Research Article

Evaluation of Antidiabetic Potential of Aqueous Extract of *Passiflora edulis* Sims on Alloxan Induced Diabetes Mellitus in Wistar Albino Rats

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ABSTRACT

The aqueous leaf extract of *Passiflora edulis (P.edulis)* Sims were investigated for its anti-diabetic effect in Wistar albino rats after induction of diabetes (150 mg/kg, of alloxan, i.p.). Aqueous extract of *P.edulis* (AEPE) at a dose of 200 mg/kg, p.o was administered as single dose per day to diabetes-induced rats for 30 days. The effect of AEPE leaf extract on blood glucose, insulin, hemoglobin and glycosylated hemoglobin, serum lipid profile, liver glycogen and protein were measured in the diabetic rats. AEPE elicited significant reduction of blood glucose, glycosylated hemoglobin, lipid parameters and serum enzymes and significantly increased plasma insulin, hemoglobin and HDL level. *P.edulis* also caused significant increases in liver protein, glycogen and normalize the carbohydrate metabolizing enzymes. From the above results, it is concluded that *P.edulis* possesses significant anti-diabetic effects in alloxan-induced diabetic rats.

Keywords: *Passiflora edulis,* Antidiabetic, Hyperglycemia, Alloxan mono hydrate

INTRODUCTION

Insulin is the principal hormone secreted by β-cells of pancreas that regulate the uptake of glucose from the blood into most of the cells. In diabetes mellitus the body cannot secrete enough insulin or it cannot use its own insulin or both which result in accumulation of sugar in blood, leading to various potential complications.[1,2]

Diabetes is a pandemic in both developed and developing countries. The incidence of diabetes is considered to be high worldwide.[3] According to the International Diabetes Federation, there are 246 million people with diabetes on the globe and this figure will rise to 380 million by the year 2025.[4] In 2000, there were 175 million people with diabetes world wide and by 2030, the projected estimates of diabetes is 354 million. The greatest relative increase is predicted in the developing countries of Middle Eastern crescent, Sub-Saharan Africa and the Indian sub continent. By the year 2030, over 85% of the world's diabetics will be in developing countries.[5]

Biguanides and Sulphonyl urea serves as oral hypoglycemic agents which are available along with insulin for the treatment of diabetes mellitus[6] but side effects associated with their uses are reported.[7,8] Herbal remedies have greater advantages because of their effectiveness, minimal side effects in clinical experience and relatively low costs hence there is a growing interest in this field.[9] Herbal drugs or their extracts are prescribed widely, even when their biological active compounds are unknown. Even the World Health Organization (WHO) approves the use of plant drugs for different diseases, including diabetes mellitus.[10]

Historical and traditional plant which is used in folklore medicine is *Passiflora edulis*, which is known as yellow passion fruit, maracuja and yellow granadilia. *P. edulis* Sims (Passion fruit) belongs to the genus Passiflora, comprising about 500 species that are distributed in warm temperatures and tropical regions. It is a vigorous climber. The leaves are evergreen and alternate, 3 lobed when mature.

Several species are grown in the tropics for edible fruits, the most widely grown being *P. edulis.* The leaves and stems of *P. edulis* have shown antiinflammatory, antianxiety, antitumour, antimicrobial activity.[11] The antioxidant activity of leaves of *P.edulis* was already documented.[12]

HPTLC finger print analysis of leaves of *P.edulis* indicated that it is an important source of phytochemicals like phenols and flavonoids.[13] In this study, evaluation of glucose lowering effect of *P.edulis* and hypolipidemic activities of leaves of *P.edulis* in alloxan diabetic rats were analysed to establish pharmacological evidence in support of the folklore use of this plant.

MATERIALS AND METHODS

Plant material

Leaves of *Passiflora edulis* were collected from a local farm and authenticated by Dr. G.V.S.Moorthy, Botanical Survey of India, Tamilnadu Agricultural University Campus, Coimbatore (Voucher No: BSI/SRC/73/5/23/09-10/Tech.-624) and the specimen was deposited in the department herbarium.

After washing with water the leaves were dried at 25°C for 10 days in the absence of sunlight and powdered coarsely using a mixer. Then they were weighed and kept in an airtight container and stored in refrigerator for future use.

