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Full Length Research Paper

Biochemical changes induced in rats by aqueous and ethanolic corm extracts of *Zygotritonia croceae*

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Zygotritonia crocea **is a medicinal plant that belongs to the plant family of Iridaceae. It is well known traditionally in the Western Nigeria for treatment of intestinal disorder associated with typhoid, diarrhoea and dysentery, and as component of anti-tuberculosis recipes. In this present study, the biochemical changes occasioned by the oral administration of aqueous and ethanolic corm extracts of the plant were considered. Forty-two female rats were grouped into two (1 and 2) of twenty-one rats per group. Each group was divided into three sub-groups (1a, 1b, 1c, 2a, 2b and 2c,) of seven rats per sub-group. 1b and 1c were treated with 100 and 200 mg/kg body weight aqueous extract respectively for twenty-one days. 2b and 2c were also treated with 100 and 200 mg/kg body weight ethanolic extract respectively for twenty-one days. 1a and 2a served as control for each group. Both aqueous and ethanolic extracts produced significant reduction ($p < 0.05$) in the plasma glucose level. Protein level was significantly increased ($p < 0.05$) with aqueous extract while it was reduced with ethanolic extract. Cholesterol level was increased while ALT, AST and ALP activities were significantly reduced ($p < 0.05$) in rats treated with aqueous and ethanolic extracts. The results obtained clearly indicate that these extracts alter biochemical parameters in animals and its effects are dose-dependent.**

Key words: Biochemical markers, *Zygotritonia crocea*, aqueous and ethanolic corm extracts, female rats.

INTRODUCTION

One area of scientific research where some remarkable progress has been made in the very recent past in Nigeria is the area of phytochemistry and bioactivity of indigenous flora (Adesina, 1998). This development has led to an increased recognition by individuals and government agencies of herbal medicine as alternative therapy in various parts of the country. According to the guidelines for assessment of herbal medicines by the World Health Organisation (WHO), herbal medicines are finished, labelled medicinal products that contain as active ingredients, aerial or underground parts of plants or other plant material, or combinations thereof, whether in the crude state or as plant preparation (WHO, 1991). However, there is still lack of information regarding the biochemical, haematological and safety assessment of many of these plants in their applications and uses.

Z. crocea stapf. known in Western part of Nigeria (Yoruba) as “Baka” and the corm as “Isu baka” belongs to the plant family known as Iridaceae (Gbile, 1984), herbs perennial (or shrubs or annuals) with rhizomes, bulbs or corms (http://zipcode, 2009). The leaves are alternate, often two-ranked but usually sword-shaped to linear, parallel veined (http://zipcode, 2009). This plant is also referred to as famine food with corms and fruits eaten during times of famine (http://wwwhort, 2009). Approximately seven species of the genus *Zygotritonia* are known: *Zygotritonia bongensis, Zygotritonia crocea, Zygotritonia giorgii, Zygotritonia gracillima, Zygotritonia hysterantha, Zygotritonia nyassana* and *Zygotritonia praecox* (http://zipcode, 2009).

Z. crocea is an anti-infective plant prominent for the

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traditional treatment of intestinal disorders particularly those associated with typhoid, diarrhoea and dysentery and also as components of traditional anti-tuberculosis recipes (Ashidi et al., 1997). There has been very scanty literature on this plant. No previous biochemical or haematological study has been reported except the work on the antimicrobial potential of the plant (Abo et al., 1999). In his study on the antimicrobial potential, Abo et al reported the presence of only alkaloid in the phytochemical screening and a significant anti-fungal activity comparable to tioconazole (an antifungal medication of the imidazole class) using the corms methanolic (MeOH) extract of the plant (Abo et al., 1999).

It is for this reason that this study is primarily designed to evaluate and compare the changes in which the aqueous and ethanolic corm extracts of this plant might probably have on basic biochemical parameters in rats.

