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EFFECT OF *CRINUM JAGUS* EXTRACTS ON LIPIDS PARAMETERS AND NON ALCOHOLIC HEPATIC STEATOSIS IN MACAPOS 1 INDUCED DIABETES RATS

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ABSTRACT

Diabetes is associated with metabolic disorder, including alterations of lipid profile and fat tissue accumulation, which causes among other pathologies, non-alcoholic fatty liver disease. The aim of this work was to investigate the effect of *Crinum jagus* extracts on lipids parameters and non-alcoholic hepatic steatosis in MACAPOS 1 induced diabetes rats. To induce diabetes, wistar rats (6-8 weeks old) were fed with high sugar diet (HSD) associated, one month after the beginning of the HSD, with dexamethasone (DXM) injection (25 µg/kg once after 2 days during 3 weeks). During 50 days, diabetic rats once daily orally received Metformine (38 mg/kg b.w.), hydroethanolic (75, 150 mg/kg b.w.) and aqueous (150 mg/kg b.w.) extracts. After the induction, diabetic rats presented, overweight, insulin resistance, polydipsia, dyslipidemia associated with increased visceral fat, liver damage lipid peroxydation associated with decrease in antioxidant defense. *Crinum jagus* extracts remarkably improved the insulin sensitivity of peripheral tissues, reduced body weight gain, seric total cholesterol, triglycerids and increased the seric level of HDL-cholesterol. These effects led to LDL cholesterol and the atherogenic index reduction. The improvement of the lipid profile was associated with the reduction of visceral fat. The plant extracts also significantly ($P < 0.01$) reduced the amount of liver TG, the activity of seric transaminases, lipid peroxydation markers (plasma, liver, aorta) and increased tissue protein levels, SOD and catalase activity. The hydroethanolic extract was in a dose-dependant manner more effective than aqueous extract and metformine. These results showed that *C. jagus* extracts possess insulin sensitizing and antidyslipidemia activities, act against non-alcoholic hepatic steatosis and improve oxidative status on MACAPOS 1 diabetic rats. The results thus support the use of *C. jagus* in African folk medicine, mostly in obesity, diabetes treatment and likely its complications.

KEYWORDS: *Crinum jagus*, diabetic rat MACAPOS 1, lipids parameters, insulin sensitivity, hepatic steatosis.

INTRODUCTION

Diabetes Mellitus (DM) is widely recognised as one of the leading causes of death in the world.[1] In 2013, 382 million people have been diagnosed with diabetes in the world, and 593 million people could have the disease by the year 2035.[2] DM is a non-communicable disease characterised by chronic hyper glycemia, altered metabolism of lipids, carbohydrates and proteins which results from either the relative impairment in pancreatic insulin secretion or varying degrees of peripheral resistance to the action of insulin or both. Insulin deficiency has been known to stimulate lipolysis in the adipose tissue and give rise to hyperlipidemia and fatty liver. Thus, in diabetes, hypercholesterolemia and hypertriglyceridemia often occur.[3] The imbalance of lipid metabolism is one of the causes of the accumulation of fat (steatosis) in the liver, the premature appearance of atherosclerosis and increased susceptibility to lipid peroxidation. Lipid peroxidation impairs membrane functions by decreasing membrane fluidity and changing the activity of membrane-bound enzyme and receptors. Its products (lipid radicals and lipids peroxides) are harmful to the cells in the body and associated with atherosclerosis and damage to liver and other tissue. Hyperglycemia and dyslipidemia as well as oxidative stress generally coexist in diabetic patients.[4,5] As the knowledge of diabetes heterogeneity increases, more appropriate therapy is needed. 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 pathological aspects of diabetes, as well as the enormous cost and poor availabilities of the modern therapies for rural populations in developing countries.[6, 7] According to traditional medicine practitioners in Western and Eastern regions of Cameroon, *Crinum jagus* (Amaryllidaceae) is used as antidiabetic, antiobesity, antidiarrheoal remedy and also again poison and in general body detoxification.

This study was to ascertain the effect of *Crinum jagus* aqueous and hydroethanolic extracts on lipids parameters and non-alcoholic hepatic steatosis of MACAPOS 1 induced diabetic rats.

MATERIALS AND METODS

Plant extracts

Fresh *Crinum jagus* plants were collected from Batié (Western Region of Cameroon) during the month of April. The species was confirmed by the National Herbarium of Yaounde-(Cameroon), compared 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, 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.

Animals of experiment

For the experiment, Male albino Wistar rats (6-8 weeks old) 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 rodent chow. The animals were acclimatized to laboratory condition for one week before the experiment starts. Before testing the blood glucose level, the rats were fasted overnight but had free access to water. Animal housing and in vivo experiments were performed according to the Guidelines of the European Union directive on Ethical Evaluation of Animal Experiments (CEE Council 86/609)[8] and ethically approved by the Institutional Committee of the Ministry of Scientific Research and Innovation of Cameroon.

