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**PJBS**

ISSN 1028-8880

**Pakistan  
Journal of Biological Sciences**

**ANSI***net*

Asian Network for Scientific Information  
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

## The Effect of *Equisetum arvense* L. (Equisetaceae) in Histological Changes of Pancreatic $\beta$ -Cells in Streptozotocin-Induced Diabetic in Rats

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**Abstract:** In view of alleged, the methanolic extract of *Equisetum arvense* was analysed for its antidiabetic activity in streptozotocin-induced diabetic rats. The blood glucose lowering activity of the methanolic extract was determined in streptozotocin-induced (50 mg kg<sup>-1</sup>, i.p.; dissolved in normal saline) diabetic rats, after oral administration in doses of 50 and 250 mg kg<sup>-1</sup> daily for 5 weeks. The data was compare statistically using one-way ANOVA tukey test. The results showed methanolic extract of *Equisetum arvense* produced a significant antidiabetic activity at doses 50 and 50 mg kg<sup>-1</sup>/b.wt. Concurrent histological studies of the pancreas of these animals showed comparable regeneration by methanolic extract which were earlier, necrosed by streptozotocin.

**Key words:** Diabetic rats, *Equisetum arvense*, extract, blood sugar, pancreas

### INTRODUCTION

It is well known that diabetes mellitus is the commonest endocrine disorder that, according to the World than 176 million people world wide, in Mexico the WHO estimates that the Health Organization, affects more number of diabetic patients will increase from more than 2 million in 2002 to more than 6 million in 2030, which would imply that in a few decades Mexico may have highest rate of diabetes in the world. Because of the complications linked to diabetes like heart disease, retinopathy, kidney disease and neuropathy, it also is a common cause of chronic morbidity and disability among the working population. The term diabetes mellitus describes a metabolic disorder of multiple a etiologies and is characterized by chronic hyperglycaemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both. The causes of type 2 diabetes are either insulin resistance with relative insulin deficiency or predominantly an insulin secretory defect with or with out insulin resistance (Soleimani *et al.*, 2007).

Recently, there has been increasing interest in use of medicinal plants. The plant kingdom has become a largest for the search by multinational drug and biologically active lead compounds. Ethnobotanical in formation indicates that more than 800 plants are used as traditional remedies for the treatment of diabetes. The hypoglycaemic activity of a large number of these plant has been evaluated and confirmed in different animal models (Eidi *et al.*, 2005). Enhancement of the division of *E. arvense* protoplasts in culture by activated charcoal

and their further development (Akira *et al.*, 1990) and the hepatoprotective effect of *E. arvense* has been reported by Katikova *et al.* (2002). Hepatoprotective and free radical scavenging activities of phenolic petrosins and flavonoids isolated from (L) extract reported by Oh *et al.* (2004). Antidiabetic effect of *Equisetum arvense* in diabetic rats reported by Soleimani *et al.* (2007). The aim this study was to investigate the hypoglycemic effects of methanolic extract and histological change in Streptozotocin (STZ) induced rats.

### MATERIALS AND METHODS

***Equisetum arvense* L. subsp. *arvense* (Equisetaceae) pharmacopeia:** IName Equiseti herba is a traditional plant. Traditional use of the plant was recorded at market in 2005; by our selves of the plant for the treatment of kidney problems and diabetes in the Ardabil. Our own ethnopharmacological studies were performed in the community in Urmia university in 2005. Diabetic people were identified by the local health services and local healers. All informations were obtained about the plant and its special usage based on structured and unstructured interviews with the traditional healers and the diabetic people, respectively. The data were referred to plant samples (mini-herbarium) collected at its natural habitats and stored as herbarium vouchers for exact identification.

**Plant material:** *Equisetum arvense* is mainly found in northwestern regions of Iran especially Ardabil. This plant is considered an herbal drug and is used for renal

disorders and diabetic related illness. This powdered from of this plant was purchased from the Herbal medicine research institute of Tabriz university, Iran and their identity was confirmed and voucher specimens were deposited at this Herbarium in Tabriz (No. 313).

**Extraction:** The dried plant was than milled to fine powder and 500 g of this powder was packed in to Soxhlet apparatus and extracted with methanol. The extract were dried at 45°C in hot air oven till solid to semisolid mass was obtained and were stored in airtight containers in refrigerator below 10°C.

