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Evaluation of Acute Toxicity and Hypoglycaemic and Hypolipidaemic Effects of *Cyathula prostata* (Linn.) Blume Weeds on Adult Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Author SOO designed the experiment, the protocol for the study and also partook in the manuscript preparation and statistical analysis. Author GOM undertook the tissue processing and analysis as well as partook in the write up and final editing of the manuscript. Author OEA managed the literature searches. Author DAO perform the spectroscopy analysis. Author TAA managed the experimental process as well as identified the plant species. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Objectives: The study aimed at evaluating the acute toxicity and hypoglycaemic and hypolipidaemic effects of *Cyathula prostata* plant used locally in the treatment of various diseases including diabetes.
Materials and Methods: The toxicity of the extract was evaluated in Swiss albino mice by feeding the animals with the graded doses of the extract between 1.0 to 20.0 g/kg body weight (bwt) and continuously observed for the first 4 h, then hourly for the next 12 hand 6 hourly for the next 56 h (72 h, acute toxicity). Diabetes was induced in the male and female Wistar rats with alloxan monohydrate, at the dose of 150 mg/kg, dissolved in normal saline and administered intraperitoneally (i.p). The plasma glucose levels of the induced animals were monitored with a

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Glucometer (MERCK) after 72 h. The animals with plasma glucose level ≥200 mg/dl were classified as diabetic and were included in the study. The diabetic animals were randomly distributed into five groups of 5 animals each. The first group was normal not treated but received acacia 2% w/v solution. The second group was induced but not treated while the remaining four groups were treated with the reference drug, glibenclamide, (600 µg-1 kg bwt)/ the extract in graded doses of 75, 150 and 250 mg/kg bwt respectively for 30 days.

Results: The animals that received 20 g/kg bwt of the extract survived beyond 24 hrs, therefore the LD_{50} could be assumed to be above 20 g^{-1} kg bwt. The plasma level of alanine aminotransferase (ALT) was found to have significantly ($p \leq 0.05$) increased in the extract treated compared to the negative control whereas aspartate aminotransferase (AST) level showed marked decrease with dose. However, hepatic tissue histology at the highest extract dose treatment showed mild portal hepatitis with hepatocytes vacuolization and sinusoidal congestion. There were no significant changes ($p \geq 0.05$) in protein and creatinine levels. There were significant ($p \leq 0.05$) reduction in the plasma glucose, cholesterol, triglycerides and LDL levels while a significant increase in HDL levels was observed. The tissue histology of pancreas showed significant survivor of beta cells in the extract treated groups.

Conclusion: The extract was observed to have beneficial effect on alloxan-damaged pancreas as beta cells showed signs of recovery.

Keywords: Acute; toxicity; hypoglycaemic; hypolipidaemic; Cyathula prostata.

1. INTRODUCTION

Diabetes mellitus (DM) is one of the ravaging diseases affecting many lives world over. It is now recognized as one of the leading causes of death in the developing countries where the high prevalence of the disease could be attributed to improved nutritional status coupled with a gross lack of modern facilities for the early diagnosis of the disease [1]. Diabetes, a metabolic disorder of multiple aetiology, is characterised with disturbances of carbohydrate, fat and protein metabolism resulting from absolute or relative lack of insulin or both leading to hyperglycaemia [2], and in general is characterised with some symptoms such as increased thirst, polyuria, blurring of vision and weight loss [3]. They are basically divided into type 1 diabetes resulting from beta-cell destruction, usually leading to absolute insulin deficiency and type 2 also known as Non-Insulin Dependent DM (NIDDM). In type 2 diabetes, insulin is secreted but not being utilized by the tissues or an organ of the body [4].

DM has been conventionally treated with orthodox medicines that function as hypoglycaemic agents and as insulin production modulators or lipoprotein lowering agents [1]. Sulfonylurea and metformin have been invaluable in the management of hyperglycaemia in NIDDM but they are often unable to lower blood glucose concentrations to within the normal range, or to reinstate a normal pattern of glucose homeostasis [5]. Even when effective glycaemic control is achieved, the use of these drugs is restricted by their pharmacokinetic properties, secondary failure rates and accompanied undesirable effects [6,7]. In addition, they are not suitable for use during pregnancy [5,8]. Since the therapy is life long, therapeutic agents devoid of the above side effects would be appreciated and one of such approaches is the use of a popular alternative system of medicine, herbal medicine [9].

