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Original Article

EFFECT OF EXTRACTS AQUEOUS OF PHRAGMITES AUSTRALIS ON CARBOHYDRATE METABOLISM, SOME ENZYME ACTIVITIES AND PANCREATIC ISLET TISSUE IN ALLOXAN-INDUCED DIABETIC RATS

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ABSTRACT

Objective: The present study was aimed to investigate the effects of the aqueous extract of rhizome of *phragmites australis* on carbohydrate metabolism, some enzyme activities and pancreatic tissue in alloxan induced diabetic rats.

Methods: Wistar rats were divided into three groups (n=6) as control, diabetic group and test groups (Diabetic+AEPA). Diabetes in rats was induced by alloxane using a single peritoneal injection of 150 mg/kg dose. Aqueous extract of *Phragmites australis* was supplemented (200 mg/kg b. w) orally for three weeks. The aqueous extract of *Phragmites australis* was prepared and phytochemical were analyzed by using standard methods. Blood glucose level, pancreas histology and various biochemical parameters were assessed.

Results: The results of the phytochemical analysis of aqueous extract of *Phragmites australis* (AEPA) revealed the presence of tannin, terpenoids, glycosides and flavonoids. Compared with the control, a significant decrease in the body weight gain ($p<0.01$) and increase in food intake ($p<0.001$) were noticed in the diabetic group. The biochemical evaluation showed significantly higher values for glucose ($p<0.001$), lipid profile ($p<0.05$), transaminases ($p<0.05$), amylase and alkaline phosphatase ($p<0.001$) activities in diabetic group compared with the control. Histology of the pancreas showed congestion of vessels and focal area of necrosis in diabetic untreated rats. However, treatment with aqueous extract of *Phragmites australis* significantly normalized blood glucose, serum biochemical profile and pancreas histomorphology.

Conclusion: It is suggested that aqueous extract of *Phragmites australis* exhibits a benefic effect in rat to a great extent in attenuating and restoring the damage sustained by diabetes.

Keywords: *Phragmites australis*, Diabetes, Enzymes marker, Pancreatic Islet, Alloxan

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INTRODUCTION

Diabetes mellitus is a global health crisis, which has been affecting the humanity irrespective of the socio-economic profile and geographic location of the population [1]. Diabetes is a group of metabolic alterations characterized by hyperglycemia resulting from defects in insulin secretion, insulin action or both [2]. It has already been established that chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction and eventually failure of organs, especially the eyes, kidneys, nerves, heart and blood vessels [3]. Therapy has been based on insulin or drugs that stimulate insulin secretion (sulphonylureas and rapid-acting secretagogues), reducing hepatic glucose production (biguanides), delaying digestion and absorption of intestinal carbohydrate (alpha-glucosidase inhibitors), or improving insulin action in thiazolidinedione's [4]. Unfortunately, all of these therapies have various side effects such as gastrointestinal upset, weight changes, hypoglycemia, joint stiffness, kidney complications, and skin alterations [5]. A multitude of plant materials has been described for the treatment of diabetes throughout the world [6]. The medicinal plants provide a useful source of oral hypoglycemic compounds for the development of new pharmaceutical leads as well as a dietary supplement to existing therapies [7]. Some of the plants which are being used for the treatment of diabetes have received scientific or medicinal scrutiny and even the world health organization (WHO) expert committee on diabetes recommends that this area warrant further attention [8]. There are no pharmacological studies so far reported the hypoglycemic potential for an aqueous extract from the rhizome of Phragmites australis to date. The promising preliminary results reported with the use of Phragmites australis in disease components of metabolic syndrome (like Hepatoprotective and antioxidant activities) have prompted us to investigate the effect of aqueous extract from rhizome of Phragmites australis on carbohydrate metabolism, some enzymes activities and pancreatic tissue in alloxan induced diabetic rats, which is an important contributor to the metabolic syndrome.

