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Effect of *Equisetum arvense* L. (*Equisetaceae*) in Microalbuminuria and Creatinine Excretion in Streptozotocin-Induced Diabetes in Male Rats

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Abstract: In the present research, the methanolic extract of *Equisetum arvense* was analysed for its antidiabetic activity in streptozotocin-induced diabetic rats. The efficacy of the extract was also evaluated for protection of renal defects in diabetic rats. The blood glucose lowering activity of the methanolic extract was determined in steptozotocin-induced (50 mg kg^{-1}, i.p.; dissolved in normal saline) diabetic rats, after oral administration in doses of 50, 100, 250 and 500 mg kg^{-1} daily for 5 weeks. Urine samples were collected before the induction of diabetes and at the end of 5 weeks of treatments and analyzed for urinary microalbumin and creatinine level. The data was compare statistically using one-way ANOVA tukey test. The results showed that in different doses of methanolic extract blood sugar decreased significantly in comparison with the treatment and control groups of diabetic rats. Also the weights of methanolic-extract treatment group were higher than the other treatment groups. The present studies clearly indicate a significant antidiabetic and renoprotective effect with the methanolic extract of *Equisetum arvense* and lend support for its traditional usage.

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

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 (WHO, 2004), 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 (WHO, 1999). Associated changes in kidney function in terms of increased levels of blood urea, nitrogen and distinct proteinuria have been reported in streptozotocin induced diabetic rats (Pallavi *et al*., 2003). Detection of microalbuminuria or other evidence of the microvascular implications of diabetes mellitus (DM) is unusual before the onset of puberty, even though structural changes are occurring (Pascale *et al*., 2004; Masateru *et al*., 2005). STZ-induced diabetes in rats had been shown to be associated with functional and or morphological changes in the kidney and liver (Urmila *et al*., 2003). After the introduction of insulin therapy the field of herbal medicines research has been gaining significant importance in the last few decades and the demand to use natural products in the treatment of than 400 plant species showing anti diabetic activity, although some of these many remain to be scientifically established (Nalamolu *et al*., 2006). In north western regions of Iran *Equisetum arvense* used anti-diabetic herbal remedy. *Equisetum* is a Asia and European herb which grows in moist waste places throughout temperate regions of the world and is cultivated in Iran. It is a member of a very primitive family of plants. In spring a spore-bearing stem, resembling a thin asparagus shoot, rises 15-20 cm once shed, this is replaced by a pale green bush with erect hollow jointed stems with longitudinal furrows and with sharply-toothed sheaths covering each joint; from the sheaths of the central stem arise

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whorls of fine branches, each giving off finer whorls, the whole sometimes extending up to 60 cm in height, but usually less. Thiaminase activity in *Equisetum arvense* extracts has been reported by Fabre *et al.* (1993). Urinary metabolites of flavonoids and hydroxycinnamic acids in humans after application of a crude extract from *Equisetum arvense* reported by Graefe and Veit (1999). 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 *E. Arvense* (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 in streptoztocin (STZ)-induced rats. We have found that administration of *E. arvense* to streptozitocin-induced diabetic rats for month lowers the level of serum glucose, urinary creatinine and microalbuminuria.

MATERIALS AND METHODS

Equisetum arvense L. subsp. *arvense* (*Equisetaceae*) pharmacopeial Name Equiseti herbars 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 42 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 12 hour's 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^{-1} body weight) in acetate normal saline. Two days later, induction of diabetes was con firmed by measuring the blood glucose level. One day prior to sacrifice, the rats were placed in metabolic cages to determine 12 h urinary microalbumin and creatinine excretion.

Experimental groups: The diabetic animals were classified into 6 groups (1-6) 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^{-1} bw.) in the same vehicle

Groups (4-5-6 and 7) received (50, 100, 250 and 500 mg kg^{-1} bw.) 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).