Herbal medicine, an oldest mode of therapy, is of great interest as it has been observed to be effective even in small doses for their physiological benefits and is perceived to have minimal side effects. Herbal medicine has been reported to be effective in the treatment of certain diseases such as diabetes where orthodox medicine could only proffer palliative effect. Several herbs have been reported in folk medicine to be successfully employed in the management of DM especially Type 2 diabetes (non-insulin dependent diabetes) [10]. The popularity of herbs and natural products in developing countries could be attributed to their being a cheaper source of healthcare compared to conventional medicines and are also associated with lesser side effects [11].

Cyathula prostata (*C. prostate*) is being used by traditional herbalist in the treatment of diabetes singly or in combination with other herbs. The aim of this study is, therefore, to scientifically evaluate *C. prostata* acute toxicity, hypoglycaemic and hypolipidaemic activity as well as its cardiovascular risk benefits giving credence to its local use.

2. MATERIALS AND METHODS

2.1 Plant Materials

The *C. prostate* (Linn.) Blume (*Amaranthaceae*) plant material was obtained from a traditional herbalist based in Lagos, Nigeria. The plant sample was identified and authenticated by a taxonomist in the Department of Botany of the University of Lagos where the voucher specimen was (LUH 3161) deposited in the Department Herbarium.

The dried powder of *C. prostata* plant (870 g) was soaked in 10 L of boiled (100℃) distilled water over night, with regular stirring. The extract was clarified by filtration with sterilized muslin cloth [12] and lyophilized to yield 122.5 g gel (14.1% yields).

2.2 Acute Toxicity Study

The toxicity study was carried out using twenty-five (25) male and female Swiss albino mice weighing between 20 – 25 g. The animals were randomly distributed into one control group and four treated groups, containing five animals per group. They were maintained on Animal feed, (Livestock feed Nigeria Ltd) provided with water *ad libitum* and were allowed to acclimatize to the laboratory conditions for seven days before the experiment. After overnight fast of the animals received acacia 2% w/v solution orally, while each treated group received orally solution of the extract in the doses of 5 g, 10 g, 15 g and 20 g/kg body weight (bwt) respectively. The stock solution was prepared by dispersing 16 g of the gel with 7 ml of the acacia solution in a 100 ml beaker and then transferred to a 20 mL volumetric flask. The volume was made to mark with the acacia solution to give a stock solution of 800 mg/mL (80% w/v). For mice of average weight of 22.5 g were administered 20,000 mg/kg bwt (20 mg/g), the total volume consumed was 0.56 mL (450÷800 mL) while for 15,000 mg/kg 15 mg bwt (/g) the total volume received was 0.42 mL [13].They were closely observed in the first 4 hours and then hourly for the next 12 hours followed by 6 hourly intervals for the next 56 hours (overall 72 hr) after the drug administration to observe any death or changes in general behaviour and other physiological activities [14,15] (Table 1).

2.3 Determination of LD_{50}

Since all the animals that received 20 g/kg bwt survived beyond 24 hrs, the median lethal dose (LD_{50}) was assumed to be more than 20 g/kg bwt [16]. The results obtained is shown in Table 1.

2.4 Diabetic Study

Healthy Wistar rats of both sexes weighing 120±15 g were used. The animals were fed on Animal feed (Livestock feed, Nigeria Ltd), and provided with water *ad libitum*. Diabetes was experimentally induced after fasting the animals overnight by administering intraperitoneally (i.p) alloxan monohydrate at 150 mg/kg bwt [17] dissolved in normal saline. After 72 hr, the blood sugar levels were monitored with a glucometer (Accu-Chek, Roche Diagnostics) and the rats with plasma glucose ≥ 200 mg/dl were classified as diabetic and were used in the study. A total of six groups containing five animals per group were used. Five groups comprising groups 2-6 were diabetic while group 1 was used as positive control. Treatment was daily for 30 days as follows:

Group 1: Normal rats not induced with diabetes to serve as positive control but received acacia 2% w/v solution.
Group 2: Alloxan-induced diabetic rats not treated, to serve as negative control.
Group 3: Alloxan-induced diabetic rats treated with Glibenclamide 600 µg/kg bwt dissolved in acacia 2% w/v solution [18]
Group 4: Alloxan-induced diabetic rats treated with 250 mg/kg bwt extract
Group 5: Alloxan-induced diabetic rats treated with 150 mg/kg bwt extract
Group 6: Alloxan-induced diabetic rats treated with 75 mg/kg bwt *e*xtract

The animals were initially weighed and subsequently weighed every seven days from the beginning to the end of the treatment to observe variations in the body weights. On the 31st day, after overnight fast they were sacrificed under mild diethyl ether and blood was obtained via cardiac puncture and distributed into heparinized, EDTA and fluoride containers respectively for biochemical, haematogical and plasma glucose studies. The blood was centrifuged within 5 min of collection at 4000 rpm for 10 min to obtain plasma which was analysed for glucose level, total cholesterol, total triglycerides, high density lipoprotein (HDL) cholesterol levels by precipitation and modified enzymatic procedures from Sigma Diagnostics [19]. Low density lipoprotein (LDL) and cholesterol levels were calculated using

Friedwald equation [20]. The results are given in Table 2.

