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Research Article

ACETONE EXTRACT OF *CRINUM JAGUS* BULBS (LILLIACEAE): AN ACUTE AND SUB CHRONIC TOXICOLOGICAL EVALUATION IN ALBINO RATS

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ABSTRACT
The bulbs of *Crinum jagus* bulbs are used in the treatment of various ailments in traditional medicine. This study was aimed at evaluating the toxicity of acetone extracts of *Crinum jagus* bulbs on albino rats following acute and sub-chronic exposure on haematological and biochemical parameters. The LD_{50} of this plant was estimated to be more than 5000 mg/kg. Phytochemical screening of the bulbs extract revealed the presence of flavonoids, tannins, saponins, steroids, alkaloids, carbohydrates, reducing sugars and saponins. In the repeated dose 28 days oral toxicity study, administration of 250 and 500 mg/kg of body weight of extracts revealed increase in body weights. The extract produced a significant ($P < 0.05$) increase in urea level and bicarbonate ion .There was a significant decrease ($P < 0.05$) in the levels of alanine amino transferase (ALT), and aspartate amino transferase (AST)) in the group treated with 250 mg/kg of extract when compared with the control. No significant ($P > 0.05$) changes in alkaline phosphatase, total bilirubin, total cholesterol, conjugated bilirubin, total protein and haematological parameters of the treated groups compared with control were observed. The plant extract is however, not safe for long term use.
Keywords: Toxicity, *Crinum jagus*, haematological, biochemical, parameters

INTRODUCTION

Plants and their derived products having been used in traditional medicine as therapeutic agents against various human, animal and even plant diseases has made plants a sine qua non to human and animal lives[1,2]. The World Health Organization (WHO) reported that as many as 80 % of the world's people depend on traditional medicine for their primary health care needs[3]. The use of herbal remedies especially in the form of teas or extracts for the treatment of various diseases is gaining increasing popularity. Their increasing popularity may likely be due to their advantages of being efficacious and a cheap source of medical care[4]. The WHO Expert Committee[5] recommends appropriate use of herbal medicines and encourages the use of herbal drugs that have been proven to be safe and effective. Herbal medicines are prepared and dispensed by traditional medicine practitioners. Only a few of these preparations have been tested and evaluated scientifically for safety[4]. There is therefore the need for a scientific evaluation of plants used in traditional medicine for safety. Secondly, there is a growing disillusionment with modern medicine and also misconception that herbal remedy being natural may be devoid of adverse and toxic effects often associated with allopathic medicines[6]. An increase in the morbidity and mortality associated with the use of herbal or traditional medicines has raised universal attention in the last few years[7,6]. Upon exposure, the clinical toxicity may vary from mild to severe and even life threatening making the safety and toxicity evaluations of these preparations necessary. Therefore, the potential toxicity of medicinal plants employed in the treatment of diseases over a long period of time demands that the practitioners should be kept abreast of the reported incidence of renal and hepatic toxicity associated with the ingestion of medicinal herbs[4,8]. For a plant or herbal preparation containing active organic principles to be identified for use in traditional medicine especially for long term treatment, a systemic approach is required for the evaluation of efficacy and safety through experiment and clinical findings[9]. The study was designed to evaluate the safety of acetone extract of *Crinum jagus* bulbs on haematological and biochemical parameters in albino rats.

MATERIALS AND METHODS

Plant Collection and Identification

The bulbs of *Crinum jagus* were collected from Ikono, Akwa-Ibom State. The plant was identified and authenticated in the herbarium of the Department of Pharmacognosy by Prof. Mrs Bassey, Faculty of Pharmacy and University of Uyo, where a voucher specimen of the plant has been deposited in the herbarium (UUH 012/12).

Preparation of Plant Extract

Extraction of the plant material was carried out using cold maceration method with 96 % (v/v) acetone (Sigma, Aldrich) for 72 hours. The filtrate was concentrated to dryness with a Rotary evaporator at 60^0C. The crude extract was further dried over a water bath set at 40°C. The obtained extract was weighed, and stored in a refrigerator (4^oC) till it was needed.