Preparation of extract

About 50g of shade dried powdered material was added with 100 ml of water. The container was shaked for every half an hour for period of 24 hours. The extract was filtered, concentrated and dried. This dried viscous material obtained was used for the analysis.

Animals

The adult albino rats of both sexes, weighing about 150-180 g were procured from animal house of Karpagam University, Coimbatore and used for the study. Rats were housed at constant temperature of 22±5°C with a 12-hour light, 12-hour dark cycle. The rats were fed on pellets with free access to tap water. All the experiments were carried out according to the guidelines recommended by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India.

Induction of Diabetes

Diabetes induction was done by single intra peritoneal injection of alloxan monohydrate (150 mg/kg) in saline. The hyperglycemia was confirmed after 72 hrs by the elevation of blood glucose and the behavioral changes (Excess thirst and frequent urination).The rats with blood glucose level more than 250 mg/dl were used for the study.

Experimental Design

A total of 25 rats (15 diabetic surviving rats, 10 normal rats) were used. The rats were divided in to five groups of five animals each. Group I served as untreated control. Group II was diabetic induced. Group III and Group IV was diabetic rats received standard oral hypoglycaemic agent, glibenclamide (5mg/kg) and *P.edulis* aqueous extract (200mg/kg) respectively. Group V was normal rats treated with aqueous extract of *P.edulis* (200mg/kg) alone. The treatment groups were given the *P. edulis* extract and the standard drug through oral gastric tube for a period of 30 days.

Blood collection

After 30 days, the animals were kept overnight fast and sacrificed under light chloroform anesthesia. Blood was drawn from the ventricles, centrifuged and the serum was used immediately for various biochemical estimations. Pancreas was excised immediately, washed with ice cold saline stored in 10% formalin and 0.9% saline, for histopathological and biochemical studies respectively.

Biochemical Estimations

Commercial diagnostic kits were used to estimate blood glucose level colorimetrically. (Sigma Diagnostics Pvt Ltd, Baroda, India). Estimation of protein in serum and liver was carried out with Lowry's method.[14] Serum lipid profile, protein and albumin content were determined by standard procedures in an auto analyzer using Ecoline kits (E.Merck, Mumbai, India). Liver glycogen was estimated by Morales method.[15] Carbohydrate metabolic enzymes of liver like glycogen phosphorylase,[16] glucose-6-phosphatase[17] and hexokinase[18] were also analysed. From the lipid profile, atherogenic index (AI)[19] and coronary risk index (CRI)[20] were calculated.

$$Atherogenic\ index\ (AI) = \frac{LDL\ cholesterol}{HDL\ cholesterol}$$

$$Coronary\ risk\ index\ (CRI) = \frac{Total\ cholesterol}{HDL\ cholesterol}$$

Statistical Analysis

Results are expressed as Mean ± SD of five individual experiment and the statistical significance was evaluated by one way analysis of variance (ANOVA) using SPSS version (10.0) and the individual comparisons were obtained by the Duncan multiple range test (DMRT).[21] A value of $p<0.05$ was considered to indicate a significant difference between groups.

RESULTS AND DISCUSSION

Changes in Body Weight

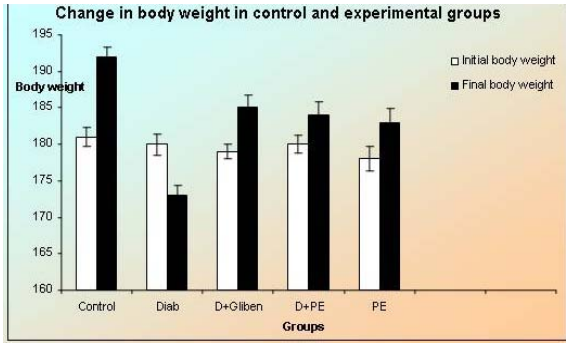

Figure 1: Effect of *P.edulis* leaf extract on the on the body weight of control and experimental rats

Values are given as Mean ± S.D for 5 rats in each group.

* - Significant at 5% level (t<0.05), compared between initial and final body weight.

Diab -Diabetic control;

D+Gliben - Diabetes +Glibenclamide treated;

D+PE -Diabetes+ *P. edulis* treated;

PE - *P. edulis* alone treated;

The results of body weight indicated that the final body weight was significantly increased in the normal control rats when compared to initial body weight, whereas in the diabetic control rats there was a significant decrease in the body weight (Figure 1). The decrease in body weight observed in diabetic rats is reason out as the result of degradation of proteins (muscle wasting). Structural proteins are known to contribute to the body weight.