Plasma was analysed for alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and creatinine by standard enzymatic assay analysis and the plasma protein and glucose contents were determined using enzymatic spectroscopic methods [21]. The results are shown in Table 3.

2.5 Phytochemical Evaluation of the Crude Extracts

Phytochemical screening of the extract was performed for the presence of secondary metabolites according to the method of Farnsworth, [22] and Harborne, [23].

2.6 Tissue Histology

The organs were fixed in 10% formal saline for ten days before embedding in paraffin wax. Each organ tissue was sectioned at 5µm and stained with Haematoxylin and Eosin (H and E) stain [24]. The slide specimens were examined under light microscope at high power magnification for changes in organ architecture and photomicrographs were taken.

2.7 Statistical Analysis

Differences in total blood glucose, total plasma cholesterol, total plasma triglyceride, LDL-cholesterol and HDL-cholesterol levels, AST, ALT, creatinine levels and body weight variations for all treated and control rats were determined using an analysis of variance (ANOVA). Significant differences were determined using a Student's t-test and differences were considered significant if $p \leq 0.05$. All data are expressed as mean ± standard error of the mean.

3. RESULTS AND DISCUSSION

Herbal medicines have received greater attention as alternative to clinical therapy in recent times leading to subsequent increase in their demands [8,18]. In rural communities, the exclusive use of herbal drugs in the treatment of various diseases is still very common. Preparations with different parts of *Cyathula prostata* has been used by herbal practitioners in the treatment of various diseases including DM.

The acute toxicity results are shown in Table 1. The median lethal dose (LD_{50}) value of the extract was observed to be higher than 20 g/kg bwt. According to Ghosh [25] and Klaasen et al. [26], the extract can be classified as being non-toxic, since the LD_{50} by oral route was found to be much higher than WHO toxicity index of 2 g/kg.

The postprandial plasma sugar activity is shown in Fig. 1. The extract was observed to demonstrate a significant ($p \leq 0.05$) postprandial plasma sugar lowering effect explaining its' α-glucosidase activities. The weight variations (Fig. 2) results showed the effect of the extract on the body weight of the diabetic and normal rats. Also the effect of the reference drug, glibenclamide, on the diabetic rats was shown. There was a decrease in the weight of the animals treated with the least dose (75 mg/kg) of the extract in the first seven days and subsequently, weight gain was observed compared to the control. The decrease in body weight of the untreated rats was observed throughout the experiment. The body weight of the other treated groups increased marginally but was still lower than those treated with the reference drug at day 7. However, all showed progressive decrease to the last day of the treatment. This showed that the extract might be lacking obesity forming tendency, which is one of the draw backs associated with sulphonylureas.

The result of the biochemical parameters are shown in Table 2. In the extract treated animal groups, the plasma level of ALT was found to have significantly ($p \leq 0.05$) increased compared to the negative control whereas AST level showed marked decrease with dose. An elevation in plasma concentration of ALT is usually due to liver damage while increase in AST level could be linked to damage to either cardiac or hepatic tissues or damage to both [19,20]. The photomicrograph of hepatic tissue at the highest dose of extract treatment showed mild portal hepatitis with hepatocytes vacuolization and sinusoidal congestion while the histology of cardiac tissue treated with the extract indicated no pathological changes. It was therefore obvious from the hepatic tissue morphology that the extract had deleterious effect on the liver at the highest dose of administration. Also, the extract at the highest dose showed significant ($p \leq 0.05$) increase in the ALP levels and the reason for this cannot be readily ascertained.

Table 1. Acute toxicity of aqueous *C. prostata* leaves extract in mice

Doses of the drug (g/kg)	Log dose	Number of mice used	Number of mice dead	%Cumulative number of mice dead
0.50	-0.30103	5	0	0
1.0	0	5	0	0
2.5	0.39794	5	0	0
5	0.69897	5	0	0
10	1.0000	5	0	0
15	1.1761	5	0	0
20	1.3010	5	0	0

Control group received orally 0.3ml acacia solution 2% w/v

Table 2. Plasma glucose level and other biochemical profiles of untreated diabetic rats, diabetic rats treated with the extract and glibenclamide respectively and the normal control rats

