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EFFECT OF ETHANOL-WATER EXTRACT OF *CRINUM JAGUS* ON GLYCEMIA REACTIVITY IN DEXAMETHASONE-INDUCED DIABETIC RAT MACAPOS 1

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Abstract: *Crinum jagus* is a plant traditionally used by the population of the western region of Cameroon in the management of diabetes. The effects of ethanol/water extract of *C. jagus* were evaluated on diabetic rats. Diabetes was induced on 6-8 weeks old wistar rats with high sugar diet (HSD) associated with dexamethasone (DXM) injection (25 µg/kg once after 2 days during 3 weeks) one month after the beginning of the HSD. Diabetic rats were treated with 150 mg/kg b.w. extract during 20 days. The extract effects were investigated on body weight gain, food and water intakes, fasting glycemia and insulin sensitivity, compared with glibenclamide. Diabetic rats were obtained after 8 weeks of induction, characterized by hyperglycemia (148.78 ± 3.35 mg/dL), significant body weight gain and insulin resistance. The extract of *C. jagus* significantly ($P< 0.01$) reduced the fasting glycemia in diabetic rats (-42.62 %). The extract remarkably ($P< 0.01$) and more than the glibenclamide improved insulin sensibility (+55.47 % vs +27.97 %). Diabetic rats treated with the extract exhibited significant body weight decrease and a slight food intake increase. The phytochemical analysis of the extract revealed the presence of antidiabetic substances such as triterpenes, flavonoïds, phenols, saponins, anthocyanidines and coumarins. These results indicated that the DXM reduced the MACAPOS 1 diabetes induction duration to 8 weeks and also showed that the ethanol/water extract of *Crinum jagus* could have an antihyperglycemic activity and could improve peripheral insulin sensitivity. This might justify its traditional use in the management of diabetes.

Keywords: *Crinum jagus*, diabetic rat MACAPOS 1, dexamethasone, glycemia, insulin sensitivity.

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INTRODUCTION

Diabetes mellitus (DM) is widely recognized as one of the leading causes of death in the world.[1] In 2013, the global prevalence of diabetes worldwide was 382 million people constituting around 8.3% of the world population.[2] DM has become a real public health problem in developing countries, where its prevalence is increasing steadily and adequate treatment is often expensive or unavailable. This disease is a metabolic disorder characterized by fasting hyperglycemia and alterations in carbohydrate, fat and protein metabolism, associated with absolute or relative deficiencies in insulin secretion and/or insulin action.[3] As the disease progresses, pancreatic function falters and is no longer able to meet peripheral demands. As a result, insulin levels fail to keep up with the body requirements.[4] Control of plasma glucose concentrations is vital to decrease the incidence and severity of long-term diabetic complications. Management of diabetes with minimized side effects is still a complicated medical challenge. Dietary changes, oral hypoglycemic agents, or insulin injections, utilized either alone or in combination to prevent hyperglycemia cannot restore normal blood glucose homeostasis, and many limitations exist in their use. There is a growing interest in herbal remedies due to the side effects associated with orthodox therapeutic agents, the inability of existing modern therapies to control all the pathological aspects of diabetes, as well as the enormous cost and poor availability of the modern therapies for rural populations in developing countries. Traditional knowledge should be used for the learning of the pharmacological actions of plants commonly used in treating different diseases.[5] *Crinum jagus* (Amaryllidaceae) is a plant widely used in Africa as antitumoral, antiviral, antiparasitary and immunostimulator. It is also used against mental trouble and snake bite [6]. According to traditional medicine practitioners in the Western Region of Cameroon, this plant is efficient in DM management and also against poison. On diabetic rats MACAPOS 1,[7] which induction duration was reduced with the dexamethasone, investigations were carried on to study the effect of ethanol-water extract of *Crinum jagus* on fasting glycemia and insulin sensibility.