Experimental Animals

The internationally accepted principles for laboratory animal use and care were adopted, Ethical Clearance for research was obtained from the University of Port Harcourt Ethics Committee and the protocols were strictly adhered to. Albino rats were obtained from the Animal House of the Department of Pharmacology, Faculty of Basic Medical Sciences and University of Port-Harcourt. The animals were kept in cages at a constant room temperature and allowed free access to feed (Vital feeds, UAC PLC) and water *ad libitum* in the animal house of the Department of Pharmacology, Faculty of Pharmaceutical Sciences and University of Port Harcourt.

The animals were allowed to acclimatize for two weeks. The animals were deprived of food and water overnight prior to the experiment.

Acute Toxicity Determination

A total of 18 albino rats of either sex weighing 150-200 g were used in the determination of the acute toxicity of the acetone extract of *Crinum jagus* Linn. The animals were randomly distributed into 6 groups of three animals each and the first three groups were administered with 10 mg/kg, 100 mg/kg and 1,000 mg/kg of the extract respectively through the oral route. They were observed for 24 hours for signs of toxicity or death. After 24 hours, no animal died nor showed signs of toxicity. Subsequent doses of 1,600 mg/kg, 2,900 mg/kg and 5,000 mg/kg of the plant extract was administered to the other three groups of three animals each and observed as above[10].

Experimental Protocol

Thirty healthy albino rats of both sexes were used for the sub-chronic study. They were divided into three groups of 10 rats per group with each having equal numbers of male and female rats. Group I was administered with distilled water only. Group II animals were administered with 250 mg/kg of acetone extract of *Crinum jagus* while group III were administered with 500 mg/kg of the extract. All administrations of the plant extract were done with the aid of an oral cannula for a period of 28 days. The body weights of the animals were evaluated on day O and day 28 of the experiment. At the end of the experimental period, they were anaesthetized in ether saturated chamber, one at a time in a desiccator[11]. While under anaesthesia, blood samples were obtained by cardiac puncture from each rat by means of a 5 ml hypodermic syringe and needle. The blood samples were introduced into clean dry bottles (EDTA bottles) for haematological parameters while heparinized tubes were used to collect blood for biochemical estimation. The haematological parameters performed were according to standard methods. Measurement of Haemoglobin was done by using cyanomethaemoglobin technique. Packed cell volume of each sample was determined by using a Hawksley micro-haematocrit centrifuge at 12,000 rpm for five minutes[12]. Total white blood cells were counted using the improved Neubauer haemocytometer[13]. For biochemical assays, commercial test kits obtained from Roche Diagnostics, GmbH, Mannhein, Germany were used for all biochemical parameters measured using Uniscope 23D spectrophotometer, England. The following biochemical parameters were carried out in plasma: alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were estimated using[14] method; alkaline phosphatase (ALP) was carried out using the phenolphthalein monophosphate method[15]; total bilirubin was estimated using[16] method; total protein was calculated using biuret method[17]; urea was analysed using the Bethlot Searcy' method[18]; creatinine was determined by Jaffe's method described by[19]. Serum electrolytes were determined by established methods; sodium and potassium concentration by flame photometry and biocarbonate concentration by titrimetric method[20,21].

Statistical Analysis

Data are expressed as mean ± SEM. Statistical analysis of data was done using Microsoft excel. Student's t-test was done to determine the significance of difference between the control groups and the treated groups. P-values < 0.05 were considered to be statistically significant.

RESULTS

There were observable changes in the body weight of treated and untreated rats. Treatment of rats with the polyphenol fractions of *Crinum jagus* was associated with weight gain even though the percentage increase was lower but there was no statistically significant differences ($P < 0.05$) when compared to the control (Table 1). There was a significant decrease ($P < 0.05$) in the levels of Alanine amino transferase (ALT) and Aspartate amino transferase (AST)) in the group treated with 250 mg/kg of extract when compared with the control. However, no significant ($P > 0.05$) changes in alkaline phosphatase, total bilirubin, total cholesterol, conjugated bilirubin and total protein of the treated groups compared with control (Table 2) were observed. The effect of sub-chronic administration of *Crinum jagus* on kidney function is presented in (Table 2). The urea level in treated rats was significantly ($P < 0.05$) higher compared to the control group. There were however, no significant ($P > 0.05$) changes in creatinine and sodium of the treated groups compared with control. There was an increase in potassium (K) and bicarbonate (HCO_3) levels that was statistically significant ($P < 0.05$) in rats treated with the plant extract when compared with the control. The packed cell volume, haemoglobin and the red blood cell count of the group treated with 500 mg/kg extract was lower than the group treated with 250 mg/kg extract and the control. The white blood cell count of the group administered with 500 mg/kg of the extract was slightly higher than the other groups (Table 3). The results of the packed cell volume, haemoglobin, red blood cell count and white blood cellcount showed that there was no significant difference ($P > 0.05$) between the control group and the extract treated groups after oral administration of the extract of *Crinum jagus* for 28 days.