MATERIALS AND METHODS

Chemicals and reagents

All chemicals used were of analytical grade and purchased from Sigma-Aldrich, Mo, USA. Commercials Kit obtained from Spinreact, Spin.

Collection, identification and extraction of plant material

Fresh rhizomes of the plant were collected from a village in Touggourt of Ouargla state, Algeria and were identified by a botanist at the herbarium in the Department of biology, the University of El Oued, Algeria (voucher specimen no: FSNV/DB/consult/2016/78-15-02). The rhizome was washed, with distilled water, and used immediately. The extraction methods described by Mamta and Parminder (2013) [9] were adopted using distilled water separately. After extraction, the solvents were removed using a rotary evaporator, to get gel-like extracts.

Phytochemical screening

The methods of Sasidharan *et al.* (2011) [10] and Usman *et al.* (2009) [11] were used to identify the following phytochemicals in the extracts: alkaloids, saponins, tannins, anthraquinones, flavonoids, terpenoids and glycosides.

Animals

Eighteen adult male albino rats, weighing 224–240 g, were brought from the animal house of Pasteur institute, Algeria. They were placed in three groups of six rats in each and kept in animal's house

of molecular and cellular biology department, university of El Oued, Algeria. Standard rats food and tap water were available ad libitum for the duration of the experiments unless otherwise noted [11]. Animals were acclimated for two weeks under the same laboratory conditions of photoperiod (12h light/12 h dark) with a relative humidity 64.5 % and room temperature of 22±2 C°. The experimental procedures were carried out according to the national institute of health guidelines for animal care and approved by the ethics committee (n °: BCM 022/2016) of our institution.

Induction of diabetes

Diabetes was induced by a fresh alloxan monohydrate solution. Alloxan was administered intraperitoneally at a dose of 150 mg/kg body weight dissolved in citrate buffer (0.01 M, pH 4.5). Blood glucose was measured 7 d after induction of diabetes in samples taken from the tail vein [12]. The diabetic state was established when the glucose concentration exceeded 14 mmol/l, confirmed by a glucometer (Accu-Check; Roche Diagnostics, Paris, France).

Acute toxicity test

The test was performed using healthy albino rats of Wistar strain weighing between 200 and 220 g. The animals were divided into five groups of three rats each and administered 0, 100, 2000, 5000 mg/kg orally. Animals were observed after dosing at least once during the first 30 min, periodically during the first 24 h with special attention given during the first 4 h, and daily thereafter for a total of 14 consecutive days [13].

Experimental design

The adult Wistar albino rats were randomly divided into three groups, each containing 6 rats.

Group I: Control group was given normal diet (served as control);

Group II: Diabetic group given normal diet (diabetic);

Group III: Diabetic treated groups were given normal diet plus aqueous extracts of *Phragmit australis* (200 mg kg^{-1} d^{-1}) administered orally (diabetic+AEPA).

All the groups of animals had free access to water and diet. Food intake and body weight was monitored weekly.

Blood collection and preparation of tissue samples

At the end of 3 w of AEPA treatment, rats were fasted for 16 h, decapitated and blood samples were transferred into ice-cold centrifuge tubes. The serum was prepared by centrifugation, for 10 min at 3000 revolutions/min and utilized biochemical assays. The blood glucose was measured by glucometer. The pancreas from each rat was removed immediately and preserved in a sample bottle containing 10% formalin solution. The pancreas was processed by the paraffin technique. Sections of 5 µm thickness were cut and stained by hematoxylin and eosin for histological examination.

Biochemical parameters

The activities of glutamate-oxaloacetate transaminase (GOT), glutamate pyruvate transaminase (GPT), alkaline phosphatase (PAL) and amylase were determined using commercial kits from Spinreact (Girona, Spain) (refs: GOT-1001161, GPT-1001171, ALP-1001131 and amylase-41201). Triglyceride (TG), total cholesterol (TC), high-density lipoprotein (HDL) concentrations were also measured using commercial kits (Spinreact) (refs: TG-1001311, TC-1001090, HDL-C-1001096, total proteins-1001291). Low-density lipoprotein (LDL) and very low-density lipoprotein (VLDL) level were calculated indirectly by formula as per Friedwald *et al.*, (1972) [14].