Diabetes induction

To induce type 2 diabetes, the rats were submitted to high sugar diet (HSD), 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 as formally described.[9] In order to reduce the duration of diabetes induction, the animals received, one month after the beginning of HSD, the glucorticoid (Dexamethasone (DXM) Rotex Medica Laboratory, Germany: 25 mg/kg b.w. *i.m.*) once every 2 days during 3 weeks. After 10 weeks of diet, the animals with fasting total blood glycemia ≥ 126 mg/dL were considered as diabetic and were selected for the next stage of experiment.

Effect of plant extracts on lipids parameters

The rats were divided into 6 groups of 5 animals each: normal controls (NC), diabetic controls (DC), diabetic rats treated with Metformine (38 mg/kg bw: Met38), diabetic rats treated with 75, 150 mg/kg bw of *C. jagus* hydroethanolic extract (Cjh75; Cjh150) and diabetic rats treated with 150 mg/kg bw of *C. jagus* aqueous extract (Cja150). The animals were treated once daily by intra-gastric gavages for 50 consecutive days. During the treatment, body mass was assessed every five days, food and water intakes were recorded every two days. At the end of the experimental period, all the rats were fasted over 24 hours.

At the end of treatment, the peripheral insulin resistance of all rats was assessed through the insulin tolerance test (ITT).

Under mild ether anaesthesia, all the rats were thereafter sacrificed. Blood was collected into heparinised and centrifuge tubes. The collected blood from heparinised tubes was used for the preparation of plasma and the one from 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 (seric lipids parameters, transaminases). The liver and aorta were excised immediately, thoroughly washed in ice cold saline and used for the preparation of tissue homogenates. Visceral and peritesticular white adipose tissues were removed and weighed; carcass was also taken and weighed.

- Insulin Tolerance Test

Rats received, after fasting (24 hrs), acute insulin (Actrapid Human HM, Ordinary, Novo Nordisk Laboratory, Bagsvaerd, Danemark, 2 UI/kg bw) by intramuscular injection. Glycemia was estimated at 0 (before insulin administration), 10, 20, 30, and 60 min after insulin administration using a glucometer Glucoplus™. Blood sample was obtained from the tail tip of fasted rats.

- Seric lipid profile

Analyses of supernatant serum total cholesterol (TC), triglycerides (TG) and HDL-Cholesterol (HDL-C) were performed, through colorimetric methods with commercially available test kits according to the manufacturer's recommendations (Fortress diagnostic UK). LDL-Cholesterol (LDL-C) level was calculated with the standard formula.[10]

$$\text{LDL-C} = \text{TC} - (\text{HDL-C} + \frac{TG}{5})$$

Atherogenic index (AI) was calculated as follows:[11]

$$\text{AI} = \text{Total Cholesterol} - \frac{\text{HDL cholesterol}}{\text{Total Cholesterol}}$$

Biochemical parameters of diabetic hepatosteatosis and biochemical analysis

Hepatic triglycerides and proteins, serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were estimated, and the ratio of AST to ALT was calculated. Hydroperoxides, malondialdehyde (MDA) and antioxidant enzymes (SOD, catalase) activity were also assessed at the end of treatment in plasma, liver and aorta homogenates. To prepare tissue homogenate, liver and aorta were homogenized respectively in 50 mM Tris-HCl buffer (pH 7.4) and in Mc Even solution (147 mM NaCl ; 5.6 mM KCl ; 2.6 mM CaCl2; 0.66 mM NaH2PO4; 11.9 mM CO3NaH ; 0.24 mM MgSO4 11 mM glucose, pH7.4), and then centrifuged at 10,000 rpm for 15 min; the super-natant obtained was used for various biochemical estimations. Superoxide dismutase (SOD) activity was determined according to the method of Mirsa and Fridovich.[12] Catalase activity was assessed using Sinha method.[13] Tissue total protein concentration was estimated by the Biuret method using Fortress test kit. Hydroperoxydes were estimated as describe by Jiang *et al*.[14] Malondialdehyde was determined using Yagi method.[15] Serum AST was determined according to Tietz and Shuey method.[16] Serum ALT was assessed using the method of Bergmeyer *et al*.[17]

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

Effect of aqueous and hydroethanolic extracts of *C. Jacus* on diabetics rats

- Peripheral insulin sensitivity

During the insulin sensitivity assessment, the glycemia of NC and diabetic rats treated with plant extracts or metformine significantly decreased as from 10 min with hydroethanolic extract and 20 min for others. The decrease was more marked in Cjh75 than in Cjh150, Cja150 and Met. At 60 min -56.59 ± 2.4 % for Cjh75, -42.91 ± 2.79 % for Cjh150, -33.75 ± 1.73 % for Cja150 and -37.70 ± 3.75 % for Met. The DC glycemia on a contrary remained high during the insulin sensitivity assessment (Fig. 1).

- Lipemia, atherogenic index

In diabetic control rat (DC), the seric levels of total cholesterol (TC), triglycerides (TG), cholesterol LDL (LDL) significantly ($P< 0.01$) increased while the HDL-cholesterol level (HDL) remarkably ($P< 0.01$) decreased, compared to NC. After 50 days of treatment, *C. jagus* hydroethanolic extract, in a dose dependent manner and more than aqueous extract and metformin, significantly ($P< 0.01$) reduced TC and TG level to normal values and LDL under normal value. Concomitantly, *C. jagus* extracts and metformin significantly ($P< 0.01$) increased HDL level to normal value (in Cja150 and Met) and over normal value (in Cjh75 and Cjh150) (Fig. 2A).