MATERIALS AND METHODS

Plant material

Fresh corms of *Z. crocea* were collected during the month of May 2008 at Obantoko Market, Abeokuta, Ogun State, Nigeria. They were identified and authenticated at the Forestry Research Institute of Nigeria (FRIN), Ibadan, Nigeria where a herbarium specimen was deposited.

Preparation of aqueous corm extract

To prepare the aqueous corm extract, 200 g of fresh corm, peeled, sliced and washed was oven-dried at 60°C. 50 g of dried sample was ground to powder and added to 250 ml boiled distilled water, stirred and allowed to stand for 3 days. The decanted filtrate was oven-dried at 60°C and weighed. The extract was reconstituted in normal saline to give two final concentrations of 100 and 200 mg/ml.

Preparation of ethanolic corm extract

To prepare the ethanolic corm extract, 200 g of fresh corm was peeled, sliced and washed and oven dried at 60°C. 50 g of dried sample was ground to powder and macerated in 250 ml ethanol for 3 days. The decanted filtrate was oven-dried at 40°C and weighed. The extract was reconstituted in normal saline to give two final concentrations of 100 and 200 mg/ml.

Experimental animal

All experimental protocols and procedures involving the experimental animals were conducted in conformity to the institutional, national and international guidelines of the United States National Institutes of Health (NIH) for laboratory animal care and use (USNIH, 1985). Forty-two female albino rats (Wistar strain) weighing between 160 and 215 g, were purchased from the Animal House of College of Veterinary Medicine (COLVET), University of Agriculture, Abeokuta, Ogun State, Nigeria. The rats were kept in standard cages at room temperature (26 - 28°C) and under controlled light cycles (12 h light/dark) with humidity (54 - 57%). They were fed freely on normal laboratory chow (Ola Feed Mills, Asero, Abeokuta, Nigeria) and water *ad libitum.*

Experimental design and administration of corm extracts

The forty-two rats were randomly distributed into two main groups (1 and 2) of twenty-one rats each. Each of these groups was divided into three sub-groups (1a, 1b, 1c, 2a, 2b and 2c) of seven animals each and was allowed free access to feed and water for a period of 2 weeks to acclimatize before the experiment. The sub-groups 1a and 2a which are the controls for both aqueous and ethanolic experiments received through oral cannula 0.3 ml normal saline only. Sub-groups 1b and 1c received 100 and 200 mg/kg body weight per day of aqueous extract, respectively while sub-groups 2b and 2c received 100 and 200 mg/kg body weight per day of ethanolic extract, respectively. The experiment was carried out for a period of 21 days before the animals were sacrificed.

Sample collection

The rats were sacrificed 24 h after the last dose of extracts administration and as overnight fast under ether anaesthesia. 3.0 ml of blood samples were collected from the rats by heart puncture into clean bottles and allowed to clot. The clot blood samples were centrifuged at 2,500 rpm at 4°C for 15 min and the clear supernatant decanted remaining the plasma for biochemical assays using RANDOX laboratory reagent kits obtained from RANDOX Laboratories Ltd., Ardmore, United Kingdom.

Biochemical assays

Glucose determination

Determination of glucose level was carried out using RANDOX Glucose Assay kit with 4-amino phenazone as oxygen acceptor as described by Trinder (1969). 1.0 ml of reagent [GOD-PAP reagent made up of glucose oxidase (GOD), peroxide and 4-aminophenazone (POD)] was mixed with 0.1 ml of sample in a test-tube, 0.1 ml of prepared standard glucose was also mixed with 1.0 ml of reagent in another test-tube while 1.0 ml of reagent was measured into the third test-tube as blank. The mixtures were thoroughly mixed and incubated for 25 min at 25°C. The absorbance of the standard and the samples were measured against the reagent blank at wavelength 500 nm. Glucose concentration (mg/dl) of samples was calculated by:

$$\text{Glucose concentration (mg/dl)} = \frac{\text{Absorbance}_{\text{sample}} \times \text{standard conc. (mg/dl)}}{\text{Absorbance}_{\text{standard}}}$$