MATERIALS AND METHODS

Plant extract and phytochemical analysis

Fresh *Crinum jagus* plants were harvested in April 2008 in Bangou (Western Region of Cameroon). Botanical identification was performed at National Herbarium, Yaounde-Cameroon, with the voucher specimens HNC 14049. The whole plant was cleaned, sliced into small pieces, shade dried and powdered. Two thousand one hundred and fifty grams of this powder were macerated in 5 L of ethanol/water (4:1) mixture for 48 hours (with occasional stirring) at room temperature. After filtration, the residue was remacerated for 48 hours and filtered. The two filtrates were pooled, concentrated in a rotary evaporator (Rotavapor) at 40°C and dehydrated

to yield 195.8 g of dry dark extract. Phytochemical analysis of this extract for its active biological principles was conducted using the standard methods [8].

Animals

Male albino Wistar rats (6-8 weeks old) and Wistar mice (male and female, 8-10 weeks old) were used for the experiment. They were raised in the animal house of the Faculty of Science of the University of Yaounde I (Cameroon) under natural conditions of light and temperature, with free access to water and regular roden chow. Before testing the blood glucose level, the rats were fasted overnight but had free access to water. Animal housing and in vivo experiments were done according to the Guidelines of the European Union directive on Ethical Evaluation of Animal Experiments (CEE Council 86/609)[9] and ethically approved by the Institutional Committee of the Ministry of Scientific Research and Innovation of Cameroon.

Acute oral toxicity evaluation in mice

Albino mice were randomly allotted to five groups with ten animals each (5 males, 5 females). Mice were fasted for 12 hours. The normal control, was received the DMSO solution 3 %. Four groups received (*per os*) one of the various doses of the extract: 5, 10, 15 and 20 g/kg body weight (bw), dissolved in DMSO solution 3 %. The mice were closely observed for toxicity symptoms and behavioural changes for the first 2 hours, intermittently for the next 4, 6, 24 and 48 hours after extract administration; mortality recorded within 48 hours and the lethal dose that killed 50 % of mice (LD_{50}) was estimated.[10]

Efficiency doses and administration frequency assessment

Fasted male Wistar rats were randomly divided into seven groups containing three rats each. The different groups orally received one of the following *Crinum jagus* extract concentrations: 0, 50, 75, 100, 150, 200 and 300 mg/kg b.w. Blood glucose level was assessed at time 0 (before administration) and subsequently at time 1.5, 3, 6 and 12 hours after the administration of the extract. From the results obtained from this screening, we retained 3 doses (75, 150 and 300 mg/kg b.w.) for the determination of frequency of administration.

To determine the frequency of administration of the extract, 4 groups of 4 overnight fasted rats each were orally given respectively 1 mL/100 kg b.w. of vehicle (control animals), 75, 150 and 300 mg/kg b.w. *C. jagus* extract. Blood glucose level was assessed at 0 (before the extract administration), 6, 12, 24 and 48 hours after administration of the extract.

Results of the screening and the determination of administration frequency were taken as hypothetical reference to extrapolate a dose which will be used for evaluating the effects of ethanol/water extract of *Crinum jagus* on diabetic rats and the frequency of the administration of the extract.

Induction of experimental diabetes

To induce type 2 diabetes, adult rats were submitted to high sugar diet (HSD). Those rats also received per os 0.8 g/kg of dextrose (Gwaudan Laviretteet Cie, Glucose pure Anhydre) and 4 g/kg of sucrose (SOSUCAM, Bandjock-Cameroon) every two days.[11] In order to reduce the duration of diabetes induction, one month after the beginning of HSD, the animals received the dexamethasone (DXM) treatment (25 mg/kg b.w. *i.m.* Dexamethasone Rotex Medica Laboratory, Germany) every 2 days during 3 weeks. After 2 months of diet, all rats with fasting total blood glycemia > 110 mg/dL were considered as diabetic.[12]

Treatment of animals

Rats were divided into 4 groups of 6 animals each: normal controls (NC), diabetic controls (DC), diabetic rats treated with glibenclamide (10 mg/kg b.w.: DG10) and diabetic rats treated with 150 mg/kg b.w. of *C. jagus* extract (Cj150). The animals were treated once daily for 20 consecutive days. During the treatment, body weight gain and food intake were assessed every week. Fasting glycemia was estimated at the beginning and at the end of the treatment period.