Statistical analysis

Data were reported as mean±SEM. Results comparisons were carried out by using 1-way analysis of variance followed by the Student t test to compare means among the groups. Differences were considered statically significant at $p<0.05$.

RESULTS

Phytochemical screening

Phytochemical analysis revealed the presence of glycosides, tannins, phenolic compounds, flavonoids and terpenoids in the extract (table 1).

Initial body weight, body weight gain, food intake in control and experimental animals

Induction of type 1 diabetic state caused a decrease ($p<0.001$) in body weight and relative liver and kidney weight and an increase in food intake in the rats with diabetes compared to the rats without diabetes. Whereas *Phragmites Australis* treatment reversed these changes (table 2).

Table 1: Phytochemical composition of aqueous extracts of rhizome *Phragmit australis*

Phytochemicals	Aqueous extract of *P. australis*
Flavonoids	+
Tanins	+
Terpenoids	+
Glycosides	+
Saponins	-
Alkaloids	-

+presence–indicates

Table 2: Mean initial body weight, body weight gain and food intake of control and experimental rats

Parameters	Initial body weight (g)	Body weight gain (g/j/rat)	Food intake (g)
Control	213.6±12.2	0.42±0.03	12.40±0.08
Diabetic	213.8±15.6	0.13±0.02**	17.10±0.07***
Diabetic+AEPA	211.3±12.7	0.31±0.04[a]	15.80±0.09***[a]

** p<0.01, ***p<0.001: significantly different from control group, [a]p<0.05 significantly different from Pb group, Data are expressed as mean±SD (n=6)

Blood biochemical values

As seen from table 3, blood glucose level and serum lipids concentrations were significantly altered which increased the level of glucose ($P<0.001$), triglycerides, cholesterol, low density lipoprotein (LDL) and very low density lipoprotein (VLDL) ($P<0.01$) at the end of the treatment period in diabetic animals as compared to control. The administration of AEPA to diabetic rats restored the changes in the blood and lipid level which decreased the level of glucose (37.45%), cholesterol (8.55%), triglycerides (36.75%), low density lipoprotein (LDL) (26.19%), and very low density lipoprotein (VLDL) (36.75%) and increased the level of high density lipoprotein (HDL) (37.34%) as compared to diabetic animals.

Table 3: Mean blood glucose, lipid profile of control and experimental animals

Parameters	Glucose (mmol/l)	Triglyceride (mg/dl)	Cholesterol (mg/dl)	LDL (mg/dl)	HDL (mg/dl)	VLDL (mg/dl)
Control	4.61±0.11	49.5±2.5	73.5±0.065	30.1±5.5	30.5±0.5	9.9±0.5
Diabetic	10.2±0.41***	58.5±2.0*	79.5±3.7*	33.6±2.3*	24.5±0.8**	11.7±0.4*
Diabetic+EAPA	6.38±0.11**c	37±1.7**b	72.7±3.4a	24.8±1.3**a	31.2±2.3a	7.4±0.3**b

LDL: low-density lipoprotein; HDL: high-density lipoprotein; VLDL: very-low-density lipoprotein, *p<0.05, **p<0.01, ***p<0.001: significantly different from control, ap<0.05, b p<0.01, c p<0.001: significantly different from diabetic, Data are expressed as mean±SD (n=6).

Enzymes activities

As indicated in fig. 1 and fig. 2, diabetes resulted in significant increases (p<0.05) in serum glutamic-oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT), alkaline phosphatase (PAL) and amylase (p<0.001) activities. In addition, whereas AEPA at 200 mg/kg b. w supplementation led to the better correction of these enzymes activities.