Renal function: Urinary creatinine was measured by the jaffepicric acid procedure using Darman Kave kit absorbance at 490 nm was measured before and after addition of the acid reagent. In these and other clinical assays samples were diluted so that measurements were within the range of the standard curve. A portion of carefully timed (12 h collection period from 6:00 p.m. until 6:00 a.m., patients had avoided any physical activity) well-mixed sample of urine was used. Sample were stored at ±2 to ±8°C prior to testing. Analysis was made the same day after urine collection by immunoturbidimetric assay for urinary microalbumin Zist Chimiy on autoanalyser.

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

The result of present field study confirmed that *Equisetum arvense* is used as a hypoglycemic agent against diabetes type 1 and is also used against kidney problems. The plant locally named by its Persian name Dome asb. In general, the people drink the infusion of the plant after boiling 20-29 g in 1 L water. The tea or the macerated in drunk during the day. The dry plant is sold at the main market place in Urmia and Azarbaijans other place, with the same preparation way and a similar recommended dose, further-more an methanolic preparation was found, this preparation is recommended for the treatment of type 1 diabetes. Those results confirm the previously reported use of *E. arvense* for the treatment of diabetes type 1. STZ administration at of dosage of 50 mg kg^{-1} bw. to normal rats significantly elevated the blood glucose levels compared with rats injected normal salin alone (Table 1) as in previous reports (Andrae-Cetto *et al*., 2000; Andrae-Cetto and Wiedenfeld, 2001). In present diabetic rats, the extracts showed significant hypoglycemic effects (Table 1). The methanolic extract at doses of 50, 250 and 500 mg kg^{-1} bw. significant reduction ($p<0.0001$) of plasma glucose level compared with diabetic control from the 1 and 5 weeks of treatment. The methanolic extract at a dose of 100 mg kg^{-1} bw. did not show significant activity of plasma glucose level compared with diabetic control. Gilbenclamide (5 mg kg^{-1} bw.) 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).

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. arvense* at a dose of 100 mg kg^{-1} bw. treated not different from that of diabetic control rats. At the same time diabetic's rats treated with methanolic extracts (50, 250 and 500 mg kg^{-1} day) immediately after diagnosis of diabetes had their body weight comparable to no-diabetic control groups (Table 1).

STZ-diabetic rats exhibited significantly lower urinary creatinine level compared to their respective non-diabetic control group (Table 1). Urinary creatinine level of diabetic groups treated with doses 50, 250 and 500 mg kg^{-1} bw. extract and gilbenclamide (5 mg kg^{-1} bw.) were significantly ($p<0.001$) higher compared to their respective diabetic control group. The urinary creatinine level in diabetic rats treated with methanolic extract of *E. arvense* at a dose of 100 mg kg^{-1} bw. treated not different from that of diabetic control rats. Table 1 show data for urinary microalbumin levels for healthy and diabetic rats. urinary microalbumin levels were significantly ($p<0.05$) increased in diabetic control. The urinary microalbumin levels in diabetic group treared with doses 50, 250 and 500 mg kg^{-1} bw. extract and diabetic group treated with 5 mg kg^{-1} bw. gilbenclamide were significantly ($p<0.05$) lower compared to their respective diabetic control group.

Table 1: Effect of oral administration of methanolic extract of *Equisetum arvense* on plasma glucose concentration, body weight, urinary creatinine and microalbumin in diabetic rats

	5 weeks diabetic rats			
Treatment	Body wight (g)	Blood glucose (mg dL^{-1})±SE	Urinary reatinine (mg dL^{-1})±SE	Urinary microalbum (mg dL^{-1})
I	197.33±3.710[a]	93.00±3.511[a]	66.23±3.18†	-------
II	110.00±9.120	419.67±47.24	5.96±1.97	46.33±2.40
III	186.33±13.16[a]	193.00±5.060[a]	40.32±12.35†	12.55±2.30*
IV	186.00±10.77[a]	128.40±51.77[a]	29.65±21.11†	15.83±2.20*
V	154.61±10.84[a]	188.00±34.78[a]	11.50±1.870	29.00±2.80*
VI	205.16±14.77[a]	138.33±42.30[a]	72.39±32.59†	11.50±0.76*
IIV	204.80±7.390[a]	160.60±42.88[a]	63.73±36.55†	13.83±0.72*