Plasma protein determination

Total protein level was measured by Lowry method using bovine serum albumin (BSA) as standard (Lowry et al., 1951) with the use of RANDOX assay kit. 0.1 ml of sample (plasma) was added to 1.0 ml of reagent, 0.1 ml of prepared standard BSA was added to 1.0 ml of reagent in another test-tube while 1.0 ml was measured into another test-tube as reagent blank. The tubes were shaken and allowed to stand for 15 min. The absorbance was measured at 540 nm and the values were expressed as mg/dl.

Cholesterol determination

Cholesterol level was determined using the RANDOX Cholesterol Assay kit as described by Zak (1959). 0.1 ml of sample (plasma) was added to 1.0 ml of reagent, 0.1 ml of prepared standard cholesterol was added to 1.0 ml of reagent in another test-tube

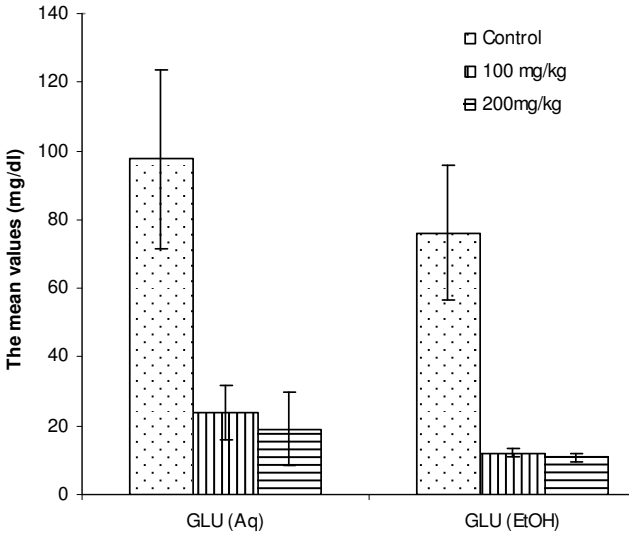

Figure 1. Effect of aqueous (Aq) and ethanolic (EtOH) corm extracts of *Zygotritonia crocea* on plasma glucose (GLU) level in rats.

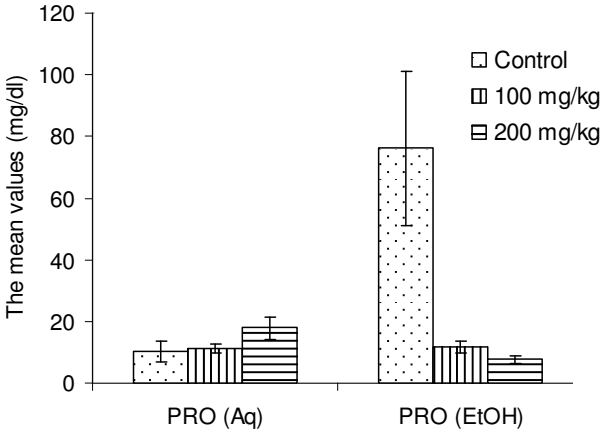

Figure 2. Effect of aqueous (Aq) and ethanolic (EtOH) corm extracts of *Zygotritonia crocea* on total protein (PRO) level in rats.

while 0.1 ml of distilled water was added to 1.0 ml of reagent as reagent blank and these were incubated at 25°C for 10 min. Absorbance was measured at 500 nm and the values were expressed as mg/dl

Alanine and aspartate aminotransferases determination

Plasma alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were estimated as described by the Randox reagent kit manufacturer using 2,4-dinitrophenylhydrazine as substrate according to the combination of methods of Reitman and Frankel (1957) and Mohun and Cook (1957).