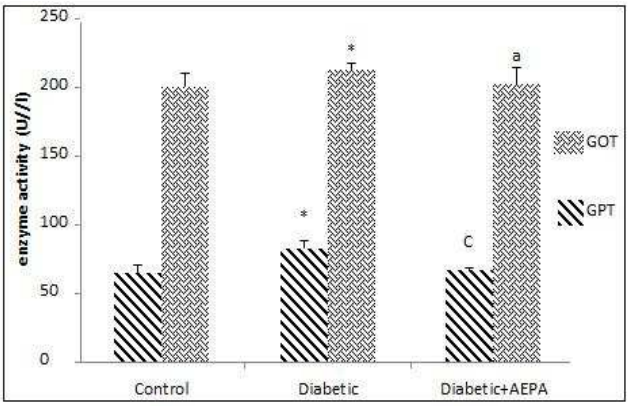

Fig. 1: Glutamate-oxaloacetate transaminase (GOT) and glutamate pyruvate transaminase (GPT) activities of control and experimental animals.*p<0.05: significantly different from control group. a p<0.05, c p<0.001: significantly different from the diabetic group. Values are mean±SEM; n=06

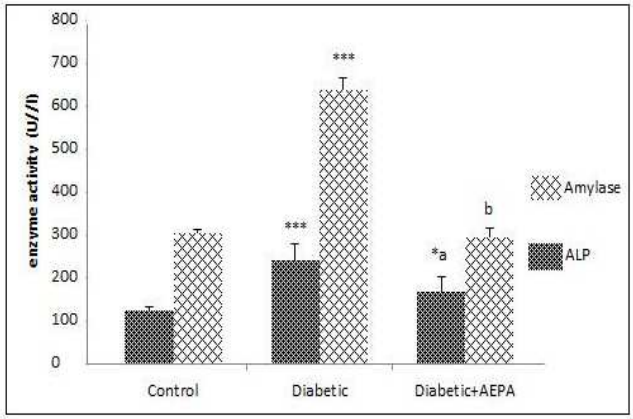

Fig. 2: Alkaline phosphatase (ALP) and amylase activities in serum of control and experimental animals. *p<0.05, ***p<0.001: significantly different from control. a p<0.05, b p<0.01 significantly different from diabetic. Values are mean±SEM; n=06

Histology of the pancreas showed the focal area of necrosis and fatty infiltration in diabetic untreated rats, but these lesions were absent in pancreas of rats treated with rhizome *Phragmites australis* extract (fig. 3).