Insulin Tolerance Test

At the end of treatment, the peripheral insulin resistance of all rats was assessed through the insulin tolerance test (ITT). For this test, rats were fasted for 12 hours before insulin challenge. Acute insulin (Actrapid Human HM, Ordinary, Novo Nordisk Laboratory, Bagsvaerd, Danemark, 2 UI/kg bw) was administered by intramuscular injection. Glycemia was estimated at 0 (before insulin administration), 10, 20, 30, and 60 min after insulin administration.

Blood glucose level

Blood samples for glucose determination were obtained from the tail tip of fasted rats. Blood glucose level was estimated using a glucometer Glucoplus.

Statistical analysis

The results are expressed as mean ($\overline{X}$) ± 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 $P< 0.05$.

RESULTS

Phytochemical analysis

The phytochemical analysis of the ethanol/water extract of *C. jagus* revealed the presence of different classes of chemical compounds such as triterpens, flavonoids, phenols, saponins, anthocyanidins, coumarins and polysaccharids.

Acute oral toxicity

Oral administration of ethanol/water (4:1) extract of *C. jagus* did not produce any observable changes in gross behavior. Mortalities were recorded in mice at higher doses (more than 5 g/kg bw), producing a LD_{50} of 10.5 g/kg b.w..

Dose efficacy and administration frequency

After single oral administration of different doses of *C. jagus* extract (50, 75, 100, 150, 200 and 300 mg/kg b.w.), glycemia progressively decreased from 1.5 hour to 12 hours. Twelve hours post dosing, the decrease of glycemia was 46.54 %, 46.80 %, 43.46 %, 47.69 %, 45.71 % and 51.46 % with different doses respectively. Three doses (75, 150, 300 mg/kg) were retained to determine the frequency of administration of the extract; the maximal decrease rate of the glycemia was observed 24 hours post dosing: -49.11 %, -54.62 %, and -33.71 % respectively (data not shown). The glycemia tended to initial normal values after 24 hours following the administration of the extract. The results achieved from this screening let to the use of 150 mg/kg bw dose for the evaluation of the effects of *C. jagus* ethanol/water extract on diabetic rats. Animals received this extract dose once daily.

Glycemia, body weight, food and water intake of diabetic rats

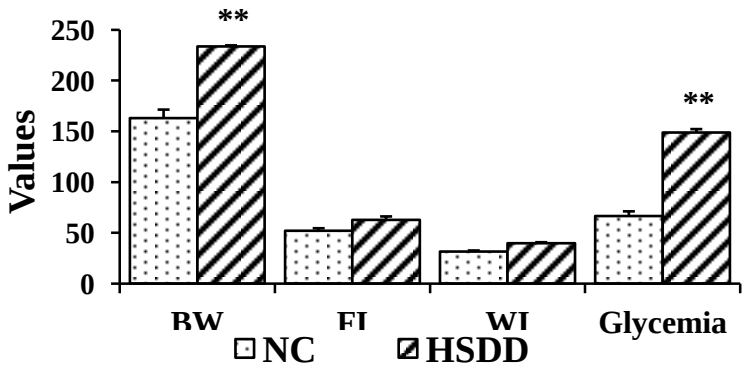

Figure 1: Anthropometric parameters (body weight, BW: g; food intake, FI: g; water intake, WI : mL; and fasting glycemia: mg/dL) after 8 weeks of diabetes induction. NC: normal control rats; HSDD: rats submitted to high sugar diet associated with glucocorticoids injection. Significant difference: **P< 0.01 compared to NC.

After 8 weeks of hypercaloric diet, the body weight of rats submitted to high sugar diet associated with dexamethasone injection (HSDD) significantly (P< 0.01) increased when compared to rats submitted to normal diet (NC). Food and water intake did not increase significantly. Furthermore, the HSDD glycemia was notably (P< 0.01) high compared to NC: 148.78 ± 3.35 mg/dL vs 66.57 ± 4.53 mg/dL (Fig. 1).