Alkaline phosphatase determination

Alkaline phosphatase (ALP) activity was estimated by colorimetric method as described by the Randox reagent kit manufacturer using p-nitrophenylphosphate as substrate according to the method of Bassey et al. (1946). To 0.1 ml of plasma, 1.0 ml of reagent was added. The solution was mixed and absorbance was measured at 405 nm and the value was expressed as unit/l.

Statistical analysis

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

RESULTS

Effect of aqueous and ethanolic corm extracts of *Z. crocea* on the level of glucose (GLU) in rats

The effect of two different doses (100 and 200 mg/kg body weight) of both extracts that is, aqueous and ethanolic of *Z. crocea* were assessed on the blood sugar level. Aqueous extract of 100 and 200 mg/kg produced significant reduction ($p < 0.05$) GLU level by 75 and 80%, respectively compared to the control while the ethanolic extract produced significant reduction ($p < 0.05$) GLU level by 85 and 86%, respectively compared to the control as shown in Figure 1.

Effect of aqueous and ethanolic corm extracts of *Z. crocea* on the levels of protein (PRO) and cholesterol (CHOL) in rats

Administration of two different doses (100 and 200 mg/kg body weight) of aqueous extract produced a non-significant increase ($p < 0.05$) PRO level of 8% and a signify-cant increase ($p < 0.05$) PRO level of 75%, respectively compared to the control while the 100 and 200 mg/kg body weight of ethanolic extract produced significant reduction ($p < 0.05$) PRO level of 85 and 90% compared to the control as shown in Figure 2. Aqueous extract of 100 and 200 mg/kg body weight produced significant increase ($p < 0.05$) CHOL level of 74% and non-significant CHOL level of 29%, respectively compared to the control while the ethanolic extract of 100 and 200 mg/kg body weight produced significant increase ($p < 0.05$) CHOL level of 30 and 60%, respectively compared to the control as shown in Figure 3.

Effect of aqueous and ethanolic corm extracts of *Z. crocea* on the levels of ALT, AST and ALP

Plasma alanine aminotransferase (ALT), aspartate amono-transferase (AST) and alkaline phosphatase (ALP) levels of animals treated with two doses of 100 and 200 mg/kg body weight aqueous extract significantly reduce ($p < 0.05$) by 34 and 48, 18 and 60, 82 and 85%, respectively compared to the control (Figures 4, 5 and 6). ALT, AST and ALP levels of treated animals with ethanolic extract

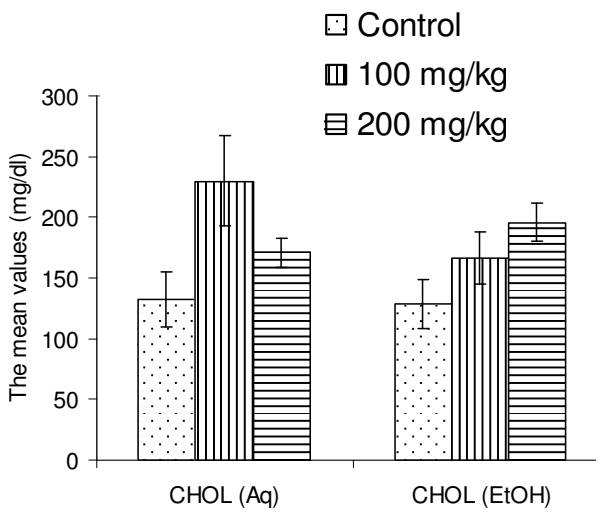

Figure 3. Effect of aqueous (Aq) and ethanolic (EtOH) corm extracts of *Zygotritonia crocea* on plasma cholesterol (CHOL) level in rats.