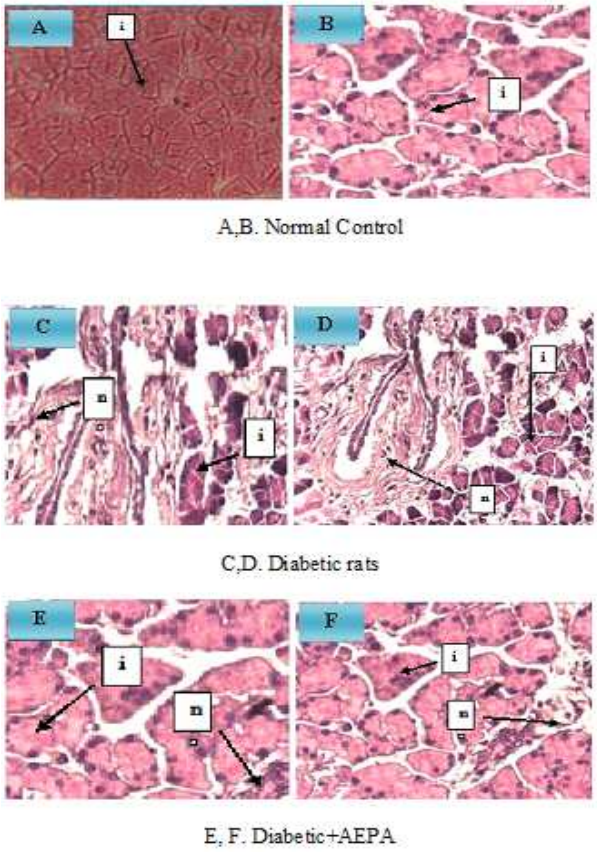

Fig. 3: Effect of aqueous extract of rhizome *Phragmites australis* on pancreas in alloxan–induced diabetic rats, fig. 3 Histology of the pancreas following induction of diabetes mellitus and treatment with *P. australis* rhizome extract in rats. (A,B) normal controls with normal Islet; (C,D) untreated diabetic group with congestion of vessels and focal area of necrosis and (E,F) diabetic treated with 200 mg/kg *P. australis* with moderate congestion of vessels, islets appear normal but few and small in size. Histologic slides were stained with hematoxylin and eosin (×400)

DISCUSSION

The results of phytochemical screening of aqueous extract of dried roots of *Phragmites australis* revealed the presence of various bioactive compounds, including phenolics, terpenoids and flavonoids. The phenolic compounds are well known as antioxidants and directed against free radicals associated with oxidative damage [15]. Acute toxicity studies revealed no mortality and no visible sign of toxicity. The LD50 was not calculated because there was no mortality at 5000 mg/kg dose level of aqueous extract of *Phragmites australis* indicating that it is relatively safe under short-term exposure. Characteristic symptoms in alloxan-induced diabetic rats included excessive water intake (polydipsia), increased food intake (hyperphagia), hyperglycaemia, and severe loss of body weight, which is consistent with some previously published reports [16, 17]. Loss body weight and increased food intake are a direct result of the accumulation of glucose in the blood and a compensatory reaction to

