Subchronic Toxicity of *Crinum jagus* Extracts on Wistar Rats

Clémence Mvongo¹*, René Kamgang²,³, J.L. Essame Oyono³

¹Department of Life Science, Higher Teacher Training College Bertoua, University of Ngaoundere, Cameroon.
²Laboratory of Endocrinology and Radiotopes, Institute of Medical Research and Medicinal Plants Studies (IMPM), Yaoundé, Cameroon.
³Animal Physiology Laboratory, Faculty of Science, University of Yaoundé I, Cameroon.

**ABSTRACT**

*Crinum jagus* is used in Cameroon western and eastern regions folk medicine in detoxification, the management of diabetes and obesity and also as antivenous and antipoison. The aim of this work was to evaluate the subchronic toxicity of *Crinum jagus* extracts on male and female Wistar rats. Subchronic toxicity of aqueous and hydroethanolic *Crinum jagus* extracts was determined on two month old normal Wistar rats. Those rats, once daily orally received, hydroethanolic (75, 150 mg/kg b.w.) and aqueous (150 mg/kg b.w.) extracts, during 90 days (tree month). In both males and females animals, the *C. jagus* effects were investigated on the evolution of weight, food and water intake, kidney and liver functioning markers (serum total cholesterol (TC), total proteins, creatinine and transaminase: AST and ALT). Both aqueous and hydroethanolic extracts in males as in females, did not cause any adverse changes in anthropometric (body mass, relative weight of liver and kidneys, food and water intakes) and seric parameters (total cholesterol, total proteins, creatinine AST and ALT activity). Instead, those extracts remarkably improved antropometric parameters, liver and kidney function and even protect again atherosclerosis. The results indicated that aqueous and hydroethanolic extracts of *Crinum jagus* did not induce toxic effect at the doses used in long term treatment; thus justifying its empiric use in detoxification and as antivenous.

1. Introduction

The enormous cost and poor availabilities of the modern therapies among others, for rural populations in developing countries led to a growing interest in herbal remedies [1, 2]. Present estimates indicate that about eighty percent of the world’s population relies on traditional medicine for health care delivery. However, a number of studies have reported the toxic effects of herbal medicines [3]. *Crinum jagus* is an Amaryllidaceae distributed in West Africa and Cameroon. In Cameroon, it is quite common from the southern border to Adamawa in the north. It is limited to fairly humid regions, especially frequent in riparian vegetation, and can even withstand flooding. *C. jagus* is found in many types of vegetation: coastal, Biafran, semi-deciduous, periforest savannah and Adamawa savannah. *Crinum jagus* is a bulbous plant [4], with multiple virtues. It is used empirically in South Africa in the treatment of several ailments and diseases [5]. In Nigeria *Crinum jagus* is used alone or in combination with *C. glaucum* in the treatment of memory loss, other mental abnormalities associated with aging and, as an antivenous [6]. It works by inhibiting acetylcholine esterase, making it effective for the treatment of Alzheimer’s disease by preventing the destruction of acetylcholine in the brain [7]. According to traditional medicine practitioners in Western and Eastern regions of Cameroon, *Crinum jagus* is used as antidiabetic, antiobesity, antidiarrheal remedy and also again poison and in general body detoxification. Aqueous and hydroethanolic extracts of this plant have been shown to be effective against hyperglycemia, oxidative stress, neurological and renal damage in type 2 diabetics, MACAP051 model [8]. In acute treatment, these extracts have been shown to be non-toxic in terms of cellular toxicity [9]. Despite of the popular use, exploring various medicinal importance of this plant, using scientific approaches, there is no report on the subchronic toxicity study of the entire plant. The present investigation was therefore carried out to study the subchronic toxicity of the hydroethanolic and aqueous extracts of *C. jagus* in Wistar rats.