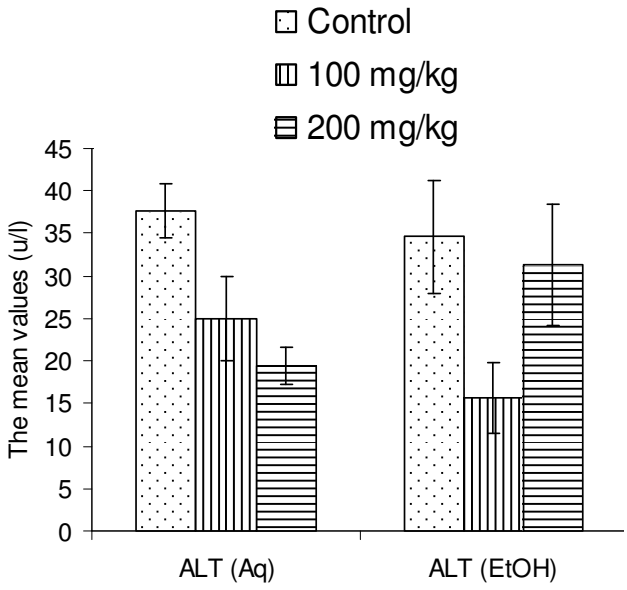

Figure 4. Effect of aqueous (Aq) and ethanolic (EtOH) corm extracts of *Zygotritonia crocea* on plasma alanine aminotransferase (ALT) activity in rats.

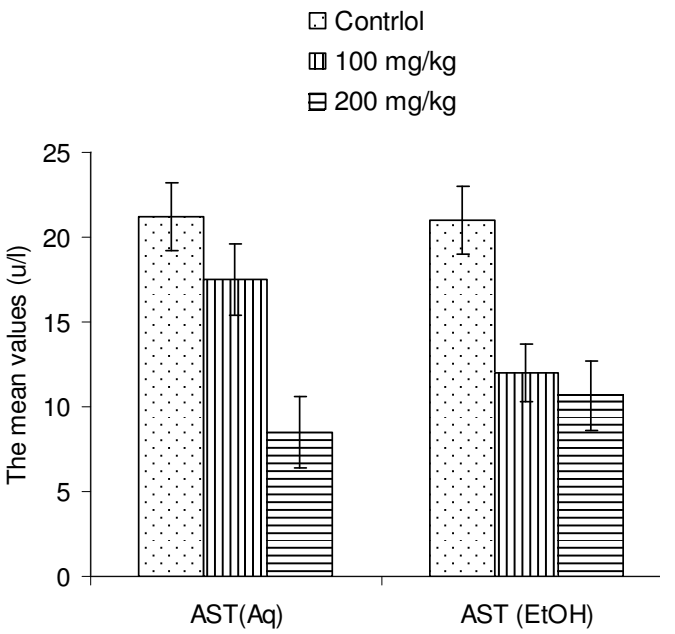

Figure 5. Effect of aqueous (Aq) and ethanolic (EtOH) corm extracts of *Zygotritonia crocea* on plasma aspartate aminotransferase (AST) activity in rats.

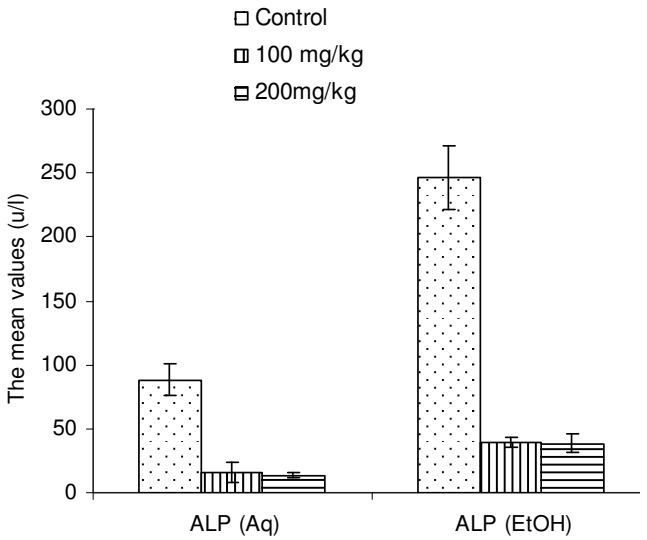

Figure 6. Effect of aqueous (Aq) and ethanolic (EtOH) corm extracts of *Zygotritonia crocea* on plasma alkaline phosphatase (ALP) activity in rats.

doses (100 and 200 mg/kg body weight) were reduced by 55 and 10, 43 and 49, 84 and 85%, respectively compared to the control (Figures 4, 5 and 6).