Atherogenic index (AI) of DC significantly ($P< 0.01$) increased compared to NC. At the end of treatment, *C. jagus* extracts more than metformin, significantly ($P< 0.01$) decreased AI to comparable normal value (Fig. 2B).

- Anthropometric parameters: body mass, visceral fat, carcass, food and water intakes

During the 50 days of treatment, DC body mass remained significantly ($P< 0.01$) high compared to NC. Meanwhile *C. jagus* extracts (hydroethanolic and aqueous) and metformin remarkably ($P< 0.01$) decreased body mass as from 10 days until the end of treatment. Yet, only *C. jagus* extracts decreased body mass to normal value from 20 Days and even under normal values à the end of treatment: 205.54±7.71 g for Cjh75, 235.48±8.84 g for Cjh150, 237.62±6.92 g for Cja150 and 248.44±5.79 g for NC (Fig. 3A).

Compared to NC, visceral fat of DC significantly ($P< 0.01$) increased. More than aqueous extract, hydroethanolic extract of *C. jagus* remarkably ($P< 0.01$) reduced visceral fat to normal values and even less in a dose dependent manner. On contrary, visceral fat of diabetics rats treated with metformin remained significantly high compared to NC (Fig. 3B).

Diabetes induced significant ($P< 0.01$) decrease of carcass weight in DC compare to NC. In a dose dependent profile, only the plant extracts significantly increased the carcass weight to normal value (Fig. 3C).

Food intake of DC significantly ($P< 0.01$) decreased as from the 20th day till the end of treatment. *C. jagus* extracts and metformin remarkably ($P< 0.01$) decreased body mass as from day 10 until the end of treatment. This decrease was more marqued with hydroethanolique extract: -49.25%, -39.45%, -23.61%, -31.36% respectively in Cjh75, Cjh150, Cja150 and Met38, at the end of treatment (Fig. 4A). Diabetes induced significant ($P< 0.01$) increase of water intake during the treatment. Cjh75, more than other extracts and metformin, remarkably ($P< 0.01$) reduced water intake of diabetic animals (Fig. 4B).

- Biochemical parameters of non-alcoholic hepatic steatosis

In diabetic rats, DC, hepatic triglycerides remarkably ($P< 0.01$) increased (212.24±4.15 mg/dL), hepatic proteins decreased ($P< 0.01$), the activity of AST and ALT significantly ($P< 0.01$) increased (140 %, 125.84 % respectively) compared to NC. The transaminases activity in DC increased above 30 %. However, the AST/ALT ratio remained below 1. After 50 days of treatment, much more than metformin and aqueous extract, hydroethanolic extract of *Crinum jagus*

significantly (P< 0.01) reduced the hepatic triglycerides. Despite the significant decrease, these values remained remarkably high (P< 0.01) with aqueous extract and metformin compared to NC. Plant extracts remarkably (P< 0.01) increased hepatic proteins to the normal values. *Crinum jagus* aqueous (150 mg/kg) and hydroethanolic (75 and 150 mg/kg) extracts remarkably (p<0.01) decreased AST and ALT activities that were compared to activity in NC: respectively -60.6 %, -59.85 %, -55.30 %, (AST), and -46.27 %, -58.71 %, -49.25 %, (ALT). The decrease of transaminases activity with metformin was not as efficient as with plant extracts (Tab. 1).

- Lipid peroxidation and antioxidant parameters

In DC, plasma, liver and aorta contents of hydroperoxydes and malondialdehyde (MDA) significantly (P< 0.01) increased while the activity of SOD and catalase in the liver and aorta drastically (p<0.01) decreased compared to NC. Aqueous (150 mg/kg) and hydroethanolic (75 and 150 mg/kg) extracts of *C. jagus* remarkably (p<0.01) decreased plasma, liver and aorta contents of hydroperoxydes. However hydroperoxydes levels remained significantly (p<0.05) high with Cjh75 compared to NC. The decrease of hydroperoxydes levels with metformin was significant (p<0.01), but the values remained remarkably above normal. *Crinum jagus* hydroethanolic extract, more significantly than metformin and aqueous extract, reduced MDA levels. *Crinum jagus* extracts as well as metformin significantly (p<0.01) enhanced SOD activity in liver and aorta. Much more than metformin, *C. jagus* extracts remarkably (p<0.01) increased the catalase activity in liver and aorta to normal values. On the other hand the catalase activity of diabetic rats treated with metformin remained significantly (p<0.05) low in liver and aorta compared to NC (Tab. 2).

Table 1: Liver and seric parameters of non-alcoholic hepatic steatosis in Rat after 50 days of once daily treatment.