Effect of the extract on diabetic rats

Body weight gain and food intake

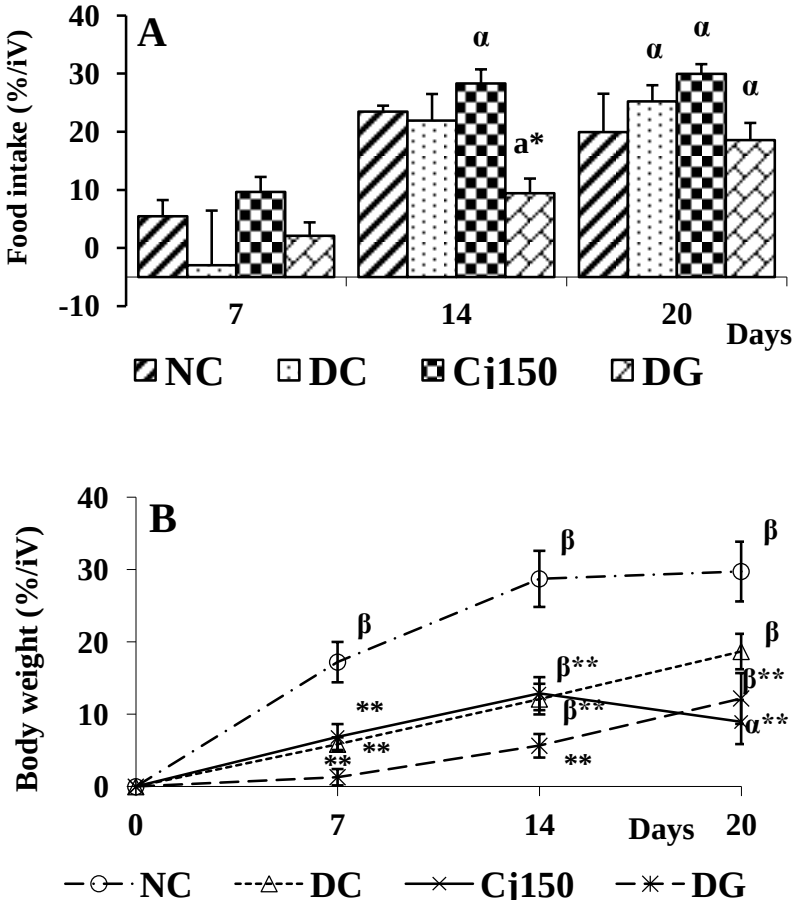

Figure 2: (A) Food intake and (B) Body weight (expressed as % of initial value iV) of rats during 20 days of once daily treatment. NC: normal control rats; DC: diabetic control rats; diabetic rats treated with: *C. jagus* extract 150 mg/kg b.w. (Cj150), glibenclamide 10 mg/kg b.w. (DG). Significant difference: $^{\alpha}$P< 0.05, $^{\beta}$P<0.01 compared to initial values; *P< 0.05, **P< 0.01 compared to NC and ^{a}P< 0.05 compared to DC. n=6 rats/group.

During the 20 days treatment, diabetic control rats (DC) body weight gain was significantly (P< 0.01) low compared to normal control rats (NC). Both extract and glibenclamide significantly (p<0.01) reduced body weight gain after 20 days of treatment: -69.92 % and -58.97 % respectively compared to NC (Fig 2B). By the end of the treatment, there was no significant difference in food intake (FI) among different animal groups. We observed a slight FI increase with DC compared to NC. FI tended to increase in Cj150 and decrease in DG (Fig 2A).

Glycemia

After 20 days treatment, the glycemia of diabetic rats treated with the extract (Cj150) or the glibenclamide (DG) remarkably (p< 0.01) decreased (-42.62 ± 1.97 % and -44.56 ± 2.28 % respectively) and was comparable to NC glycemia: 83.5 ± 4.17 mg/dL (Cj150), 82.50 ± 6.12

mg/dL (DG), 66.43 ± 2.23 mg/dL (NC). The DC glycemia remained very high: 148.78 ± 6.44 mg/dL (Fig. 3).