DISCUSSION

In this study, the aqueous and ethanolic extracts of *Z. crocea* effectively lowered the blood glucose of the treated rats compared to the normal and untreated control rats. There had been numerous studies on a variety of plant extracts that lowered blood glucose in induced diabetic animals (Kim et al., 2006; Oliver-Bever, 1986; Battu et al., 2007; Adeneye and Agbaje, 2007). The observed percentage blood glucose reduction at 100 and 200 mg/kg doses of aqueous and ethanolic extracts of

this plant is very high and not dose dependent. The reduction in level of blood glucose may suggest an increased catabolism of glucose. It is possible therefore, that these extracts possess active principles that caused the utilization of glucose and induction of enzymes which resulted in increased catabolism of glucose as alkaloids (Abo et al., 1999).

There was a contrast observation in the protein levels of aqueous and ethanolic extracts. The aqueous extract caused an increased protein level while the ethanolic extract caused a decreased protein level. This contrast observation could be due to the solubility in water components which could enhance biosynthesis of plasma protein while those components that inhibit biosynthesis of protein are alcohol soluble. It was also observed in this study that the increased cholesterol level in animals treated with these extracts, are dose dependent. The continuous use of this plant could, therefore, cause high plasma cholesterol (hypercholesterolaemia).

The plasma levels of ALT, AST and ALP showed significant decrease ($p < 0.05$) in animals treated with both aqueous and ethanolic extracts compared to the control. Liver enzymes such as ALT, AST and ALP are marker enzymes for liver function and integrity (Jens and Hanne, 2002; Adaramoye et al., 2008; Ajayi et al., 2009). It has been severally reported that liver enzymes are liberated into the blood whenever liver cells are damaged and enzyme activity in the plasma is increased (Edwards et al., 1995; Effraim et al., 2000). Elevation of these liver enzymes is also associated with cell necrosis of many tissues especially the liver (Adedapo et al., 2004). The fact that the activities of these enzymes were reduced after treatment with the extracts indicated that the plant extracts did not have necrotic effect on the liver.

Though there are many medicinal plants used in treatment of liver diseases, there are also quite some reports of liver injury after intake of herbals including those advertised for the treatment of liver diseases (Wurochekke et al., 2008). Wurochekke et al. (2008) also reported that herbs that contain pyrrolizidine alkaloids, kava, atractylis gummifera and senna alkaloids will cause liver damage. However, Abo et al., (1999) reported alkaloid as the only phytochemical component present in *Z. crocea*. Since both the aqueous and ethanolic extracts may have exhibited hepatoprotective activity, some other plant metabolites such as flavonoids and saponins (terpene glycosides) may be present because hepatoprotective activity has been attributed to these compounds (Effraim et al., 2000; Sadik and Sies, 2003; Lee et al., 2004).

Conclusion

This research work has clearly demonstrated that administration of both aqueous and ethanolic corm extracts of *Z. crocea* alters biochemical parameters such as glucose, protein, cholesterol, ALT, AST and ALP in rats leading to alteration of biochemical activity in the animal. The extent of biochemical changes occasioned by the treatment with these extracts has not been fully evaluated because the evidence at our disposal has not shown that the integrity of organs such as liver and kidney were affected by the treatment with both extracts. Further researches on phytochemical analysis and identification of those components responsible for the suppression of the blood parameters and enzymes activities are in progress.

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