than the untreated control (86.42±5.56 %) (Table 1). In the rats treated with ethanol extract, the lymphocytes was non-significantly (p>0.05) lower in 100 mg/kg body weight treated rats (85.71±6.73 %) but significantly (p<0.05) lower in 200 mg/kg body weight treated rats (78.00±7.07 %) than the control (86.43±5.50 %) (Table 2).

The platelets values was significantly (p<0.05) lower in 200 mg/kg body weight aqueous extract treated rats (219.10±8.52 x 10⁹/L) while the rats treated with 100 mg/kg body weight aqueous extract has no effect on platelets values (231.50±7.38 x 10⁹/L) compared with the control (231.50±7.39 x 10⁹/L) (Table 1). In the rats treated with

ethanol extract, the platelets values was significantly (p<0.05) higher in rats treated with both 100 and 200 mg/kg body weight extract (221.93±9.67 x 10⁹/L and 247.50±11.13 x 10⁹/L) respectively than the control (163.50±11.11 x 10⁹/L) (Table 2). The effects of treatment of aqueous and ethanol corm extracts of *Z. crocea* at 100 and 200 mg/kg body weight on the electrolyte levels of female albino rats for 21 days are shown in Tables 3 and 4 respectively.

The mean serum Na⁺ level of the control (139.01±2.79 mmol/L) in aqueous extract treated rats was lower than the rats treated with 100 and 200 mg/kg body weight aqueous extract (140.05±1.29 mmol/L and 142.00±2.65 mmol/L) respectively (Table 3). In rats treated with ethanol extract, the

control (139.10±2.79 mmol/L) was higher than rats treated with 100 mg/kg body weight (137.57±1.51 mmol/L) but lower than the rats treated with 200 mg/kg body weight (139.89±1.72 mmol/L) (Table 4). The differences observed are non-significant at ($p>0.05$). There was non-significant difference ($p>0.05$) between the mean serum K^+ level of control (5.24±0.98 mmol/L) and rats treated with 100 mg/kg body weight aqueous extract (5.22±0.29 mmol/L) but significantly ($p<0.05$) higher in the rats treated with 200 mg/kg body weight aqueous extract (6.27±0.83 mmol/L) (Table 3). However, a significant ($p<0.05$) lower level was observed in rats treated with 100 and 200 mg/kg body weight ethanol extract (5.25±0.34 mmol/L and 5.33±0.28 mmol/L) respectively than the control (10.36±0.98 mmol/L) (Table 4).

There was non-significant difference in the mean serum Cl^- level of control (99.42±3.40 mmol/L) and rats treated with 100 mg/kg body weight aqueous extract (99.75±3.30 mmol/L) but a significant increase ($p<0.05$) was noticed in rats treated with 200 mg/kg body weight aqueous extract (104.00±1.00 mmol/L) (Table 3). However, the mean serum Cl^- level in ethanol extract treated rats was non-significantly ($p>0.05$) higher in 200mg/kg treated group (102.00±2.61 mmol/L) than 100mg/kg treated group (99.29±2.43 mmol/L) and control (99.43±3.41 mmol/L) (Table 4).

The mean serum HCO_3^- level in aqueous extract treated rats was non-significantly ($p>0.05$) higher in 200mg/kg treated group (25.67±1.53 mmol/L) than 100 mg/kg treated group (23.00±1.73) and control (23.14±2.67) (Table 3). However, the mean serum HCO_3^- level in ethanol extract treated rats was non-significantly ($p>0.05$) higher in control (23.14±2.67 mmol/L) than 100mg/kg treated group (21.57±1.13 mmol/L) and 200 mg/kg treated group (22.00±1.67 mmol/L) (Table 4).

Table 4: Changes in the electrolyte levels of rats treated with ethanol corm extract *Zygotritonia crocea* for 21 days

Electrolyte levels	Control	100mg/kg body weight	200mg/kg body weight
Na^+ (mmol/L)	139.10±2.79	137.57±1.51	139.89±1.72
K^+ (mmol/L)	10.36±0.98	5.25±0.34 ^a	5.33±0.28 ^a
Cl^- (mmol/L)	99.43±3.41	99.29±2.43	102.00±2.61
HCO_3^- (mmol/L)	23.14±2.67	21.57±1.13	22.00±1.67

Values are mean ± SD (n=7). Values with ^a represent those that increased significantly at $p<0.05$ compared with the control rats.

DISCUSSION

The administration of plant extracts and chemical compounds can lead to significant alterations of haematological parameters and its assessment can be used to determine the extent of deleterious effect of these foreign compounds on blood. The health of an individual is affected by different diseased conditions, foreign bodies, drugs and drug products that get to the blood and other tissues.[16]

Administration of aqueous and ethanol extracts of *Zygotritonia crocea* to rats at two different doses of 100 and 200mg/kg body weight for 21 days produced an increase in

the mean values of Hb, PCV, RBC, MCV, MCH and Neutrophils in both extracts. Elevation of Platelets was recorded in rats treated with ethanol extract only which seems to be dose-dependent. Elevation of WBC was recorded in rats treated with 200mg/kg body weight in both extracts while it was reduced in those treated with 100 mg/kg body weight. There is reduction in the values of lymphocytes in all treated rats.

The significant increase of Hb, PCV and RBC is an indication that the extracts stimulate the production of RBC (erythropoiesis). This further implies that the extracts increased the oxygen-carrying capacity of the blood and oxygen delivered to the tissues since Hb and RBC are very important for respiratory gases transfer.[17] MCV, MCH and MCHC values are important in diagnosis of anaemia in most animals,[18] increased values of these indices imply that the extracts could be useful in the treatment of anaemia. Elevation of platelets suggests that the ethanol extract may have stimulatory effect on thrombopoietin.[19-20]

Increased circulating neutrophils is an index of bacterial infection in the body.[21] Significant increase of neutrophils in the treated rats may be due to improvement in the ability of the neutrophils to phagocytose (cellular ingestion of offending agents). Increase in WBC may also imply a boost in infection control at a higher dose. Lymphocytes are the main effectors cells of the immune system,[22] and increase in its circulating values is associated with enhanced immunological status of the body.[23] The administration of the plant extracts may indicate an inhibitory effect on the effectors cells of the immune system.

A higher level of potassium and chloride ions was recorded in rats treated with high aqueous extract dose. There was significant reduction in the potassium ions level in ethanol extract treated rats. Administration of both extracts does non-significantly affect sodium and bicarbonate ions levels. Many functions of potassium and sodium are carried out in coordination with each other and are common. High level of plasma potassium (hyperkalemia) could be due to complete shutdown of kidney function (anuria), tissue damage or violent muscle contraction and low plasma level (hypokalemia) may be due to loss of extracellular potassium into the intracellular space, loss of potassium in gastrointestinal (GI) secretions or sudden shift of potassium into intracellular fluid (ICF) causing paralysis (familial periodic paralysis).[24] The observed hyperkalemia in aqueous extract and hypokalemia in ethanol extract may suggest that the plant extracts may possess some toxic components.

CONCLUSION

Administration of both aqueous and ethanol corm extracts of *Zygotritonia crocea* stimulate erythropoiesis. However, it showed some electrolyte imbalance. Further work on the toxic effect of the plant is in progress.

Conflict of Interest

We declare no conflict of interest in this study.

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