	NC	DC	Met	Cjh75	Cjh150	Cja150
			Liver			
Triglycerides (mg/dL)	110.47±3.3	212.24±4.15**	140.95±2.77**^{b}	99.59±2.41^{b}	122.17±4.16^{b}	129.25±2.92*^{b}
Total protein (g/L)	56.96±4.01	32.38±4.27**	52.03±4.28^{*a}	56.03±3.96^{b}	58.46±4.99^{b}	55.29±3.77^{b}
			Serum			
AST (UI/L)	30.55±2.77	73.33±2.72**	53.33±1.62**^{b}	29.44±2.57^{b}	32.77±1.84^{b}	28.88±2.58^{b}
ALT (UI/L)	40.45±1.32	91.36±3.62**	69.09±1.7*^{b}	37.72±1.7^{b}	46.36±1.7^{b}	49.09±2.65^{b}
AST/ALT	0.76±0.06	0.80±0.03	0.77±0.02	0.78±0.04	0.71±0.03	0.60±0.05*

AST: aspartate aminotransferase; ALT: alanine aminotransferase; NC: normal control rats; DC: diabetic control rats; diabetic rats treated with *C. jagus* hydroethanolic 75 mg/kg (Cjh75), 150 mg/kg (Cjh150), aqueous 150 mg/kg (Cja150) extract, and metformine 38 mg/kg b.w (Met). n=5 rats/group. Significant difference: *p< 0.05; **p< 0.01 compared to NC; ^{a}p< 0.05; ^{b}p< 0.01 compared to DC.

Table 2: Plasma, liver and aorta lipids peroxidation parameters (Hydroperoxydes: Hpx, MDA), and antioxidant enzymes (SOD, catalase: CAT) of rats after 50 days of once daily treatment.

	NC	DC	Met	Cjh75	Cjh150	Cja150
			Plasma			
Hpx (nM/mg of protein)	2.75±0.18	6.41±0.15**	3.61±0.13*^{b}	2.81±0.17^{b}	2.58±0.13^{b}	3.09±0.05^{b}
MDA (nM/mg of protein)	1.61±0.06	4.86±0.04**	2.23±0.07*^{b}	2.07±0.04*^{b}	1.81±0.08^{b}	1.96±0.04*^{b}
			Liver			
Hpx (nM/mg of protein)	2.44±0.14	8.37±0.15**	3.97±0.11**^{b}	3.26±0.16*^{b}	2.7±0.12^{b}	2.83±0.09^{b}
MDA (nM/mg of protein)	1.37±0.07	6.16±0.06**	2.78±0.08*^{b}	1.75±0.06^{b}	1.63±0.06^{b}	2.17±0.07*^{b}
SOD (U/mg of protein)	3.18±0.07	0.74±0.02**	2.97±0.04^{b}	3.04±0.03^{b}	3.05±0.04^{b}	2.98±0.05^{b}
CAT (µM of H_20_2 consumed/min/mg of protein)	191.45±3.02	81.45±3.02**	170.09±3.49*^{b}	180.84±3.5^{b}	185.82±2.86^{b}	177.38±3.49^{b}
			Aorta			

Hpx (nM/mg of protein)	2.26±0.12	6.53±0.14**	3.10±0.07**^{b}	2.91±0.10*^{b}	2.63±0.12^{b}	2.80±0.14^{b}
MDA (nM/mg of protein)	1.25±0.11	5.17±0.09**	2.52±0.18*^{b}	1.71±0.17^{b}	1.50±0.18^{b}	1.83±0.16^{b}
SOD (U/mg of protein)	2.51±0.05	0.47±0.04**	2.35±0.07^{b}	2.47±0.04^{b}	2.54±0.06^{b}	2.44±0.04^{b}
CAT (µM of H_20_2 consumed/min/mg of protein)	81.48±1.22	23.48±1.22**	73.27±1.13*^{b}	76.78±1.41^{b}	80.29±1.77^{b}	76.51±1.51^{b}

NC: normal control rats; DC: diabetic control rats; diabetic rats treated with *C. jagus* hydroethanolic 75 mg/kg (Cjh75), 150 mg/kg (Cja150), aqueous 150 mg/kg (Cjh150) extract, and metformine 38 mg/kg b.w (Met). n=5 rats/group. Significant difference: *p< 0.05, **p< 0.01 compared to NC; ^{a}p< 0.05; ^{b}p< 0.01 compared to DC.