2. Experimental Methods

2.1 Plant Extracts

Fresh *Crinum jagus* plants were collected from Batié (Western Region of Cameroon) during the month of April, 2010. The species was confirmed by the National Herbarium of Yaoundé–(Cameroon), with the voucher specimens HNC 14049. The whole plant was cleaned, sliced into small pieces, shade dried and powdered. This powder was subjected to aqueous and hydroethanolic extractions. For each extract, 500 grams of the powder were macerated in 3 L of boiled water or ethanol/water (1:4) mixture, for 48 hours (with occasional stirring) at room temperature. After filtration, the water filtrate was dehydrated in a hot air oven; the residue was re-macerated for 48 hours, filtered and dehydrated and the whole dried aqueous extract obtained was weighed (91.4 g). The residue of the ethanol/water mixture, after filtration, was re-macerated for 48 hours and filtered. The 2 filtrates were pooled, concentrated in a rotary evaporator at 40 °C and dehydrated to yield 145.8 g of dry dark hydro-ethanolic extract. The hydroethanolic and aqueous extracts were kept in a well-closed container under refrigerated conditions until use.

2.2 Animals of Experiment

For the experiment, male and female albino Wistar rats (6-8 weeks old) were raised in the animal house of the Faculty of Science of the University of Yaoundé I (Cameroon) under natural conditions of light and temperature, with free access to water and regular rodent chow. The animals were acclimatized to laboratory condition for one week before the experiment starts. Animal housing and in vivo experiments were performed according to the Guidelines of the European Union directive on Ethical Evaluation of Animal Experiments (CEC Council 86/609) [10] and ethically approved by the Institutional Committee of the Ministry of Scientific Research and Innovation of Cameroon. Treatment doses were chosen based on their efficiency on type 2 diabetic rats [8].

2.3 Subchronic Toxicity Study

The rats were divided into 4 groups of 10 rats each (5 male and 5 female housed separately): normal controls (NC), normal rats treated with 75, 150 mg/kg bw of *C. jagus* hydroethanolic extract (Cjh75; Cjh150) and...
normal rats treated with 150 mg/kg bw of C. jagus aqueous extract (Cja150). Animals were treated once daily by intra-gastric gavages for 90 consecutive days. During the treatment, body mass was assessed every five days, food and water intakes were recorded every two days. The behavior of the animals (locomotion, aggressiveness and sensitivities to noise and to touch) was also assessed during the treatment.

At the end of the treatment, the animals were sacrificed under mild ether anesthesia after 12 hours of fasting. The blood collected into centrifuge tubes was allowed to clot during 5 minutes, then centrifuged (3000 rpm, 10 min) for serum separation. Clear serum obtained was used for biochemical analyses (transaminases: AST and ALT, creatinine, total serum proteins and total cholesterol). The liver and kidneys were excised immediately and weighed. The relative weight of these organs was calculated using the following formula:

Relative Weight = organ weight / animal weight

Analysé of supernatant serum total cholesterol (TC), was performed, through colorimetric method with commercially available test kits according to the manufacturer’s recommendations (Fortress diagnostic UK). Serum total protein concentration was estimated by the Biuret method using Fortress test kit. Serum AST was determined according to the method of Tietz and Shuey in 1986 [11]. Serum ALT was assessed using the method of Bergmeyer et al in 1985 [12].

### 2.4 Statistical Analysis

The results are expressed as mean (X) ± standard error of mean (S.E.M). The results were statistically analyzed by one way analysis of variance (ANOVA) associated with Turkey test followed by Dunnett test, using the computer Graphpad Instat Software. The difference between and within various groups was significant with \( p<0.05 \).

### 3. Results and Discussion

#### 3.1 Anthropometric Parameters: Body Mass, Food and Water Intakes, Relative Weight of Heart, Liver and Kidneys

During the 90 days of treatment, hydroethanolic and aqueous extracts of C. jagus slowed down weight gain in male and female rats, when compared with normal control animals. Much more marked with hydroethanolic extract (at the dose 75 mg/kg bw) in both sexes, this slowing became significant from the 10th day in male rats \((p<0.05)\) and on the 20th day in female rats \((p<0.01)\) treated with the extract (Fig. 1).