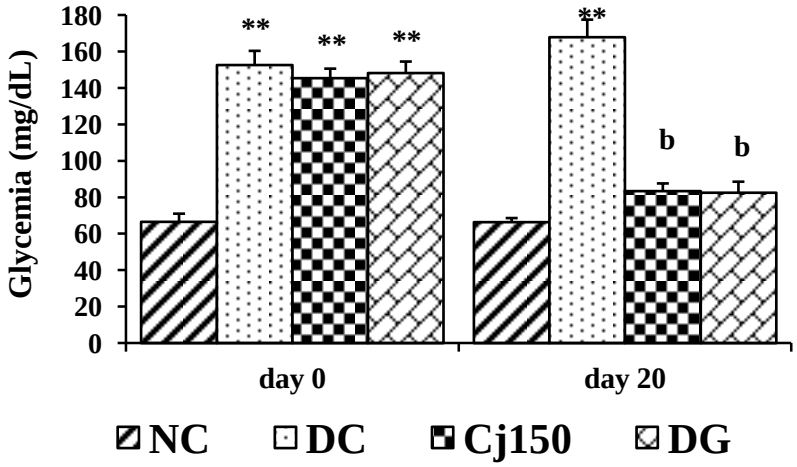

Figure 3: Glycemia (mg/dL) of rats at the end of once daily treatment. NC: normal control rats; DC: diabetic control rats; diabetic rats treated with: *C. jagus* extract 150 mg/kg b.w. (Cj150), glibenclamide 10 mg/kg b.w (DG). Significant difference: **P< 0.01 compared to NC; ^{b}P< 0.01 compared to DC. n=6 rats/group.

Peripheral insulin sensitivity

During the insulin sensitivity assessment, the glycemia of NC, Cj150 and DG significantly decreased as from 20 min, while DC glycemia rather seemingly increased: at 20 min 48.58 ± 2.95 mg/dL (-26.35 ± 4.98 %) for NC, 47.25 ± 2.50 mg/dL (-42.66 ± 5.40 %) for Cj150, 69.92 ± 6.16 mg/dL (-15.37 ± 2.21 %) for DG and 174.34 ± 18.78 mg/dL (4.30 ± 2.73 %) for DC. The decrease was more marked in Cj150 than in DG from 20 min to 60 min: at 60 min -55.47 ± 1.47 % for Cj150 and -27.97 ± 2.50 % for DG (Fig 4).

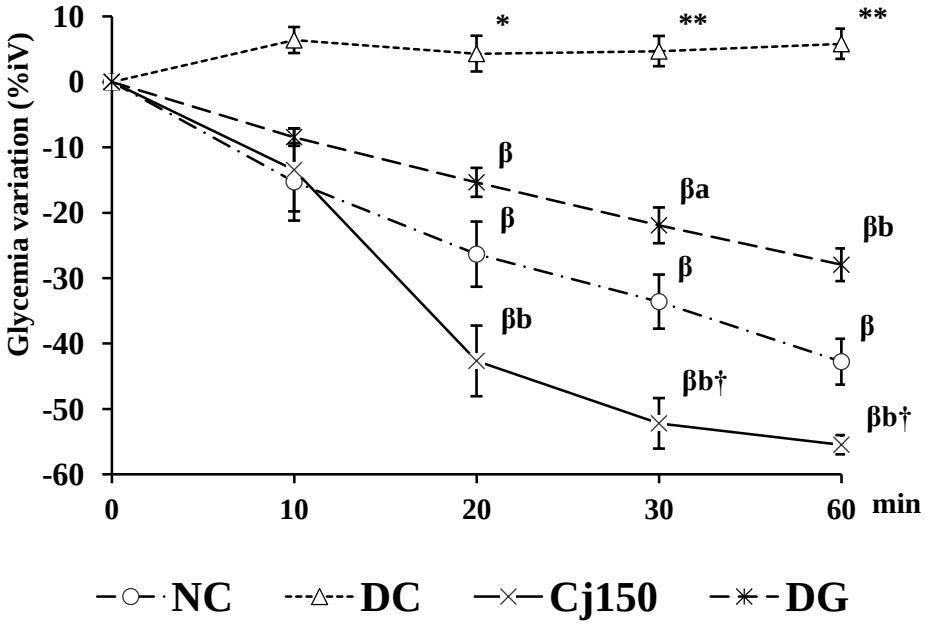

Figure 4: Insulin sensitivity (expressed as percent of glycemia variation/initial glycemia) of rats: NC: normal control rats; DC: diabetic control rats; diabetic rats treated with: *C. jagus* extract 150 mg/kg b.w. (Cj150), glibenclamide 10 mg/kg b.w. (DG). Significant difference: $^{\beta}P< 0.01$ compared to initial values; *P< 0.05, **P< 0.01 compared to NC; $^{a}P< 0.05$, $^{b}P< 0.01$ compared to DC and $^{\dagger}P< 0.05$ compared to DG. n=6 rats/group.