**Animals and experimental protocols:** A total of 30 male wistar rats were used; eight weeks old weighing 150 to 200 g was obtained from the laboratory animal center of Veterinary faculty of Urmia University. The rats were housed under controlled environmental condition (12 h dark light cycle at 22°C) and had free access to standard rat chow and water. Diabetes was induced by intravenously injecting STZ (50 mg kg<sup>-1</sup> body weight) in acetate normal saline.

**Experimental groups:** The diabetic animals were classified into 5 groups (1-5) each of them with 6 rats.

**Group 1:** As non-diabetics control received 0.03 mL physiological NaCl-solution (Vehicle).

**Group 2:** As the diabetic control received also 0.03 mL of physiological NaCl-solution (vehicle).

**Group 3:** Were given the standard oral hypoglycemic agent glibenclamide (5 mg kg<sup>-1</sup> b.wt.) in the same vehicle Groups (4 and 5) received (50 and 250 mg kg<sup>-1</sup> b.wt.) methanolic extracts, respectively.

**Collection of blood and determination of blood glucose:** Blood samples were taken from the tail vein before oral administration of the extracts or the vehicle. The glucose concentration was measured in plasma serum with Reflotron equipment and confirmed by Accutrend GC and Accu-check compact equipments (Roche).

**Histopathology evaluation:** Tissue samples from the pancreas were fixed in 10% buffered neutral formalin, embedded in paraffin, sectioned at 5 µm and stained with hematoxylin-eosin and periodic acid-schiff.

**Statistical analysis:** All the values of body weight, blood sugar and biochemical estimations were expressed as mean±Standard Error of Mean (SEM) and analyzed for ANOVA tukey test.

## RESULTS

**Activity in diabetic rats:** STZ administration at of dosage of 50 mg kg<sup>-1</sup> b. wt. to normal rats significantly elevated the blood glucose levels compared with rats injected normal salin alone (Table 1) as in previous reports (Soleimani *et al.*, 2007). In present diabetic rats, the extracts showed significant hypoglycemic effects (Table 1). The methanolic extract at doses of 50 and 250 mg kg<sup>-1</sup> bw significant reduction (p<.0001) of plasma glucose level compared with diabetic control from the 1 and 5 weeks of treatment. Glibenclamide (5 mg kg<sup>-1</sup> b. wt.) produced a significant decrease in plasma glucose (Table 1).

These results indicate that there is no significant difference between the tested plant preparations in comparison to glibenclamide (standard hypoglycemic drug).

**Animal weights:** The mean weight of diabetic animals was significantly lower than that of non-diabetic animals (Table 1) how ever; the glibenclamide regimen was sufficient both to support weight gain in the diabetic animals during the early stages and to maintain their weight at the later stages of the study. Mortality was less than 6% among diabetic animals during the study. The weights of the extracts-treated diabetic animals were statistically identical (Table 1).

The body weight of 5 weeks diabetic rats treated with methanolic extract of *E. arvensis*. At the diabetic's rats treated with methanolic extracts (50 and 250 mg kg<sup>-1</sup> day) immediately after diagnosis of diabetes had their body weight comparable to no-diabetic control groups (Table 1).

**Histopathological examination of pancreas:** Figure 1 A and B represent two islets of langerhans from a normal and a STZ-induced diabetic rats, respectively. Comparison of Fig. A and B it is clearly indicates the

Table 1: Effect of oral administration of methanolic extract of *Equisetum arvensis* on plasma glucose concentration, body weight in diabetic rats

Treatment G. No.	Body weight (g)	Blood glucose (mg dL <sup>-1</sup> ) ± SE
1	197.33±3.71 <sup>a</sup>	93.00±3.511 <sup>a</sup>
2	110.00±9.12	419.67±47.24
3	186.33±13.16 <sup>a</sup>	193.00±5.06 <sup>a</sup>
4	186.00±10.77 <sup>a</sup>	128.40±51.77 <sup>a</sup>
5	205.16±14.77 <sup>a</sup>	138.33±42.30 <sup>a</sup>