![Fig. 1](image1.png)

**Fig. 1** Body mass of males (A) and females (B) rats at the end of 90 days of once daily treatment. NC: normal control rats; normal rats treated with C. jagus hydroethanolic extract 75 mg/kg (Cjh75), 150 mg/kg (Cjh150) and C. jagus aqueous extract 150 mg/kg bw (Cja150). n=10 rats/group (5 males and 5 females). Significant difference: \( *p<0.05; **p<0.01 \) compared to NC.

Food and water intakes of normal control rats significantly \((p<0.01)\) increased during the treatment: +33.90% and +36.64% respectively in the male, +45.63% and +29.54% respectively in the female, at the end of treatment. C. jagus extracts lowered food and water intakes in both males and females. The decrease were more marked with hydroethanolic extract, and especially at the dose 75 mg/kg bw (-39.62% and -41.46%) respectively in the male (Figs. 2A1 and B1), -47.29% and -25.52% respectively in the female (Figs. 2A2 and B2), at the end of treatment.

![Fig. 2](image2.png)

**Fig. 2** (A) Food and (B) Water intakes (expressed as % of initial values) of males (1) and females (2) rats at the end of 90 days of once daily treatment. NC: normal control rats; normal rats treated with C. jagus hydroethanolic extract 75 mg/kg (Cjh75), 150 mg/kg (Cjh150) and C. jagus aqueous extract 150 mg/kg bw (Cja150). n=10 rats/group (5 males and 5 females). Significant difference: \( *p<0.05; **p<0.01 \) compared to NC.

After 90 days of treatment, the aqueous and hydroethanolic extracts of C. jagus did not cause any significant change in the relative weights of the heart, kidneys and liver compared to NC, in either males or females (Table 1).

#### 3.2 Biochemical Parameters of Liver and Kidney Function

After 90 days of treatment, the plant extracts increased serum total proteins in males as well as in females compared to NC. This increase was significant \((p<0.05)\) in male rats treated with 75 mg/kg bw of hydroethanolic extract: +15.65% (Table 2).

After 90 days of treatment, C. jagus extracts significantly \((p<0.01)\) reduced serum creatinemia in male (-26.25%, -53.18% and -45.39%) and female (-25.83%,-49.16% and -43.33%) respectively at doses of 150 mg/kg of the aqueous extract, 75 and 150 mg/kg of the hydroethanolic extract compared to the NC. This decrease was more marked with hydroethanolic extract than with aqueous extract in both male and female (Table 2).

Hydroethanolic and aqueous extracts of C. jagus lowered seric alanine amino transferase (ALT) and aspartate amino transferase (AST) activity. The reduction of ALT were significant with hydroethanolic extract at the
doses of 75 (-21.8% p<0.01) and 150 (-15.5% p<0.05) mg/kg in males and 75 mg/kg (-22.4% p<0.05) in females. For AST, this decrease was only significant for hydroethanolic extract at 75 (-28.6% p<0.01) and 150 (-20.4% p<0.05) mg/kg in males (Table 2).

After 90 days of treatment, the aqueous and hydroethanolic extracts remarkably (p<0.01) decreased seric total cholesterol level: So: 88.35 ± 2.68 mg/dL (Cjh75), 106.37 ± 2.99 mg/dL (Cjh150), 122.41 ± 2.16 (Cja150), 151.21 ± 1.98 mg/dL (NC) in males; 109.67 ± 1.85 mg/dL (Cjh75), 126.37 ± 2.79 mg/dL (Cjh150), 111.87 ± 2.39 mg/dL (Cja150), 162.42 ± 2.56 mg/dL (NC) in females. This decrease was more marked with hydroethanolic extract at the doses of 75 and 150 mg/kg in males and at a dose of 75 mg/kg in females. No changes in behavior (locomotion, aggressiveness, and sensitivities to noise and to touch) were observed in animals treated with plant extracts during treatment (Table 2).