In the absence of glucose and lipid sources, proteins are the next main source of energy in body. So it is clear that the decrease in body weight in diabetic rats were mainly because of degradation of structural proteins.[22,23]

Diabetic rats treated with *P.edulis* restored the body weight, this may be explained that the extracts improved the insulin secretion which reduces the hyperglycemia by peripheral utilization of glucose by the cells which ultimately improved the body weight.

The plant drug seemed to increase the body weight in group III which showed that the *P.edulis has* beneficial effect in maintaining the body weight.

Change in glucose, plasma insulin, hemoglobin and glycosylated hemoglobin

Table 1 shows the concentration of blood glucose, plasma insulin, hemoglobin and glycosylated hemoglobin in serum of control and experimental groups. Administration of alloxan to diabetic control group significantly increased the blood glucose level. Alloxan produce diabetes mellitus with a single dose of administration through selective necrosis of pancreatic β-cells of islets of langerhans that initiate insulin deficiency. *In vitro* studies proved that alloxan mediate cytotoxic action by production of ROS that cause destruction of β-cells.[24,25]

Oral administration of glibenclamide and aqueous extract of *P.edulis* (200mg/kg) significantly reduced ($p<0.05$) the blood glucose and increased the insulin level in diabetic rats, but not to the level of control rats. Glibenclamide, a prototype of the second generation sulfonyl urea class of the oral hypoglycemic agent is known to mediate its hypoglycemic effect by stimulating insulin release from the pancreatic β-cells. It also stimulates the release of somatostatin and suppresses the secretion of glucagons.[26]

Since, *P.edulis* brought down the blood glucose level and increase the insulin level very similar to glibenclamide, the mechanism behind the reduction of blood glucose may be of its increasing ability in releasing the insulin from the β-cells.

Numerous studies have demonstrated that a variety of plant have been reported to contain substances like glycosides alkaloids, terpenoids, flavonoids and tannin etc which have been proved to be antidiabetic by different mechanism of action.[27]

The earlier phytochemical screening of *P.edulis* indicate that it is an excellent source of phenol and flavonoid and these phyto constituents may increase the insulin secretion in this study.[12]

During diabetes mellitus, the excess glucose present in the blood reacts with hemoglobin to form $HbA1_C$. Glycosylated hemoglobin is a measure of mean blood glucose over a period of 2 months and used as marker for estimating the degree of protein glycosylation in diabetes.

It is an important parameter of chronic glycemic control in patients with diabetes mellitus and an elevated $HbA1_C$ always indicates the uncontrolled diabetes mellitus.[28,29]

Since the glucose reacts with hemoglobin, the level of hemoglobin decreased and the glycosylated hemoglobin level increased in alloxan induced diabetic rats.[30] In this study administration of the plant extracts for 30 days effectively prevented significant elevation of glycosylated hemoglobin thereby increase the level of hemoglobin in diabetic treated rats. This is due to the result of improved glycemic control exerted by the *P.edulis* extract. The serum hemoglobin concentration is an important predictor of both macrovascular and microvascular complications of diabetes mellitus including coronary mortality and lower extremity amputations.[31] Since the aqueous extract of *P.edulis* effectively prevent the $HbA1_C$ elevations, it prevents such complications. Our results are in line with the earlier reports.[32]

Significant increase in glucose, glycosylated hemoglobin and significant reduction in insulin and hemoglobin ($p<0.05$) were found in alloxan treated diabetic rats. Oral administration of the standard antidiabetic drug, glibenclamide and aqueous extract of *P.edulis* (200mg/kg) significantly reduced ($p<0.05$) the blood glucose level in diabetic rats, but not to the level of control rats. *P.edulis* extract alone treated rats did not show any significant difference with control rats.