1: Non diabetic control; 2: Diabetic control; 3: Diabetic treated with 5 mg kg<sup>-1</sup> bw glibenclamide; 4: Diabetic treated with 50 mg kg<sup>-1</sup> bw; 5: Diabetic treated with 250 mg kg<sup>-1</sup> bw extract; Values are given mean±SEM for groups of six animals each. <sup>a</sup>: p<.0001, (tukey-test), diabetic control was compared with the vehicle control and extract treated groups were compared with the diabetic control

reduction in the number of  $\beta$ -cells in the diabetic rats. As it is evident from Fig. 1B, the islets are irregularly shaped and relatively small and atrophic. Most cells of the islets are small, degranulated and dark with scanty cytoplasm. Severe vacuolation and degranulation are present in the  $\beta$ -cells of a number of islets. A exudates predominantly of lymphocytes, with a few macrophages and neutrophils is evident within and around the affected

islets. However, compared to untreated diabetic rats, histopathological examination of the plant extract treated diabetic rats revealed an increase in the number of pancreatic islets and the number of  $\beta$ -cells along with a reduction in the number of initiated lymphocytes and macrophages (Fig. 1 C and D). Restoration of normal cellular population size of islets with hyperplasia by glibenclamide (Fig. 1 C-E).

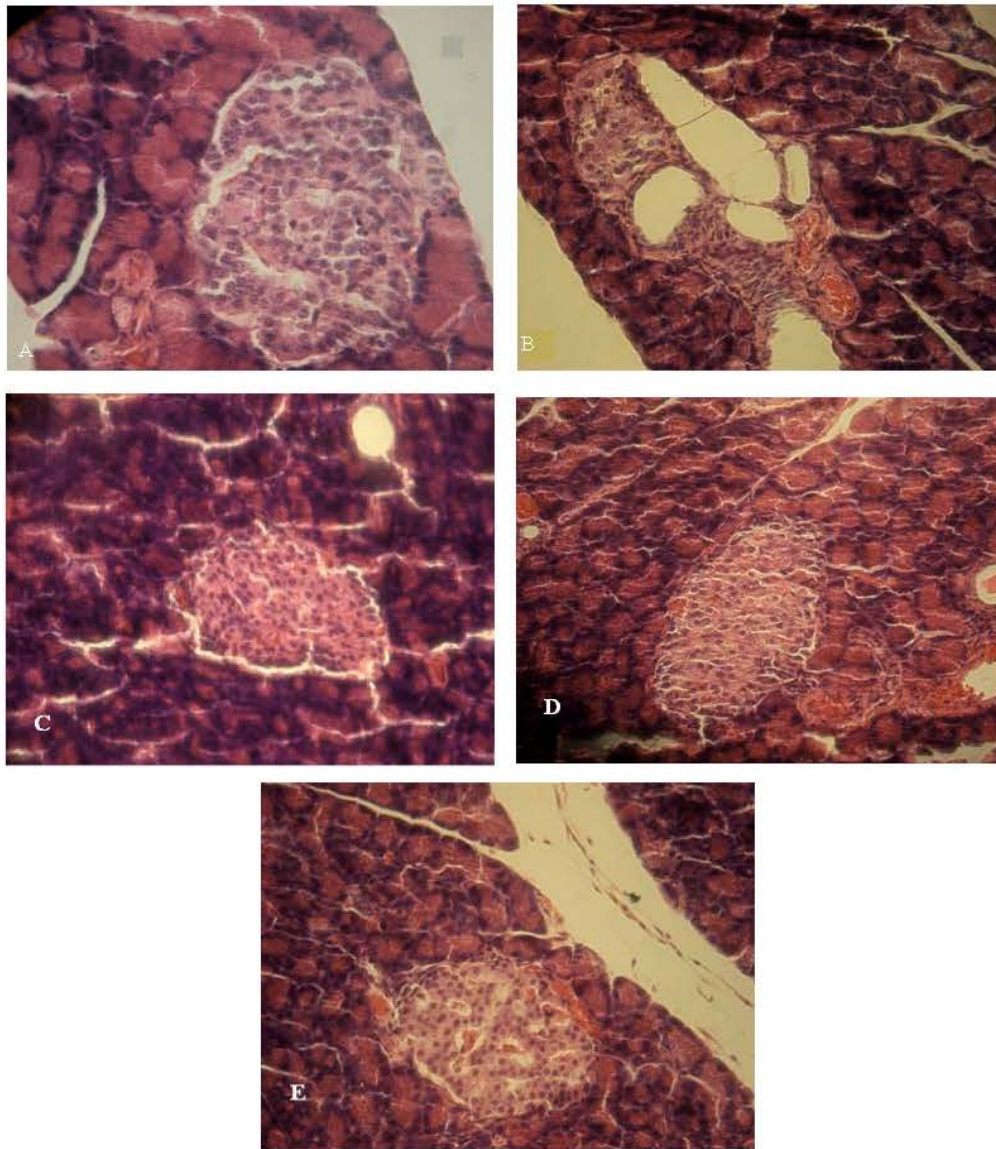


Fig. 1: Photomicrographs rat pancreas stained by hematoxylin and eosin of untreated (A) and STZ-induced diabetic (B) rats, (C) diabetic treated with 50 mg kg<sup>-1</sup> bw extract, (D) diabetic treated with 250 mg kg<sup>-1</sup> b. wt. extract and (E) diabetic treated with 5mg kg<sup>-1</sup> b. wt. glibenclamide (hematoxylin-eosin×200)

## DISCUSSION

In the present investigation, the methanolic extract of *Equisetum arvense* was investigated for its antidiabetic activity in streptozotocin-induced diabetic rats. Glibenclamide treatment (5 mg kg<sup>-1</sup>) was not as effective in reducing blood glucose in STZ-diabetic rats as in normoglycaemic rats. It has been reported that glibenclamide was not effective when destruction of  $\beta$ -cells has occurred and hence more effective in moderate diabetic rats than in severe diabetic animals (Sharma *et al.*, 1997; Andrade-Cetto *et al.*, 2000; Hosseinzadeh *et al.*, 2002). The acute hypoglycaemic effect of glibenclamide results has been shown from the stimulation of insulin release from the residual  $\beta$ -cells and inhibition of glucagon secretion (Moller, 2001). The extract might possess insulin like effect on peripheral tissues either by promoting glucose uptake and metabolism or inhibiting hepatic gluconeogenesis. The phytochemical studies of *C. pentandra* revealed the presence of epicatechin isolated from other plants has been found to stimulate insulin secretion or possess an insulin-like effect (Marles and Farnsworth, 1995; Noreen *et al.*, 1998; Kameswara *et al.*, 2001).