The results indicated that, both aqueous and hydroethanolic extracts of C. jagus did not induce toxic effect at the doses used in long term treatment. Instead, C. jagus extracts improved anthropometric parameters, liver and kidney function and even protect against atherosclerosis; thus justifying its empiric use in detoxification and as antivenomous.

Acknowledgements
The authors gratefully acknowledge the assistance from the Institute of Medical Research and Medicinal Plants Studies (IMPM), Yaoundé, Cameroon.

References
[1] R. Voeks, Disturbance pharmacopoeias: Medicine and Myth from the Humid Tropics, Ann. Am. Assoc. Geogr. 94(4) (2004) 868-888.

Table 1: Anthropometric parameters (heart, kidneys and liver relative masses: % bw.) of males and females rats at the end of 90 days of once daily treatment

|                | NC           | Cjh75        | Cjh150       | Cja150       |
|----------------|--------------|--------------|--------------|--------------|
| **Male**       |              |--------------|--------------|--------------|
| Heart          | 0.246±0.01   | 0.247±0.01   | 0.243±0.01   | 0.252±0.01   |
| Kidneys        | 0.434±0.01   | 0.440±0.01   | 0.447±0.01   | 0.437±0.01   |
| Liver          | 1.764±0.13   | 1.761±0.03   | 1.757±0.04   | 1.769±0.03   |
| **Female**     |              |--------------|--------------|--------------|
| Heart          | 0.242±0.01   | 0.241±0.02   | 0.253±0.03   | 0.239±0.04   |
| Kidneys        | 0.411±0.03   | 0.416±0.08   | 0.409±0.02   | 0.417±0.006  |
| Liver          | 1.753±0.01   | 1.762±0.09   | 1.759±0.09   | 1.749±0.04   |

Table 2: Kidney and liver function markers (serum total proteins: g/L, creatinine: mg/dL, total cholesterol (TC): mg/dL and transaminases activities (AST and ALT) of rats after 90 days of once daily treatment

|                | NC           | Cjh75        | Cjh150       | Cja150       |
|----------------|--------------|--------------|--------------|--------------|
| **Male**       |              |--------------|--------------|--------------|
| Total proteins | 70.34±3.35   | 81.34±2.36*  | 78.90±1.43   | 71.51±2.57   |
| Creatinine     | 0.414±0.013  | 0.219±0.14** | 0.224±0.017* | 0.303±0.014**|
| TC             | 151.20±1.98  | 108.35±2.68**| 106.37±2.99**| 122.42±2.16**|
| AST            | 27.22±1.62   | 19.44±1.24** | 21.61±1.04*  | 23.33±1.42   |
| ALT            | 35.45±1.36   | 27.72±1.56** | 29.95±1.54*  | 32.27±1.32   |
| **Female**     |              |--------------|--------------|--------------|
| Total proteins | 62.19±1.82   | 68.36±3.32   | 63.94±3.31   | 70.63±3.01   |
| Creatinine     | 0.355±0.012  | 0.178±0.012**| 0.198±0.009**| 0.259±0.015**|
| TC             | 162.41±2.56  | 109.67±1.85**| 126.37±2.78**| 111.86±2.39**|
| AST            | 21.11±1.11   | 17.78±1.41   | 16.67±1.24   | 18.89±1.04   |
| ALT            | 26.36±1.7    | 20.45±1.24*  | 21.36±1.15   | 22.72±1.60   |

NC: normal controls; rats treated with C. jagus hydroethanolic extract 75 mg/kg (Cjh75), 150 mg/kg (Cjh150) and C. jagus aqueous extract 150 mg/kg bw (Cja150); n=10 rats/group (5 males and 5 females); Significant difference: *p<0.05; **p<0.01 compared to NC.