Parameters	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
AST (µ/ mm^3)	115.4±0.62*	178.4±0.14	163.4±0.25*	138.2±0.58*	143.0±0.25*	154.0±0.16*
T.Bil (µmol)	1.3±0.07*	0.6±0.02	0.7±0.03	0.8±0.04	0.4±0.05	0.3±0.02
Creatinine	25.26±0.11*	33.16±0.14	26.72±0.08*	28.90±0.32	28.91±0.34	29.16±0.67
Urea (µmol)	5.6±0.50*	12.6±0.28	6.1±0.24*	7.7±0.40*	8.98±0.42*	9.7±0.16*
ALT (µ/ mm^3)	44.2±0.05	50.2±0.06	42.8±0.62*	66.5±0.36*	59.5±0.15*	59.2±0.67*
Glu (mmol/ mm^3)	3.2±0.16*	13.5±0.14	6.3±0.10*	7.4±0.06*	10.5±0.10*	14.3±0.05
ALB (g/ mm^3)	34.9±0.05	38.6±0.58	35.9±0.45	36.5±0.49	37.2±0.64	37.7±0.30
T.Protein (g/ mm^3)	65.3±0.06*	75.0±0.34	62.8±0.56*	77.9±0.16	67.9±0.29*	71.9±0.31
HDL (mmol/ mm^3)	1.3±0.06	1.5±0.03	1.3±0.02	1.3±0.06	1.5±0.09	1.5±0.09
LDL (mmol/ mm^3)	0.05±0.02	0.41±0.03	0.30±0.02	0.31±0.03	0.34±0.05	0.40±0.01
T.Chol(mmol/ mm^3)	2.03±0.06	2.31±0.06	1.20±0.13	1.40±0.02	1.70±0.12	2.00±0.08
T.G (mmol/ mm^3)	0.72±0.02	1.73±0.02	0.72±0.07	0.88±0.06	0.92±0.05	0.88±0.07
ALP (µmol)	288.6±0.35*	364.3±0.69	415.8±0.29*	434.9±0.28*	377.0±0.24*	470.6±0.26*

Group 1: Normal rats not induced with diabetes to serve as positive control received acacia 2% w/v solution. Group 2: Alloxan-induced diabetic rats not treated, to serve as negative control. Group 3: Alloxan-induced diabetic rats treated with Glibenclamide 600 µg/kg bwt. Group 4: Alloxan-induced diabetic rats treated with 250 mg/kg bwt extract. Group 5: Alloxan-induced diabetic rats treated with 150 mg/kg bwt extract. Group 6:Alloxan-induced diabetic rats treated with 75 mg/kg bwt extract

Table 3. Haematological values of control and diabetic rats treated with the *C. prostata* extract at different doses and the reference drug glibenclamide and normal control rats

Parameters	GRP 1	GRP 2	GRP 3	GRP 4	GRP 5	GRP 6
WBC (10^9/mm^3)	6.1±0.09*	18.4±0.26	8.5±0.12*	10.4±0.20*	11.2±0.21	11.6±0.05
RBC (10^{12}/ mm^3)	7.18±0.06	6.70±0.05	6.47±0.23	7.09±0.10	7.00±0.13	7.94±0.05
HB (g/dl)	12.6±0.10	11.2±0.12	10.8±0.15	13.0±0.10	13.4±0.13	13.7±0.34
PCV (%)	41.5±0.09	13.8±0.35	40.8±0.14*	40.0±0.08*	32.7±0.28*	21.4±0.33*
PLT (10^9/ mm^3)	813±0.18*	116±0.24	802±0.67*	776±0.46*	421±0.32*	255±0.03*
MCV (fL)	57.9±0.57	54.6±0.12	57.9±0.52	56.5±0.75	58.4±0.13	59.1±0.28
MCH(pg)	17.5±0.28	17.1±0.03	17.0±0.21	18.3±0.16	19.1±0.51	19.0±0.46
MCHC (g/dl)	32.8±0.10	29.6±0.38	32.5±0.41	32.4±0.19	31.5±0.53	30.3±0.13
NEU (%)	28.3±0.35*	48.8±0.76	33.1±0.02*	35.5±0.14*	40.6±0.28*	42.0±0.31
LYMPH (%)	32.6±0.10*	71.6±0.39	42.8±0.22*	44.4±0.20*	48.7±0.28*	55.5±0.19*

Group 1: Normal rats not induced with diabetes to serve as positive control received acacia 2% w/v solution. Group 2: Alloxan-induced diabetic rats not treated, to serve as negative control. Group 3: Alloxan-induced diabetic rats treated with Glibenclamide 600 µg/kg bwt. Group 4: Alloxan-induced diabetic rats treated with 250 mg/kg bwt extract. Group 5: Alloxan-induced diabetic rats treated with 150 mg/kg bwt extract. Group 6: Alloxan-induced diabetic rats treated with 75 mg/kg bwt extract