In light of the results, present study indicates that *E. arvense* have good antidiabetic activity. Methanolic extract of *E. arvense* exhibited significant anti-hyperglycemic activities in streptozotocin-induced hyperglycemic rats with out significant change in body weight. They can also improve the condition of DB as indicated by parameters like body weight. Among them methanolic extract produce a hypoglycemic effect in rats. Similar finding reported by Soleimani *et al.* (2007).

The plant extract treated diabetic samples histopathologically approach the corresponding healthy pancreatic samples. The regeneration of the  $\beta$ -cells of the STZ-destroyed islets is probably due to the fact that pancreas contain stem cells which have the capacity of regeneration. There fore, the surviving cells can proliferate to replace the lost cells (Yazdanparast *et al.*, 2005). The renewal of  $\beta$ -cells in diabetes has been studied in several animal models. The total  $\beta$ -cells mass reflects the balance between the renewal and loss of these cells. It was also suggested that regeneration of islet  $\beta$ -cells following destruction by alloxan may be the primary cause of the recovery of alloxan-injected guinea pigs from the effects of the drug (Nagappa *et al.*, 2003). Similar effects in streptozotocin-treated diabetic animal were reported by pancreas tonic (Rao *et al.*, 1998), ephedrine (Xiu *et al.*, 2001) and *Gymnema sylvestre* leaf extracts (Shanmugasundaram *et al.*, 1990). In present studies, the damage of pancreas in STZ-treated diabetic control rats (Fig. 1B) and regeneration of  $\beta$ -cells by glibenclamide (Fig. 1E) was observed.

## CONCLUSIONS

In conclusion, our histopathological investigation along with the biochemical evaluations suggests the possibility of the islets regeneration upon plant extract treatment. Further research is required to explore exactly the mechanism of islet regeneration by the plant extract.

## ACKNOWLEDGMENTS

I would like to thank the colleagues in the Faculty of Science for their cooperation. Appreciation also goes to Habib Shojaei who helped me in this study.

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