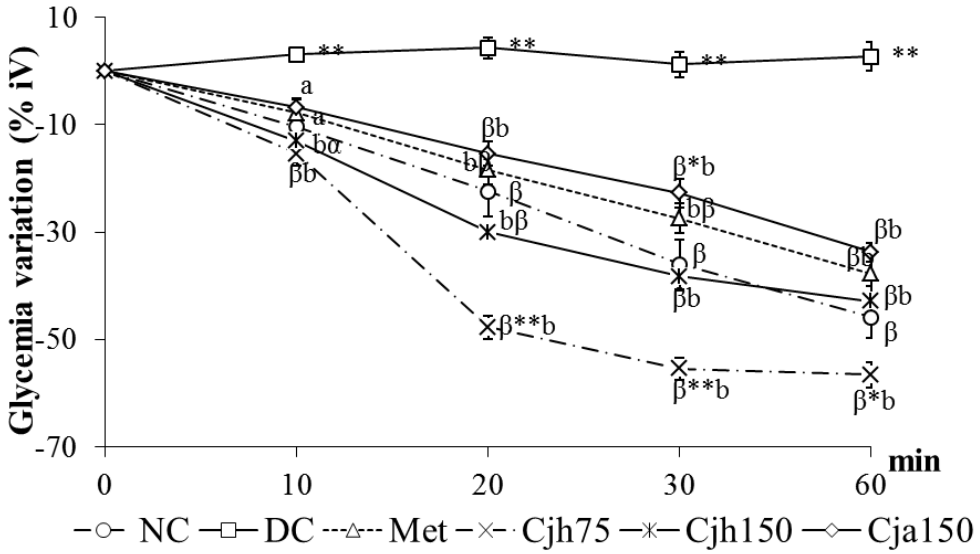
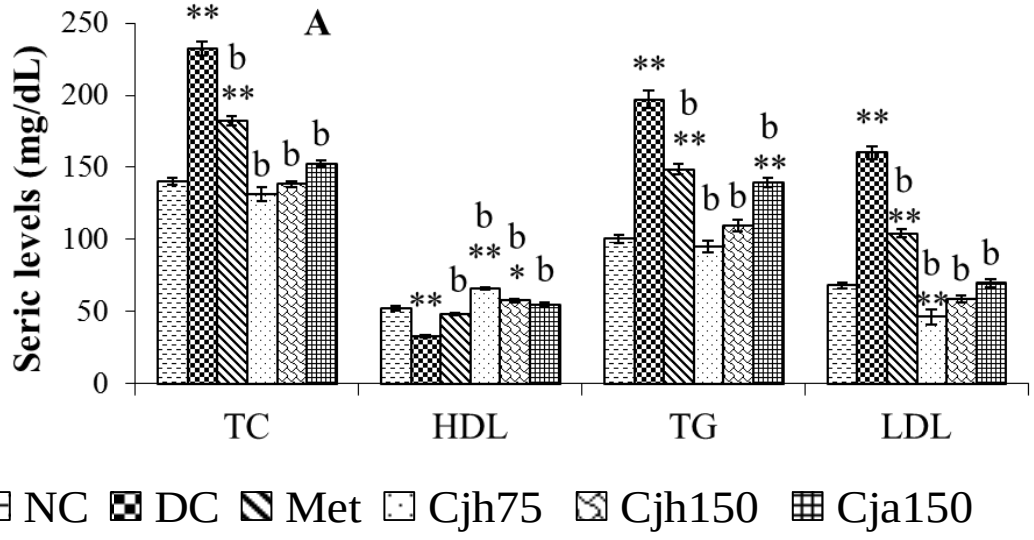


Figure 1: Insulin sensitivity (expressed as % of glycemia variation/initial value) of rats after 50 days of once daily treatment. NC: normal control rats; DC: diabetic control rats; diabetic rats treated with *C. jagus* hydroethanolic 75 mg/kg (Cjh75), 150 mg/kg (Cja150), and aqueous 150 mg/kg (Cjh150) extract, and metformine 38 mg/kg b.w (Met). n=5 rats/group. Significant difference: $^{\alpha}$p< 0.05; $^{\beta}$p< 0.01 compared to initial value; *p< 0.05; **p< 0.01 compared to NC; ^{a}p< 0.05; ^{b}p< 0.01 compared to DC.



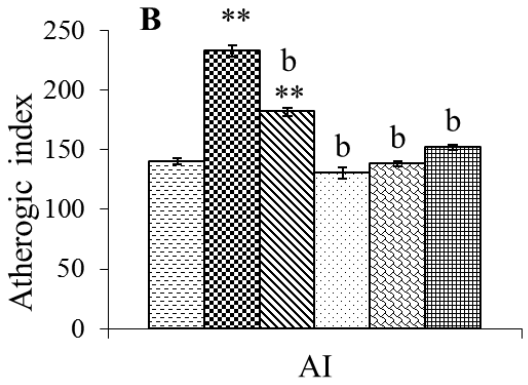
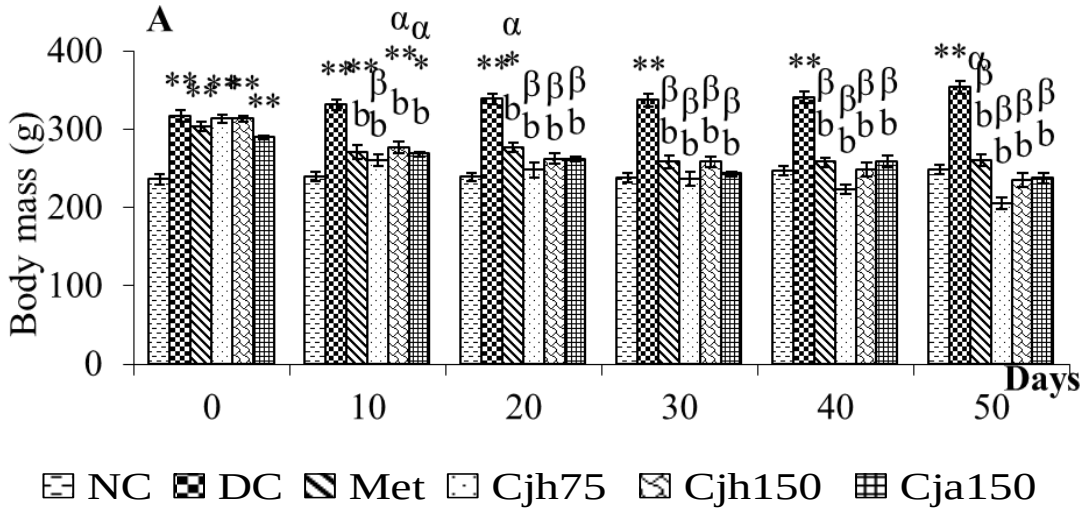