a lack of combustible fat associated with a metabolic disturbance during diabetes mellitus [18]. After the treatment time, the animals that received *Phragmites australis* showed reversion partial of this less body weight and food intake, due probably the improvement of behaviour and biochemical parameters. The treatment with aqueous extract of the roots of *Phragmites australis* for 21 d shows a significant decrease in blood glucose in diabetic rats. The anti-diabetic activity of medicinal plants has been ascribed to their phytochemical compounds [19] that, may delay the development of diabetic complications and regulate the associated metabolic abnormalities through a variety of mechanisms [20]. Phenolic compounds of *Phragmites australis* may affect the metabolism of glucose by several mechanisms such as the inhibition of carbohydrate digestion and absorption of glucose in the intestine, stimulation of insulin secretion by pancreatic beta cells, modulating the hepatic glucose release, activation of the insulin receptor and modulation of utilization hepatic glucose [21, 22]. Alkaloids are reported to retard the absorption of glucose via inhibition of enzymes, such as α-glucosidase, in the digestive organs [23]. Flavonoid contents that were reported to have anti-diabetic activity, so it's substances accelerate the functioning of the intracellular enzymatic machinery, responsible for the uptake of extracellular glucose and storage as glycogen or converted into fatty acids in the liver [24, 25]. The elevated cholesterol, triglycerides, low-density lipoprotein (LDL) and very low-density lipoprotein (VLDL) levels and decreased high-density lipoprotein (HDL) levels were reported in diabetic rats. Hypercholesterolemia is a common complication of diabetes mellitus and elevated VLDL and triglycerides (TG) reduce levels of cardioprotective high-density lipoprotein (HDL), with attending consequence of a reduction in antioxidant activities [26]. In this study, administration of aqueous extract of *Phragmites australis* significantly reduced elevated total cholesterol, triglycerides, low-density lipoprotein (LDL) and very low-density lipoprotein (VLDL) levels in diabetic rats. Also, increased level of high-density lipoprotein (HDL) was observed in diabetic rats treated with *Phragmites australis* compared to diabetic rats. These probably due to a rapid mobilization of triglycerides (TG) and consequent increased plasma free fatty acids levels [27]. This action of *Phragmites australis* renders its lipid lowering activity in diabetic condition and hence prevents diabetic-associated complications. The results of this study corroborate earlier reports that most hypoglycemic plants have potentials of ameliorating diabetes-associated abnormalities of lipid metabolism *in vivo* [28]. The presence of hyperlipidemia compounds in aqueous extract of *Phragmites australis* that may act as inhibitors for some enzymes such as hydroxy methyl glutaryl-CoA reductase, which participates in cholesterol synthesis or reduce intestinal absorption of cholesterol [29] or the plant extract might stimulate the production of insulin which in turn inhibits lipoprotein lipase activity or reduces lipid peroxidation [30]. On the other hand flavonoid contents in aqueous extract of *Phragmites australis* have a role in enhancing absorption of low-density lipoprotein (LDL) by increasing LDL receptors, increasing the activity of lecithin-cholesterol acyltransferase (LCAT) and tissue lipases and/or inhibition of acetyl-CoA carboxylase [31,32]. In the present study, the significant rises in serum glutamate-oxaloacetate transaminase (GOT), glutamate pyruvate transaminase (GPT), amylase and alkaline phosphatase (ALP) activities that were observed in alloxan-induced diabetic rats when compared to the control. These is as a result of tissue injury or changes in the permeability of liver membranes, hence the concentration may increase with acute damage to liver cells [33]. In another hand, the release of glutamate-oxaloacetate transaminase (GOT) and glutamate pyruvate transaminase (GPT) enzymes into the serum could relate to excessive accumulation of amino acids (glutamic and alanine) in the serum of diabetic animals as a result of amino acid mobilization from protein stores [34]. These excessive amino acids are then converted to ketonic bodies (alpha-ketoglutaric and pyruvate), for which the enzymes GOT and GPT are needed, leading to increased enzyme activity [35]. However, the aqueous extracts of *Phragmites australis* treated rats showed a significant reduction in the levels of these enzymes when compared to the diabetic group, thus an indication of the protective effects of the aqueous extracts over alloxan-induced liver damage which is in agreement with the other investigation [36]. Pancreatic Islets are especially vulnerable to oxidative stress mediated injuries because the antioxidant defence system of the pancreas is considerably weaker than those of other tissues [37]. There is a complex interaction between antioxidants and oxidants such as reactive oxygen species, which modulates the generation of oxidative stress [38]. In this study, the histopathological evaluation revealed a focal area of necrosis of islet cells in diabetic rats. However, pancreatic islet cells of diabetic rats treated with aqueous extracts of the rhizome of *Phragmites australis* appear normal. This suggests an inhibition of the toxic mechanisms involved in the alloxan-induced oxidative stress mediated destruction of pancreatic Islet cells by the antioxidant-rich, flavonoid-containing *Phragmites australis* extract preserving Islet cells integrity.

CONCLUSION

The present study clearly concluded that aqueous extract of the rhizome of *Phragmites australis* possesses the ability to control blood glucose in diabetes, its antioxidant and protective action on pancreatic β-cells, which in turn improve glucose metabolism and diabetic cardiovascular complications. Further studies on different animal and human models are essential to ensure the beneficial effects of aqueous extract of the rhizome of *Phragmites australis*. However, more studies also are required for the isolation and characterization of the active principles responsible for these activities.

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AUTHORS CONTRIBUTION

Derouiche Samir: Introduction, decapitation of rats, dissection, collection of blood sample and organs doing biochemical and tissue analyzes, making the results and discussion and conclusion and references

Manel Azzi: Handling rats and measure weight variation and intake food and control humidity and temperature all the long of the experiment, conducted of plant extraction methods and all the phytochemical analyses

Abir Hamida: Conducted all biostatistical and computer analysis.

CONFLICT OF INTERESTS

All authors have none to declare

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