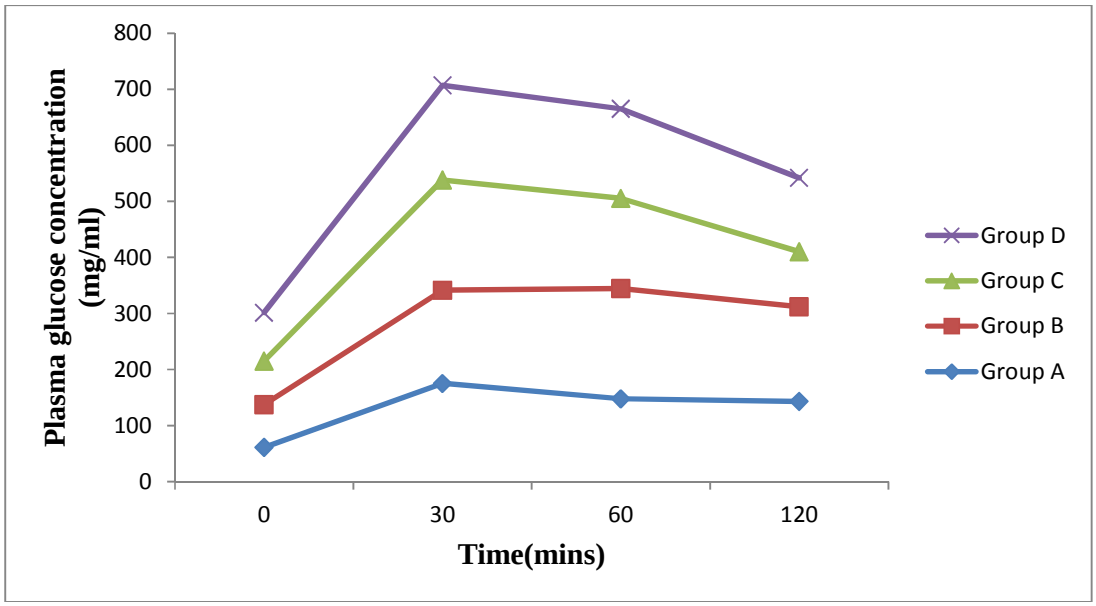


Fig. 1. Evaluation of *Cyathula prostata* extract at different doses and control effects on Post-pandrial glucose level in rats

Group A: Control animals given acacia 2% w/v solution. Group B: Cyathula prostate aqueous extract 140 mg/150g body weight, Group C: Cyathula prostata aqueous extract at 70 mg/150 g body weight, Group D: Cyathula prostata aqueous extract at 7 mg/150 g body weight

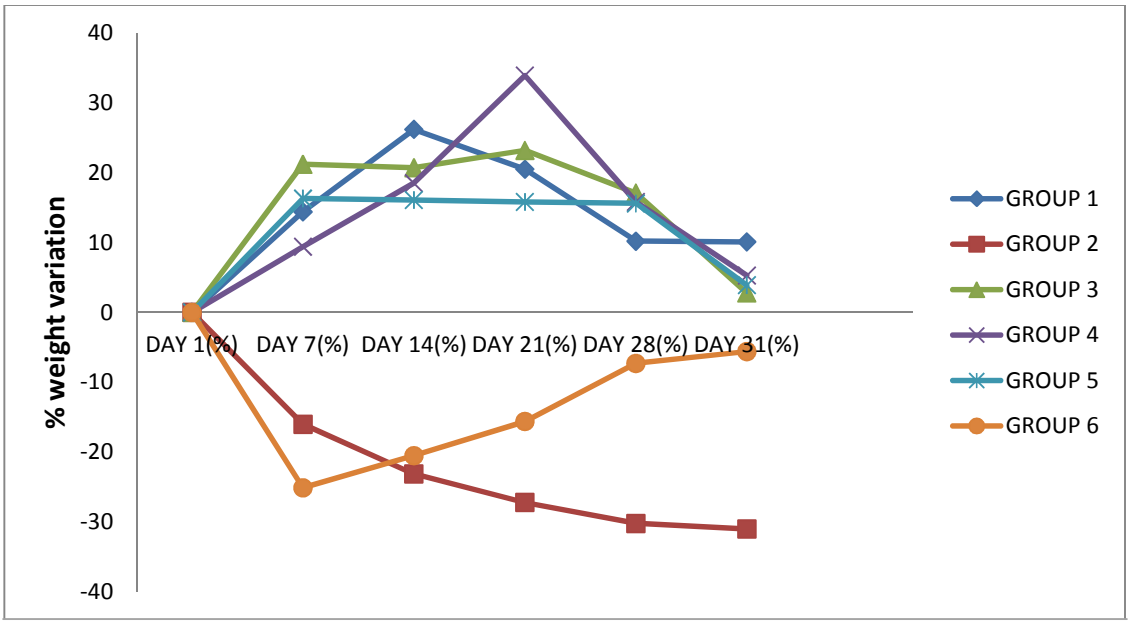


Fig. 2. Percentage weight variations of animals treated with various extract doses