Figure 2: (A) Lipemia (HDL-cholesterol: HDL, Triglycerides: TG, LDL-cholesterol: LDL, Total Cholestérol: TC) and (B) Atherogenic index (AI) of rats after 50 days of once daily treatment. NC: normal control rats; DC: diabetic control rats; diabetic rats treated with *C. jagus* hydroethanolic 75 mg/kg (Cjh75), 150 mg/kg (Cja150), aqueous 150 mg/kg (Cjh150) extract and metformine 38 mg/kg b.w (Met). n=5 rats/group. Significant difference: *p< 0.05; **p< 0.01 compared to NC; [b]p< 0.01 compared to DC.



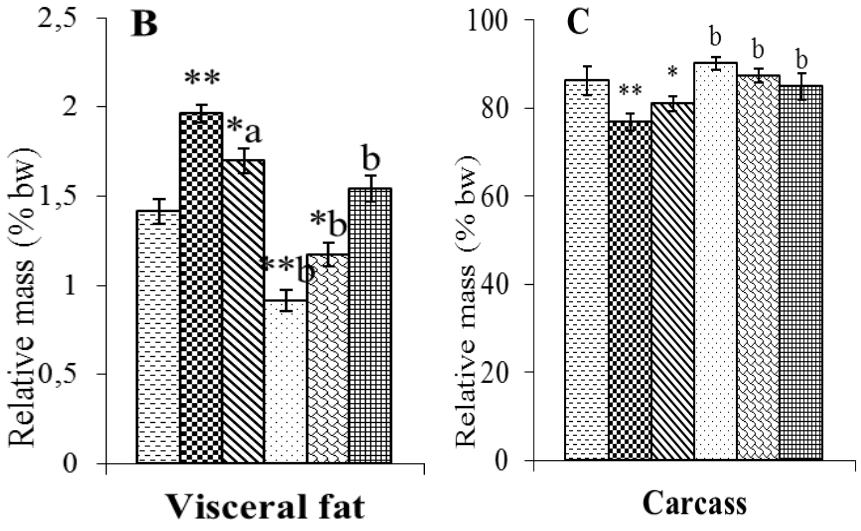


Figure 3: Value of (A) Body mass during, (B) Visceral fat and (C) Carcass of rats at the end of 50 days once daily treatment. NC: normal control rats; DC: diabetic control rats; diabetic rats treated with *C. jagus* hydroethanolic 75 mg/kg (Cjh75), 150 mg/kg (Cjh150), aqueous 150 mg/kg (Cja150) extract, and metformine 38

mg/kg b.w (Met). n=5 rats/group. Significant difference: $^{\alpha}p<0.05$, $^{\beta}p<0.01$ compared to initial values; *p< 0.05, **p< 0.01 compared to NC and $^{a}p< 0.01$, $^{b}p< 0.01$ compared to DC. n=5 rats/group.