DISCUSSION

Crinum jagus is a plant traditionally used in the treatment of diabetes. In the present study, we investigated on diabetic rat MACAPOS 1, the effects of *C. jagus* ethanol/water (4:1) extract on glycemia and peripheral resistance to insulin.

Diabetes was induced by high sugar diet (HSD) associated with dexamethasone (DXM) injections. The results observed in the previous works with 16 weeks HSD alone, were obtained in this study just after 8 weeks with HSD associated with DXM injection: peripheral resistance to insulin, fasting hyperglycaemia and body weight gain.[7, 11] During the diabetes induction, diabetogenic effect of DXM (exogenous glucocorticoid) is due to its effect on corticoid biological receptor, leading to peripheral resistance to insulin by inhibiting the secretion and expression of insulin-sensitizing adiponectin gene, by reducing the glucose carrier number and activity, and by stimulating a neoglucogenesis from amino acids released from proteins degradation.[13, 14] The glucocorticoids stimulate lypolysis leading to the increase of plasmatic levels of non-esterified fatty acids which can induce resistance to insulin by masking insulin receptors. Moreover, hyperlipidemia induced by HSD modifies the membrane fluidity and makes corticoid

entrance into the cell easier, thus amplifying their effects.[15] Thus, the combined effects of HSD and DXM justify the shortening of the duration of diabetes induction to 8 weeks.

The glycemia of diabetic rats treated with *C. jagus* extract significantly decreased in value similar to glibenclamide treated diabetic rats. This result could suggest that, *C. jagus* extract as the glibenclamide might stimulate the release of insulin which activates the mechanisms leading to the decrease of glycemia.[16, 17] This glycemia decrease activity could result from some chemical compounds found in the extract, such as flavonoids, triterpenes, phenols, saponins, polysaccharides, anthocyanidines, and coumarine, which are known for their antidiabetic properties.[18, 19] So, the possible mechanism of action of the plant extract might be the stimulation of insulin release and/or insulin mimetic action of these compounds. However, in peripheral insulin sensitivity test, the glycemia decrease was more marked on diabetic rats treated with the extract than on those treated with glibenclamide. These results led us to think that the extract could also act on insulin peripheral sensitivity, by other means than those of glibenclamide. The peripheral effect of the plant extract might then be more pronounced than its insulin release effect. *C. jagus* extract might also have an antihyperglycemic effect since the decrease of glycemia did not bring its values under the normal range.

Diabetic rats treated with plant extract and glybenclamide presented a decrease of body weight. In glibenclamide treated rats, the increase of peripheral use of glucose might annihilate cellular energizing needs; this could explain the decrease of food intake and consequently the body weight gain decrease. On diabetic rats, plant extract apparently increased food intake while reducing body weight gain, and moreover improved the peripheral insulin sensitivity. These results suggest that the *C. jagus* extract might stimulate the appetite and at the same time energy expenditure.

In the preliminary acute toxicity test, the effects of ethanol/water extract of *Crinum jagus* neither induced behavioral changes nor mortality in mice when orally administered at single dose less than 10 g/kg. However, dose dependent mortality within a range 10-20 g/kg was recorded, giving 10.5 g/kg LD_{50}. The LD_{50} per oral route in experimental animals, and the first lethal dose (10 g/kg), which are greater than 5 g/kg, suggest that *C. jagus* extract is non or very little toxic. [20, 21]

CONCLUSION

The results indicated on one hand, that dexamethasone associated with HSD reduced the diabetes induction period from 16 to 8 weeks. On the other hand, *C. jagus* ethanol/water extract at the used dose was effective in decreasing glycemia, ameliorated the peripheral insulin sensitivity in induced diabetic rats and was not toxic. This is important to justify the use of this plant in the treatment of diabetes.

CONFLICTS OF INTEREST

The authors have none to declare.

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