Group 1: Normal rats not induced with diabetes to serve as positive control received acacia 2% w/v solution. Group 2: Alloxan-induced diabetic rats not treated, to serve as negative control. Group 3: Alloxan-induced diabetic rats treated with Glibenclamide 600 µg/kg body weight. Group 4: Alloxan-induced diabetic rats treated with 250 mg/kgbody weight extract. Group 5: Alloxan-induced diabetic rats treated with 150 mg/kg body weight extract. Group 6:Alloxan-induced diabetic rats treated with 75 mg/kg body weight extract

The plasma creatinine and urea levels have been established as markers of glomerular filtration rate (GFR), though plasma creatinine is a more sensitive index of kidney function compared to plasma urea level because creatinine fulfils most of the requirements for a perfect filtration marker [27]. A significant ($p \leq 0.05$) decrease in creatinine, urea and protein levels were observed in the treated diabetic animals which showed the extract might not have any damaging effects on the kidney. The tissue histology of kidney indicating no pathological changes was confirmatory to the observation.

Table 4. Phytochemical screening evaluation

Alkaloids	-
Anthraquinones	-
Flavonoids	+
Glycosides	+
Saponins	++
Steroids	+
Tannins	+
Terpenoids	+

The photomicrographs of histological studies (Fig. 3) indicated that the pancreatic beta cells of the diabetic animals treated with the extract exhibited considerable beta cells recovery that was dose depended compared to the diabetic untreated (negative control). The reference drug however exhibited comparably better activity.

There was also observed significant ($p \leq 0.05$) decrease in the plasma levels of LDL, triglycerides, total cholesterol and an increase in HDL level. The extract therefore had a reducing effect on the cardiovascular risk factors suggesting good anti-diabetic and anti-atherogenic activities. Many anti-diabetic plants have shown to have beneficial effect on cardiovascular system [16,28,29].

Haematological studies shown in Table 3 indicated increase in Hb and PVC levels. The increase in their levels following extract treatment may be due to changes in the rate of the RBCs production. The calculated RBC indices, MCH, MCV and MCHC were not significantly altered in the extract treated animals which suggested that the activity of the extracts had minimal effect on the size of RBC and therefore did not cause anaemia which might lead to cardiac failure.

The preliminary phytochemical screening result as shown in Table 4 demonstrated the absence of alkaloids and anthraquinones. However, there was presence of tannins, flavonoids, saponins which may occur as glycosides. There was also the presence of polyphenols. These biological active components have been observed in plants with strong hypoglycaemic property [30]. It has been reported that several plant sterols reduce serum cholesterol by the inhibition of intestinal cholesterol absorption [8].