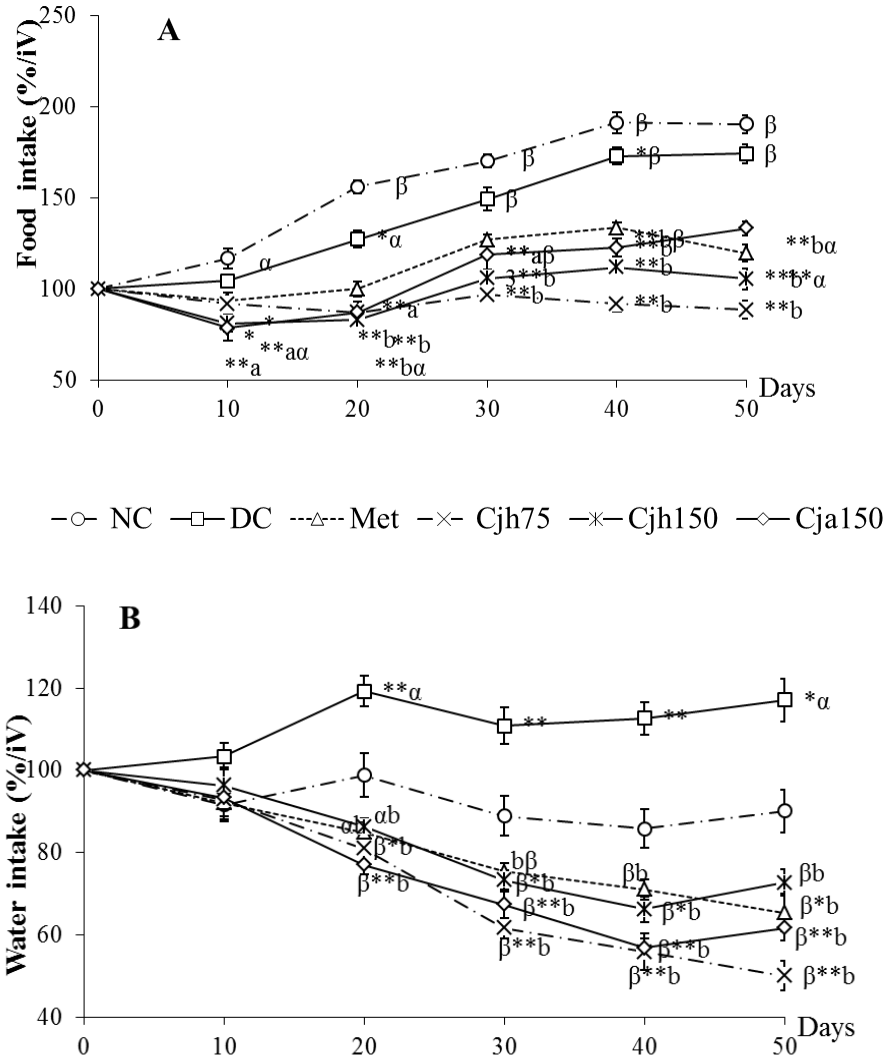


Figure 4: Food (A) and Water (B) intakes (expressed as % of initial values) of rats during 50 days of treatment. NC: normal control rats; DC: diabetic control rats; diabetic rats treated with *C. jagus* hydroethanolic 75 mg/kg (Cjh75), 150 mg/kg (Cjh150), and aqueous 150 mg/kg (Cja150) extract and metformine 38 mg/kg b.w. (Met). n=5 rats/group. Significant difference: $^{\alpha}p<0.05$, $^{\beta}p<0.01$ compared to initial values; **p< 0.01 compared to NC and $^{b}p< 0.01$ compared to DC.

DISCUSSSION

The present work aimed to investigate the effect of *Crinum jagus* extracts on lipids parameters and non-alcoholic hepatic steatosis in MACAPOS 1 induced diabetes rats. Diabetes was induced by high sugar diet (HSD) associated with dexamethasone (DXM) injection.[18] Resistance to insulin induced by the combined effect of HSD and DXM was associated with dyslipidemia. More than metformin, *Crinum jagus* extracts notably improved peripheral insulin sensitivity. During the assessment of peripheral insulin sensitivity, the best decrease in blood glucose level was observed with 75 mg / kg hydroethanolic extract. This result suggests that the peripheral insulin sensitizing pathways of *C. jagus* are far more effective than those of metformin. The insulin-sensitizing effect of plant extracts could result from some chemical compounds found in those extracts such as polyphenol, flavonoid, triterpen, saponnin, antocyan, coumarin[18] which are known for their antidiabetic properties.[19,7,20] Different efficiency of *C. jagus* extracts on insulin sensitivity improvement might be linked to various proportions of chemicals in these extracts.[18] The improvement of insulin sensitivity observed in diabetics treated rats was correlated with improvements in serum and tissue lipid status in these animals. The decrease in triglyceride, total cholesterol, LDL-cholesterol and selectively visceral fat levels observed in diabetics treated with plant extracts is associated with an increase in HDL-cholesterol. The improvement in the peripheral sensitivity of tissues to

insulin would therefore be a result of the lowering of triglycerides by the extracts. In fact, the increase of triglycerides in particular will lead to an increase in the plasma level of NEFAs which have the capacity to induce insulin resistance.[21, 4] By lowering lipemia and selectively visceral fats, the extracts improve insulin sensitivity, destroying adipocytes, preventing the accumulation or" burning" the fats of visceral adipose tissue capable of secreting adipokines such as TNF- α (Tumor necrosing factor-α) and resistin which have the capacity to induce severe insulin resistance.[22] This would also explain the fact that the best insulin-sensitizing activity is observed with the hydroethanolic extract which remarkably reduced lipemia and visceral fat. The effect of *C. jagus* on serum and tissue fats could also be explained by the presence in extracts of saponins, flavonoids, polyphenols and anthocyanidins which have lipid-lowering properties.[10] Saponins have lipolytic activity and reduce the intestinal absorption of cholesterol.[23, 24] Flavonoids have the ability to reduce adipogenesis and decrease fat deposition. They also decrease the level of plasma triglycerides.[25] The decrease in visceral fat, which is an adipose tissue responsible for the pronounced effect of insulin resistance through the excessive production of NEGAs,[26] the decrease in serum levels of LDL-cholesterol and total cholesterol could confer to *C. jagus* extracts, protective properties on the vessels. Protective properties which would have resulted not only in the significant decrease of atherogenic index, but also in the increase of serum HDL-cholesterol levels, in animals treated with plant extracts. Also called good cholesterol, increasing serum HDL cholesterol levels reduce cardiovascular risks.[25] The significant decrease in fat is believed to be the cause of the remarkable weight loss observed in the diabetics treated.