Table 1: Effect of acetone extract of *Crinum jagus* on body weights after 28 days

Group	Dose mg/kg	Day 0	Day 28	% Increase in body weight
Crinum jagus	250	257 ± 14.17	272 ± 17.27	5.84
Crinum jagus	500	251 ± 17.65	269 ± 24.70	7.17
Distilled water	10	247 ± 18.09	278 ± 17.97	12.55

Table 2: Effects of acetone extract of *Crinum jagus* bulbs on serum biochemical parameters in rats treated orally for 28 days

Parameters	Distilled water	*Crinum jagus*	*Crinum jagus*
	5 ml/kg	250 mg/kg	500 mg/kg
AST iu/L	62.8 ± 4.31	41.2 ± 3.22*	53.0 ± 5.6
ALT iu/L	24.8 ± 1.50	13.8 ± 1.71*	23.8 ± 0.92
ALP iu/L	147 ± 19.01	169 ± 40.6	157 ± 17.80
TB µmol/L	3 ± 0.45	3.2 ± 0.49	3.2 ± 0.58
CB µmol/L	0	0	0
TC µmol/L	1.22 ± 0.1	1.4 ± 0.05	1.12 ± 0.07
TP g/L	68.2 ± 3.99	63.8 ± 3.22	58.8 ± 3.68
Ur mmol/L	6.7 ± 0.31	7.10 ± 0.70	7.68 ± 0.15*
Cr µmol/L	62.2 ± 2.85	72.6 ± 3.42	64.4 ± 1.60
Na mmol/L	130 ± 1.08	131 ± 0.4	128 ± 2.38
K mmol/L	5.82 ± 0.06	4.82 ± 0.1*	4.92 ± 0.13*
HCO_3 mmol/L	27 ± 2.39	20.8 ± 1.24*	19.8 ± 1.36*

Values are expressed as mean ± S.E.M of 10 animals;
P < 0.05 vs. control group (student's t-test).
*significantly different from control, P < 0.05

Table 3: Effects of acetone extract of *Crinum jagus* bulbs on haematological parameters in rats treated orally for 28 days

Parameters	Distilled water	*Crinum jagus*	*Crinum jagus*
	5 ml/kg	250 mg/kg	500 mg/kg
PCV	41.4 ± 0.68	43 ± 1.05	39.6 ± 1.6
HB	13.9 ± 0.24	14.3 ± 0.31	13.3 ± 0.54
RBC	4.52 ± 0.07	4.72 ± 0.12	4.36 ± 0.16
WBC	87.6 ± 15.65	80.4 ± 16.9	88.8 ± 6.89

Values are expressed as mean ± S.E.M of 10 animals;
P < 0.05 vs. control group (student's t-test)