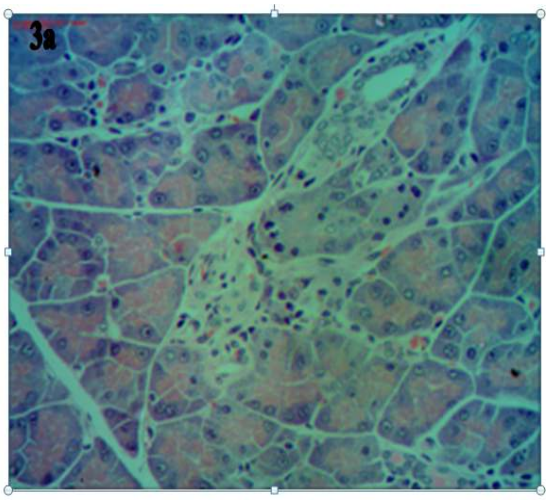


Fig. 3a. Pancreas (diabetic) not treated X400

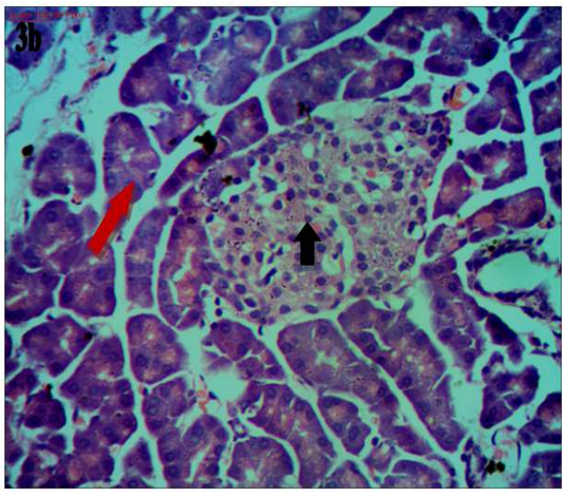


Fig. 3b. Pancreas (diabetic) treated with 250 mg extract X400

Fig. 3. Pancreatic tissue

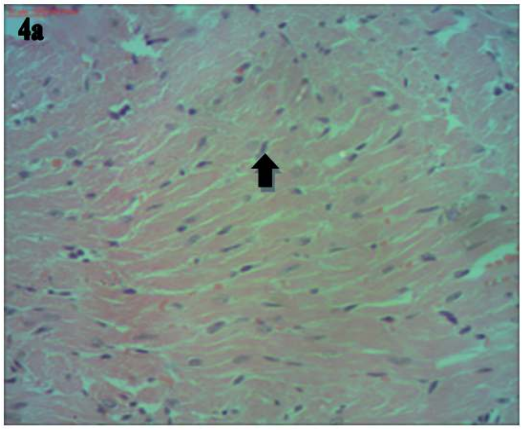


Fig. 4a. Heart (diabetic) not treated X400

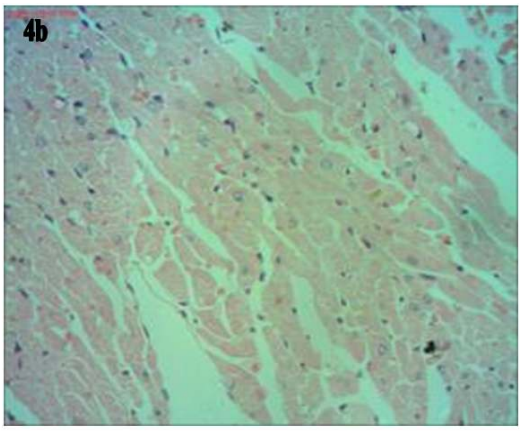


Fig. 4b. X400 Heart (diabetic) treated with 250 mg extract X400

Fig. 4. Cardiac tissue

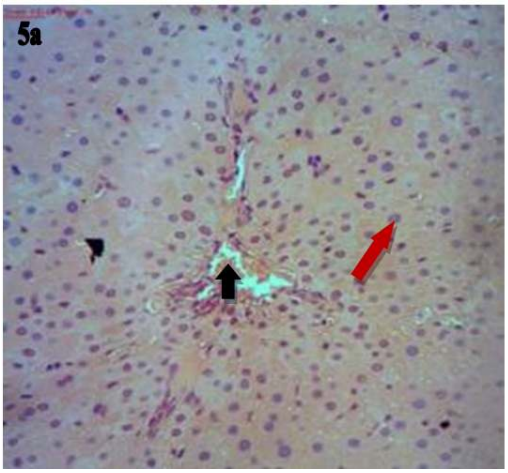


Fig. 5a. Liver (diabetic) not treated X400

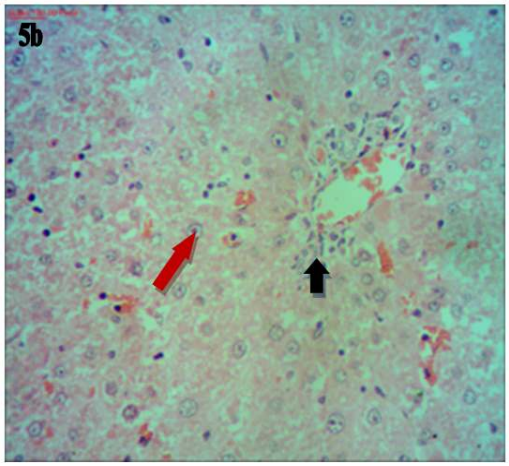


Fig. 5b. Liver (diabetic) treated with 250 mg extract X400

Fig. 5. Hepatic tissue

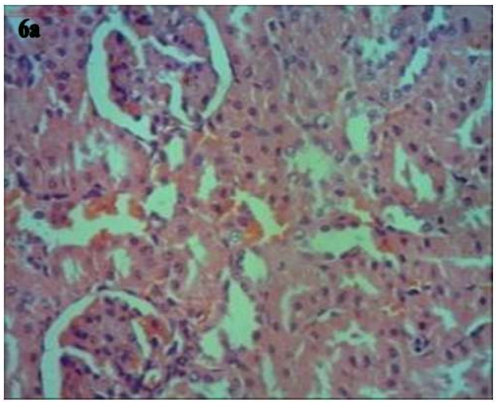


Fig. 6a. Kidney (diabetic) not treated X400

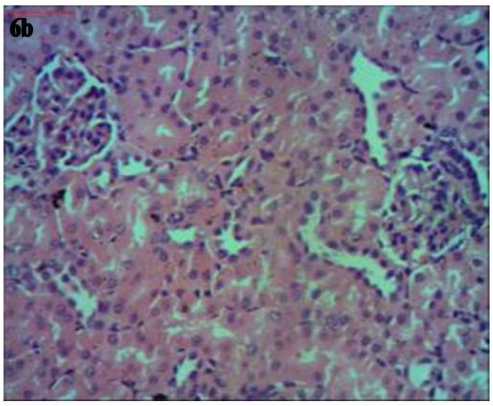
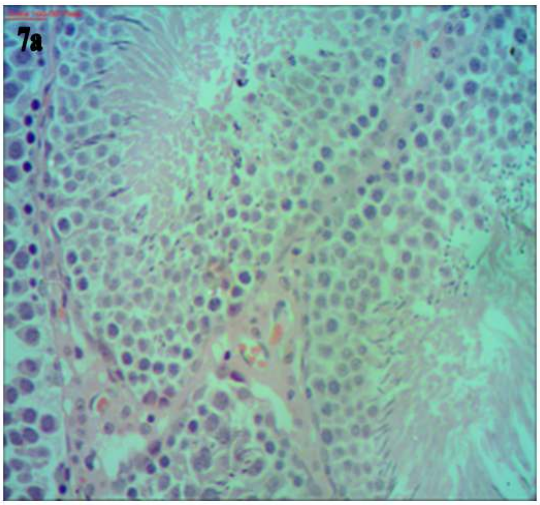


Fig. 6b. Kidney (diabetic) treated with 250 mg extract X400

Fig. 6. Renal tissue



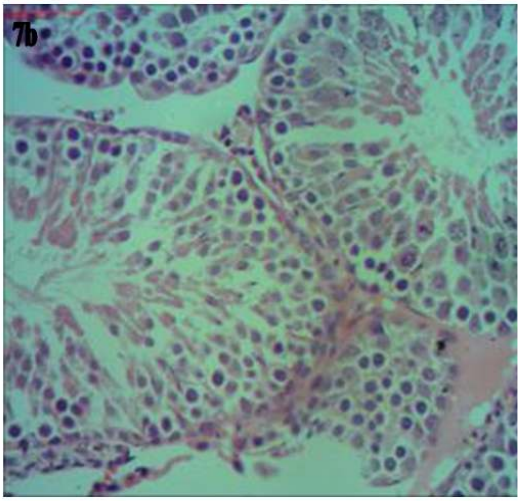


Fig. 7a. Testis (diabetic) not treated X400

Fig. 7b. Testis (diabetic) treated with 250 mg extract X400

Fig. 7. Testicular tissue

Histological studies of some organs- pancreas, kidney, liver, testes. (3a): the photomicrographs of normal Islet cells, the black arrow points to the beta cell while the red to the pancreatic acni (H & E stain) mag. X400. (3b): Photomicrograph showed scanty survivor beta cells. (4a) Photomicrograph of a cross section of myocardium (heart) showing myocytes (thick arrowed) separated by an unremarkable interstitium. (4b): Photomicrograph of a cross section of myocardium showing no lesion. (5a): Photomicrograph of a cross section of hepatic tissue of the control showing portal tract (thick arrowed) and normal hepatocytes. (5b): The hepatic tissue of animals treated with high dose of the extract exhibited mild portal hepatitis, hepatocytes vacuolization and sinusoidal congestion. (6a): Photomicrograph of a cross section of cortical region of the renal tissue of the control indicating renal corpuscles and convoluted tubules. (6b): The cross section of cortical region of the renal tissue showed no lesion. (7a): The photomicrograph of untreated testicular tissue showed densely packed spermatogenic cells. (7b): Photomicrograph of diabetic animal treated with the extract indicated normal appearance

4. CONCLUSION

The study showed that *C. prostata* extract demonstrated a good hypoglycaemic activity and also had desirable effects on the cardiovascular risk factors. Treatment of the diabetic animals with *C. prostata* extract did not induce weight gain in the animals compared to the reference drug used, glibenclamide, indicating its advantage over synthetic hypoglycaemic agents.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee of our Institution". All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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