Change in Cholesterol, triglycerides, HDL, LDL and VLDL

The levels of cholesterol, triglycerides, HDL, LDL, VLDL on various experimental groups were given in Table 2. Significant elelvation of TC, TG, LDL, and VLDL along with a decrease in HDL cholesterol in diabetic rats and its reversal by the administration of aqueous extract of *P.edulis* was noticed. Insulin activates the enzyme lipoprotein lipase that hydrolyses the triglycerides under normal circumstances. Insulin deficiency in diabetes results in failure to activate this enzyme thereby cause hyperlipidemia. Total cholesterol, triglycerides and VLDL and LDL are biomarkers of hyperlipidemia and atherosclerosis and high proportion of HDL is antiatherogenic. A significant decrease in total cholesterol and rise in HDL-Cholesterol is a desirable factor which prevents atherosclerosis and ischemic conditions.[33] Treatment of *P.edulis* with diabetic rats significant reduced the atherogenic and cardiac risk index supports that *P.edulis* will prevent cardiovascular complications.

Literatures also reported that the flavonoids, alkaloids and tannins are responsible for hypoglycemic and hypolipidemic effect[27] and hence the presence of high amount of flavonoids in *P.edulis* cause hypolipidemia which removes the LDL cholesterol from blood by increasing the LDL receptor densities in the liver and by binding to lipoprotein B.[9] The results of this study reveal that a continuous administration of *P.edulis* for 30 days effectively prevents the elevation of serum lipids which are secondary diabetic complications. The hypolipidemic effect of *P.edulis* can be explained as a direct action on insulin that result in the restoration carbohydrate and lipid metabolism.

Table 1: Effect of *P. edulis* leaf extract on the concentration of glucose, insulin, hemoglobin and glycosylated hemoglobin of control and experimental groups

Particulars	Control	Diabetic control	Diabetic+Glibenclamide	Diabetic + *P.edulis*	*P.edulis*
Glucose(mg/dl)	106.27±1.44^{ad}	240.33±0.89^{b}	104.±0.89^{a}	110.13±1.8^{ce}	105.33±2.25^{ad}
Insulin (mU/L)	0.42±0.03^{a}	0.25±0.05^{b}	0.49±0.0^{a}	0.42±0.04^{a}	0.42±0.03^{a}
Hemoglobin(%)	16.2±1.18^{a}	14.23±0.13^{b}	15.73±0.14^{c}	15.27±0.19^{cd}	15.67±0.29^{c}
Glycosylated Hemoglobbin	9.16±0.02^{a}	15.7±0.09^{b}	9.07±0.05^{a}	10.23±0.05^{c}	9.12±0.01^{a}

Values are expressed as Mean ± S.D of five individual experiments; Values not sharing a common superscript letter differ significantly (DMRT)

Table 2: Effect of *P. edulis* leaf extract on the concentration of cholesterol, triglycerides, HDL, LDL and VLDL in serum of control and experimental groups

Particulars	Control	Diabetic control	Diabetic+ Glibenclamide	Diabetic + *P.edulis*	*P.edulis*
Cholesterol (mg/dl)	121.03±1.47^{a}	266.33±1.36^{b}	117.73±1.72^{c}	119.13±0.9^{a}	120.73±1.45^{a}
Triglycerides (mg/dl)	80.67±1.05^{a}	218.8±1.17^{b}	87.47±1.22^{c}	90.53±1.84^{d}	82.2±1.53^{a}
HDL (mg/dl)	42.07±1.35^{a}	29.73±1.02^{b}	40.13±1.61^{ac}	44±1.71^{d}	38.33±1.34^{c}
LDL(mg/dl)	58.73±1.50^{a}	191.33±2.07^{b}	58.53±1.27^{a}	57.67±1.97^{a}	58±1.79^{a}
VLDL(mg/dl)	20.5±1.16^{a}	45.53±1.52^{b}	18.8±1.25^{ac}	17.73±1.34^{ac}	19.47±1.02^{ac}
AI	1.40± 0.11^{a}	6.44±0.23^{b}	1.46±0.15^{c}	1.31 ± 0.14^{d}	1.34±0.09^{a}
CRI	2.88± 0.18^{a}	8.96±0.27^{b}	2.93 ± 0.13^{c}	2.71 ± 0.17^{d}	2.86±0.08^{a}

Values are expressed as Mean ± S.D of five individual experiments; Values not sharing a common superscript letter differ significantly (DMRT)

Table 3: Effect of *P. edulis* leaf extract on the concentration of total protein, albumin, globulin and A/G ratio in serum of control and experimental groups