DISCUSSION

The use of traditional medicine has maintained greater popularity all over the developing countries and is increasing rapidly[22-25]. Although there are many traditional herbal medicines available, only a few have been verified by clinical trials, their efficacy and safety are still questioned by consumers[26]. Preliminary phytochemical screening shows that the extract contains; steroids (triterpenes), tannins, flavonoids, phlobatannins, saponins; most of which are phenolic compounds with carbohydrates, reducing sugars and alkaloids. The result of the current study showed that the LD_{50} of the acetone extract of the plant was found to be greater than 5000 mg/kg, which may be considered as relatively safe[10]. In this study, there was a progressive increase in the body weights of the rats treated with different sub-chronic doses of the acetone extract of *Crinum jagus* bulbs. This may be an indication that the plant does not affect the feed utilisation ratio of the animals. This corresponds to the reports that the body weights of animals treated with sub-chronic doses of aqueous extracts of *Boerhavia diffusa*[27]and *A. chevalieri* leaf[28] increased progressively. Serum marker enzymes are biochemical parameters associated with health indices and are of diagnostic significance in routine clinical evaluation of the state of health. Alanine amino transaminase (ALT) and Aspartate amino transaminase (AST) are used in the assessment of liver damage by drugs or any other hepatotoxin[29]. The liver and heart release ALT and AST and an elevation in their plasma concentrations are indicators of liver and heart damage[30,9]. However, ALT is more specific to the liver and is thus a better parameter for detecting liver injury[31]. The significant decrease observed in the level of ALT and AST in the group administered 250 mg/kg body weight of extract may suggest that the plant extract may not possess hepatotoxic effect[32]. This protective effect may be the result of stabilization of plasma membrane thereby preserving the structural integrity of cell as well as the repair of hepatic tissue damage[33]. The observable increase in the level of alkaline phosphatase (ALP) in the extract treated groups may be as a result of congestion or obstruction of biliary tract, which may occur within the liver. ALP activity on the other hand is related to the functioning of hepatocytes and an increase in its activity may be due to its increased synthesis in the presence of increased pressure[34]. There were no significant changes in total protein in rats treated with the plant extract, which may likely indicate that there was no sign of impaired renal function[35]. Total protein measurements can reflect nutritional status and may be used to screen for and help diagnose kidney and liver diseases and many other conditions[36]. The non- significant changes in the levels of total cholesterol observed in groups treated with the extract may be attributed to the presence of hypolipidemic agents in the plant extract[37]. The non- significant changes in serum creatinine level may suggest that the extract was not toxic to the kidney. Creatinine is the major catabolic products of the muscle and it is excreted in the kidneys. Creatinine levels are used as indicator of renal failure[38]. The increased level of urea observed is an indication of azotaemia. High blood urea is associated with increased tissue protein catabolism, excess breakdown of blood protein and diminished excretion of urea[39]. Albumin is quantitatively the most important protein in plasma synthesized by the liver and is a useful indicator of hepatic function. Albumin synthesis is affected not only in liver disease but also by nutritional status, hormonal balance and osmotic pressure[40]. The result of this study have revealed no significant (P < 0.05) difference in the serum albumin and total protein of the extract treated groups as compared to the control. Bilirubin is a useful index of the excretory function of the liver. However, there was no significant change in the bilirubin level. Similarly, the serum concentrations of electrolytes, uric acid and creatinine could give an insight into the effect of plant extract on the tubular and or glomerular part of the kidney. The significant decrease in the levels of potassium and bicarbonate at the doses investigated may suggest that the normal functioning of the nephrons at the tubular and glomerular levels were affected. This finding corresponds to the report of[41] that the extract of the root of *A. chevalieri* causes a significant increase in serum urea without adversely affecting the serum levels of uric acid, creatinine. Assessment of haematological parameters can be used to determine the extent of deleterious effect of foreign compound including plant extract on the blood. It can also be used to explain blood relating functions of chemical compound/plant extract. Such laboratory investigations have been reported to be highly sensitive, accurate, and reliable and it remains the bedrock of ethical and rational research, disease diagnosis, prevention and treatment[42,43]. The results of the packed cell volume, haemoglobin, red blood cell and white blood cells showed that there was no significant difference (P > 0.05) between the control group and the extract treated groups after oral administration of the extract of *Crinum jagus* for 28 days. This corresponds to the reports of[44] which stated that aqueous extracts of leaves, stem bark and root bark of *Eugenia jambolana* have no significant (p > 0.05) effect on packed cell volume, red blood cell, white blood cell suggesting that the plant might not compromise the functional capacity of the blood. Saponins are high molecular weight glycosylated plant secondary metabolites, consisting of a sugar moiety linked to a triterpene or steroid aglycone[45]. Detergent properties are the typical characteristics of saponins. They are also known to possess pesticidal activity[46] and also used in the treatment of contaminated water[47]. They are also known for their health benefits such as cholesterol lowering and anticancer properties[48,49]. Alkaloids are low molecular weight nitrogenous compounds. It is contained in 20 % of plant species; mainly involved in plant defense against pathogens and herbivores. Alkaloids have been found to possess analgesic, anti malarial, antibacterial, antihypertensive[50] properties. Thus, the phytochemical constituents of this plant extract may be likely responsible for the results of this study. The results have demonstrated that the acetone extract of *Crinum jagus* bulbs seems not to affect the haematopoetic system adversely but had effects on some of the biochemical parameters. However, this study has shown that the plant extract is however, not safe for long term use. Further studies are needed to evaluate the chronic toxicity effects of this plant.

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