4. Conclusion

The present investigation was carried out to study the subchronic toxicity of hydroethanolic and aqueous extracts of Crinum jagus on Wistar rats. During the 90 days of subchronic treatment of the rats, C. jagus extract decreased weight variation compared to the normal control animals. This decrease in weight variation was associated with the decrease of food and water intakes observed in these animals without distinction of sex. This could be beneficial in the prevention of metabolic diseases related to overweight. The development of adipose tissue characterized by an increase not only in the size but also in the number of adipocytes is a good indicator of weight gain and vice versa. In addition, an excess of energy accumulated in the form of fat leads to obesity as well as to insulin resistance and consequent hyperglycemia [13, 14]. By reducing food and water intake and weight change in both sexes during 90 days of treatment, hydroethanolic and aqueous extracts of C. jagus could prevent obesity as well as type 2 diabetes among others. The decrease in weight variation was also associated with the significant decrease in total cholesterol in animals treated with plant extracts regardless of sex. This result suggests that the extracts might prevent atherosclerosis by reducing seric total cholesterol, thus protecting animals against cardiovascular disease. These effects could be linked to the presence in the extracts of lipid-lowering agents such as flavonoids, polyphenols, anthocyanidins [15, 16].

The extracts did not cause any significant change in the relative weights of the heart, kidneys and liver in either sex. This result suggests that the extracts have no effect on animal growth. In addition, in toxicity studies, relative organ weight is a relatively sensitive indicator of toxicity for certain organs [17]. The insignificant change in the relative weight of these organs suggests that the extract has no negative impact on these organs. No changes in behavior (locomotion, aggressiveness, and sensitivities to noise and to touch) suggest innocuity of those extracts at the doses used. The serum biochemical parameters (alanine aminotransferase: ALT, aspartate aminotransferase: AST, total proteins, and creatinine) allowed us to assess possible alterations in the liver and kidneys under the influence of aqueous and hydroethanolic extracts of C. jagus at the doses used. The evaluation of the kidney and liver function is essential in toxicity studies of drug or plant extracts because these two organs are necessary or even essential, for the survival of the organism [18]. Increased levels of transaminases (ALT and AST) are the major indicator of liver disease or hepatotoxicity in both humans and animals. Any damage to hepatocytes results in an increase of seric transaminases [19]. The decrease in serum transaminase activity in male and female rats treated with plant extracts compared to that of normal control rats suggests that subchronic oral administration of aqueous and hydroethanolic extracts did not provoke hepatocyte damage; instead, these extracts would have repaired damages linked to the aging of the body and regenerated the hepatic cells. This regenerative effect could be due to the presence in the extracts of antioxidant compounds such as flavonoids, polyphenols capable of inactivating or destroying the free radicals which are responsible of the aging of the organism [20, 16]. Furthermore, an increase in serum proteins was observed in animals of both sexes treated with plant extracts, suggesting that these extracts would indeed have improved the synthetic function of hepatic cells. Decreased total serum protein indicates impaired synthetic function of liver cells [17]. The kidney plays an important role in removing waste products from the bloodstream; its functional state can therefore be assessed by measuring the serum concentration of certain metabolites eliminated in the urine, in particular creatinine, which is a good functional indicator of the kidney [21]. In rats of both sexes, C. jagus extracts lowered the serum creatinine level: C. jagus would rather have improved the glomerular filtration function of the kidney, thus reducing creatinemia by urinary excretion of creatinine; since any increase in creatinemia reflects a functional impairment of the nephrons [22]. The improvement in serum biochemical parameters, markers of renal and hepatic function could also be explained by the presence in these extracts of antioxidant compounds such as polyphenols, flavonoids, anthocyanidins [16], which strengthen immunity and reduce the toxicity of drugs that often generate free radicals in their metabolism [23].