Particulars	Control	Diabetic control	Diabetic+Glibenclamide	Diabetic + *P.edulis*	*P.edulis*
TotalProtein (g/dl)	8.23±0.14^{a}	4.47±0.19^{b}	8.03±0.22^{c}	8.02±0.22^{c}	8.2±0.05^{a}
Albumin (g/dl)	5.1±0.27^{a}	2.13±0.25^{b}	4.7±0.06^{c}	4.7±0.09^{c}	5.02±0.04^{a}
Globulin (g/dl)	3.13±0.21^{a}	2.03±0.12^{b}	3.33±20.052^{c}	3.31±0.04^{c}	3.15±0.05^{a}
A/G Ratio	1.65±0.08^{a}	1.25±0.05^{b}	1.41±0.01^{c}	1.42±0.07^{c}	1.63±1.01^{a}

Values are expressed as Mean ± S.D of five individual experiments; Values not sharing a common superscript letter differ significantly (DMRT)

Table 4: Effect of *P. edulis* leaf extract on the activities of liver enzymes glycogen phosphorylase, glucose-6-phosphatase and hexokinase in control and experimental rats

Particulars	Control	Diabetic control	Diabetic+Glibenclamide	Diabetic + *P.edulis*	*P.edulis*
Hexokinase (µmoles of glucose phosphorylated/min)	2.61±0.03^{a}	1.52±0.02^{b}	2.90±0.10^{c}	2.76±0.12^{d}	2.68±1.15^{e}
Glucose-6- phosphatase n moles of phosphorus liberated/ mg protein)	0.20±0.05^{a}	0.47±0.01^{b}	0.19±0.05^{a}	0.18±0.57^{a}	0.19±0.05^{a}
Glycogen phosphorylase (mg/g of fresh tissue)	8.94±0.05^{a}	11.57±0.57^{b}	8.24±1.02^{c}	8.80±0.16^{a}	8.87±0.25^{a}

Values are expressed as Mean ± S.D of five individual experiments; Values not sharing a common superscript letter differ significantly (DMRT)

In diabetic rats the serum level of cholesterol, triglycerides, LDL, VLDL were significantly increased that ultimately reflected in atherogenic and coronary risk index. Diabetic rats treated with *P.edulis* extract and the glibenclamide showed a significant increase in HDL and significant decrease in the level of other lipid parameters with concomitant decrease in atherogenic and cardiac risk index when compared with diabetic rats. It is to be noted that the administration of *P.edulis* reduced the atherogenic and coronary risk index significantly than that of glibenclamide group. In *P.edulis* alone treated rats, the lipid profile pictured no significant change to that of the control rats.

Change in total protein, albumin and globulin

The levels of total protein and albumin in various experimental groups were illustrated in Table 3. The total protein and albumin showed a significant decrease in alloxan treated diabetic rats when it is compared with control rats. Significant increases in these parameters

were observed on treatment with *P.edulis* extract and glibenclamide. The protein profile showed no significant change with control rats.

Improvement in protein profile was observed in *P.edulis* extract and glibenclamide treated diabetic rats, it may due to marked change in amino acid metabolism.[34] *P.edulis* by improving the secretion of insulin exert the protein sparing effect and reverse the altered protein profile which was very similar to that of the drug glibenclamide. Significant increase in urinary excretion of protein, albumin, glucose and urea levels indicates the impaired renal function in diabetes and the treatment with herbal remedies prevent the diabetic nephropathic complications which was already well established.[35]

Change in carbohydrate metabolizing enzymes

The level of glucokinase (Table 4) decrease significantly in alloxan treated diabetic rats where as the level of glucose-6-phosphatase and glycogen phosphorylase were increased significantly in the same group. They were restored to their normal level in *P.edulis* extract and glibenclamide treated rats. Control rats showed no significant change in these enzymes.

Changes in hepatic enzymes of carbohydrate metabolism

Liver is the candidate organ involved in glucose homeostatis. It functions as a "glucostat" and plays a vital role in the maintenance of blood glucose level and hence holds the centre of interest. It is the main site for glycogenesis, glycogenolysis and gluconeogenesis.[36] So evaluation of activity of enzymes like hexokinase, glucose-6-phosphatase and glycogen phosphorylase will be of great value in determining some of the fact of carbohydrate metabolism.