The excess weight observed in diabetic animals may be due to the storage of energy in fatty form at the cost of lean mass, justifying the significant decrease of carcass weight while visceral fat increased compared to normal animals on which the carcass weight is high and the fat low. This decrease of carcasses weight could result from a lean mass destruction and the osteoporosis that might be due to dexamethasone.[27] *C. jagus* may act in favor of the reconstitution of the lean mass and the bone mass of diabetic animals at the cost of fats. This could justify the increase of carcass weight, the decrease of fat and consequently the remarkable reduction in the body weight of treated animals. In all diabetics treated rats, the decrease of body weight was also associated with a remarkable decrease of food intake. The low effect of metformin, an insulin sensitizer, on the carcass suggests that *C. jagus* could reconstitute protein and bone mass by many other ways than strengthening the anabolic action of insulin, by improving insulin sensitivity. The decrease in water intake observed in both diabetics treated with metformin and those treated with *C. jagus* extracts may result from the restoration of glucose homeostasis.[28]

Most of type 2 diabetics suffer from fatty liver disease.[29,30] The increased serum alanine aminotransferase (ALAT) and aspartate aminotransferase (AST) activity, hepatic triglycerides and the decrease in hepatic protein level observed in diabetic animals confirm that the combined effect of the high sugar diet and dexamethasone induced liver damage in these animals, more specifically steatosis associated with cell necrosis (steatohepatitis or non-alcoholic fatty liver disease).[31, 30] Aqueous and hydroethanolic extracts of *C. jagus* remarkably reduced transaminases activity, hepatic triglycerides, and increased hepatic proteins. Absolute or relative insulinopenia stimulates lipolysis and leads to an increased release of fatty acids in order to supply the body with energy. These fatty acids are used in hepatic gluconeogenesis and the excess accumulated in the liver is converted into triglyceride[4] resulting in an increase of hepatic triglycerides. This could explain the hepatic accumulation of triglycerides in diabetic animals. Plant extracts by improving the peripheral sensitivity of tissues to insulin, restore glucose homeostasis, thus reduce the release of non-esterified fatty acids (NEFA) from adipose tissue and consequently their accumulation in the liver. The drop in hepatic triglycerides could also be linked to the weight loss of diabetics treated rats. An average weight loss of 8% in type 2 diabetics is associated with 80% reduction of liver fat content.[30] The hypotriglyceridemic effect of extracts on the liver could be due to the presence in these extracts of lipid-lowering compounds such as: saponins, flavonoids, polyphenols, anthocyanidins.[10]

The increase of transaminase activity in diabetics was associated with increased lipid peroxidation parameters (hydroperoxides and malondialdehyde: MDA) in the liver. Oxidation of liver lipids induces damage and even liver cell necrosis. This justifies the increase of serum transaminase activity which is one of the diagnostic criteria of hepatitis.[32,31] The decrease of serum transaminases activity may be due to the decrease of lipids peroxidation in the liver of animals treated with plant extracts. The presence in the extracts of antioxidant compounds such as: polyphenols, flavonoids, saponins, tannins and anthocyanidins could be responsible of regenerative properties of liver cells destroyed under the effect of reactive species. By reducing lipids accumulation and peroxidation in the liver, plant extracts could remarkably improve liver function. This may justify in animals treated with plant extracts, the increase of hepatic proteins[31], could accelerate the regeneration and the protection of hepatocytes.[33, 31] Despite the fact that it decreases triglycerides and increases proteins in the liver, metformin seemed not to cure the steatosis, since the percentage of transaminases remained high and more than 30% compared to normal control, with the ratio AST/ALT less than 1.[34] Furthermore, metformin did not have a positive impact on lipid peroxidation. This is because metformin does not improve steatosis in diabetic patients.[35]

In diabetic animals, increase of hydroperoxides and MDA went along with the decrease of SOD and catalase activity. These results suggest that the combined effect of high sugar diet and dexamethasone provoked oxidative stress in these animals. The increased percentage of hydroperoxides and MDA in the plasma, liver and aorta of diabetic animals is indicative of the deep structural disorganization of the membranes of liver cells, aorta and many other cells. Thus leading to membrane hyperpermeability resulting from lipid peroxidation.[32] By decreasing plasma, liver and aorta levels of hydroperoxides and MDA, *C. jagus* extracts remarkably reduced lipid peroxidation. This could be beneficial for the restoration of liver function and even in arterial health. At the same time, plant extracts remarkably increased the activity of superoxide dismutase (SOD) and catalase (CAT), two antioxidants enzymes. *C. jagus* extracts could then protect the liver, aorta, and many other tissues from harmful effects of superoxide anion, hydrogen peroxide and many other free radicals as well as the consequent lipid peroxidation. The antioxidant activity is much more marked with the hydroethanolic extract than with the aqueous extract. This difference could be related to the proportion of polyphenols and flavonoids, which is higher in the hydroethanolic extract than in the aqueous extract.[36]

CONCLUSION

The results indicated that, both aqueous and hydroethanolique extracts of *C. jagus* at the used doses have insulin sensitizing and antidyslipidemia activities, improve anthropometric parameters, act against non-alcoholic hepatic steatosis and improve oxidative status in MACAPOS-1 induced diabetic rats. The results thus support the use of *C. jagus* in African folk medicine, mostly in obesity, diabetes treatment and likely its complications.

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