I-non diabetic control; II-diabetic control; III- diabetic treated with 5mg kg^{-1} bw. gilbenclamide; IV-diabetic treated with 50 mg kg^{-1} bw.; V-diabetic treated with 100 mg kg^{-1} bw.;VI- diabetic treated with 250 mg kg^{-1} bw.; VII- diabetic treated with 500 mg kg^{-1} bw. extract. Values are given mean±SEM for groups of six animals each. [a]$p<0.0001$, †$p<0.001$,*$p<0.05$ (tukey-test), diabetic control was compared with the vehicle control and extract treated groups were compared with the diabetic control

DISCUSSION

Diabetes mellitus is possibly the world's largest growing metabolic disorder and as the knowledge on the heterogeneity of this disorder is advanced, the need for more appropriate therapy increases (Soleimani *et al*., 2007). Hypoglycemic effect of *Equisetum myriochaetum* aerial parts has been reported on streptozotocin diabetic rats (Andrae-Cetto *et al*., 2000). Similar findings were reported by Revilla-Monsale *el al*. (2002) on type 2 diabetic patients. In the present investigation, the methanolic extract of *Equisetum arvense* was investigated for its antidiabetic and renoprotective activity in streptozotocin-induced diabetic rats. Glibenclamide treatment (5 mg kg^{-1}) 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 β-cells has occurred and hence more effective in moderate diabetic rats than in severe diabetic animals (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 β-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 (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). However the improvement in urinary microalbumin and creatinine excretion with *E. arvense* extract. Similar results were obtained with diabetic rabbits treated with *Eugenia jambolana* (Kedar and chakrabarti, 1983) and non-diabetic rats treated with *Bauhinia forficate* (Pepato *et al*., 2002). Renoprotective effect of *Terminalia chebula* in diabetic rats reported by Nalamolu and Srinivas in (2006). microalbuminuria and proteinuria typically reflect the presence of moderate and advanced lesions, respectively in kidney disease, how ever, the development of diabetic nephropathy is characterized by a progressive increase in urinary protein particularly albumin and a late decline in glomerular filtration rate, leading eventually to end-stage renal failure (Salah *et al*., 2004). The pathophysiology involves glucose that binds irreversibly to proteins in the kidney and circulation to form advanced glycosylation end products (AGEs). AGEs can form complex crosslinks over years of hyperglycemia and can contribute to renal damage by stimulation of growth and fibrotic factors via receptors for AGEs. Increased glomerular capillary pressure occurs early in diabetes and is associated with hyperfiltration at the glomerulus. The glomerular mesangium expands, initially by cell proliferation and then by cell hypertrophy. Increased mesangial stretch and pressure can stimulate this expansion, as can high glucose levels. Mediators of proliferation and expansion include platelet-derived growth factor and transforming growth factor β (TGF-β). TGF-β are particularly important in the mediation of expansion and later fibrosis via the stimulation of collagen and fibronectin. Angiotensi-II (AT-II) also contributes to the progression of diabetic nephropathy. AT-II preferentially constricts the efferent arteriole in the glomerulus, leading to higher glomerular capillary pressure. In addition to its hemodynamic effects, AT-II also stimulates renal growth and fbrosis through AT-II type1 receptors, which secondarily upregulate TGF-β and other growth factors. The extract due to its significant hypoglycemic activity may have inhibited the formation of advanced glycosylation end products. However the extract may also have effect on the above stated other mechanisms. The possible mechanisms behind the hypoglycemic activity and the inhibition of incidence of diabetic nephropathy are yet to be studied.

CONCLUSIONS

In conclusion, the present studies indicated significant antidiabetic effects with the methanolic extract of *Equisetum arvense* (L.) and support its traditional usage in the control of diabetes and its complications. Further investigations to identify the active principle(s) are obviously needed together with a detailed evaluation on the mechanisms involved in the observed activities.

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