Hexokinase

Hepatocytes contain a form of hexokinase called hexokinase D or glucokinase, which is more specific for glucose and differ from other forms of hexokinase in kinetic and regulatory properties. It is an important regulator of glucose storage and disposal.[47] The hexokinase activity of liver was shown in the Figure 16. In this study hexokinase activity was decreased significantly in alloxan induced diabetic rats which may be due to insulin deficiency. Insulin influences the intracellular utilization of glucose in a number of ways. It has been shown to be a potentiator of hexokinase and glucokinase. Glibenclamide stimulate insulin secretion, which activates the enzyme glucokinase, thereby increasing the utilization of glucose and this increased utilization leads to decrease in blood sugar level in the glibenclamide treated rats. Sameway restoration of insulin in *P.edulis* treated rat increases hepatic glycolysis by increasing the activity of several enzymes including glucokinase.

Glucose-6-phosphatase

Glucose-6-phosphatase is one of the key enzymes in the homeostatic regulation of blood glucose level whose action is controlled by insulin. When blood glucose level comes down, the liver rapidly releases glucose into the circulation by stimulating the two major pathways namely gluconeogenesis and glycogenolysis, the terminal step in both the process are catalyzed by glucose-6-phosphatase.[48] Increased activity of glucose-6-phosphatase was observed in the liver of alloxan diabetic rats (Figure 1) explains the abnormal rise in blood glucose level in alloxan treated rats is due to diminished secretion of insulin. Decrease in the activity of glucose-6-phosphatase in rats treated groups may be attained through regulation of cAMP that control insulin secretion which ultimately decrease the activity of the enzyme.[49]

Glycogen phosphorylase

In alloxan induced diabetic rats, there was an elevation in the activity of glycogen phosphorylase (Figure 1). In the absence of insulin increased glycogen phosphorylase was observed that leads to decrease in glycogenesis or increase in glycogenolysis in liver.[40] The restoration of this enzyme activity after the treatment of *P.edulis* and glibenclamide may be due to elevation in plasma insulin level. No significant changes were observed in plant extracts alone treated rats due to the normal activity of glycogen phosphorylase.

Histopathology of rat pancreas

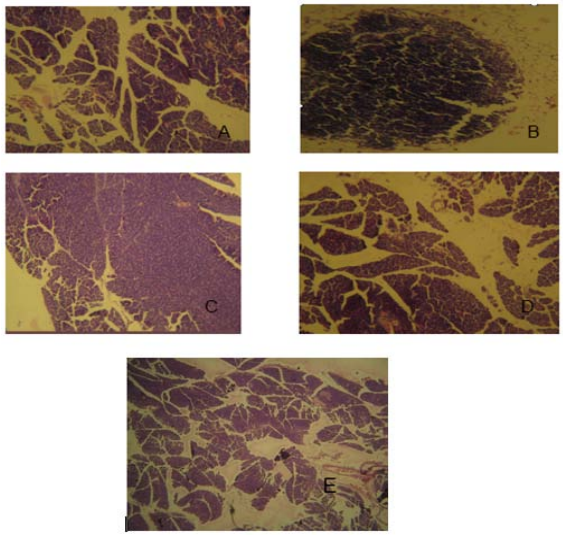

Figure 4: Effect of *P. edulis* leaf extract on the histopathology of rat pancreas control and experimental rats

A- Group I control
B- Group II Diabetic control
C- Group III Diabetes + Glibenclamide
D- Group IV Diabetes +*P.edulis*
E- Group V *P.edulis* alone

A- Normal histology of pancreas was seen in control rats with central zone of intact β-cells surrounded by other endocrine cells and parenchymal cells. **B**- In alloxan

treated rats destruction of almost all the β-cells were noted. Severe congestion of pancreatic parenchyma and mild infiltration of inflammatory cells were also seen. **C**- The rats treated with glibenclamide showed regeneration of the β-cells along with mild hyperplasia of islet cells. **D**- Pancreatic islets of diabetic rats treated with *P.edulis* indicates the regeneration pancreatic β cells. **E**- No histological changes were seen in the pancreas of rats treated with *P.edulis*.

CONCLUSION

The findings of this study pave an evidence that aqueous extract of *P.edulis* (200mg/kg) given orally to diabetic rats contain novel bioactive principles with antihyperglycaemic properties and is capable of reversing the altered carbohydrate and lipid profile in diabetes. But more concerted efforts are still needed for the isolation, characterization and biological evaluation of the active principle(s) of the extract that are responsible for this activity.

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