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Antidiabetic Effect of *Equisetum arvense* L. (Equisetaceae) in Streptozotocin-induced Diabetes in Male Rats

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Abstract: In view of alleged antidiabetic potential effect of the methanol, n-hexan and dichloromethane extracts of *Equisetum arvense* of blood sugar and body weight in streptozotocin-induced (50 mg kg^{-1}, i.p. dissolved in normal saline) diabetic rats were investigated. The blood glucose lowering activity of the methanol, n-hexan and dichloromethane extracts was determined in steptozotocin-induced diabetic rats; after oral administration in doses of 50, 100, 250 and 500 mg kg^{-1} daily for 5 weeks. The data was compare statistically using one-way ANOVA tukey test. The results showed that in different doses of methanolic extract, dichloromethane extract at doses of 50 and 250 mg kg^{-1} and n-hexan extract (50 mg kg^{-1}), 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 effect with the methanolic extract of *Equisetum arvense* and lend support for its traditional usage. Further investigation on identification of the active principles and their mode of action are needed to unravel the molecular mechanisms involved in the observed effects.

Key words: *Equisetum arvense*, extract, diabetic rats, blood sugar, body weight

INTRODUCTION

Insulin independent diabetes mellitus (IDDM), also called type 1 diabetes is defined as a chronic disease, characterized by a clinical disorder of sugar, fat and proteins metabolism caused by absence of insulin to promote sufficient glucose output from the liver (Tedong *et al*., 2006; Sanchez *et al*., 2000). Diabetes mellitus (DM) is a chronic disease caused by inherited and or acquired deficiency in production of insulin by the pancreas, or by the ineffectiveness of the insulin produced (Yazdanparast *et al*., 2005). Such a deficiency results in increased concentrations of glucose in the blood, which in turn damage many of the body's systems, in particular the blood vessels and nerves. As the number of people with diabetes multiply world wide, the disease takes an ever-increasing proportion of national and international health care budgets. It is projected to become one of the world's main disablers and killers within the next 25 years (Berlanga *et al*., 2005). Regions with greatest potentialare Asia and Africa, where DM rates could raise to two to three - folds than the present rates. Apart from currently available therapeutic options, many herbal medicines have been recommended for the treatment of diabetes. Traditional plant medicines are used throughout the world for arrange of diabetic presentations (Nagappa *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; Rai, 1995). In northwestern regions of Iran *Equisetum arvense* used anti-diabetic herbal remedy. Equisetum is an 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 whorls of fine branches, each giving off finer whorls, the whole sometimes extending up to 60 cm in height, but usually less.

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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.*, 1999) 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 *Equisetum arvense* (L) extract reported by Oh *et al.* (2004).

However, no scientific investigation has so far been conducted on the antidiabetic activity of *Equisetum arvense*. The aim this study was to investigate the hypoglycemic effects of methanolic, n-Hexan and dichloromethane extracts in streptoztocin (STZ)-induced rats. We have found that administration the methanolic extract of *Equisetum arvense* to streptozitocin-induced diabetic rats for month lowers the level of serum glucose.

MATERIALS AND METHODS

Equisetum arvense L. subsp. *arvense* (Equisetaceae), pharmacopeial Name: 'Equiseti herba' is a traditional plant. Traditional use of the *Equisetum arvense* 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 (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 (500 g). This powder was packed into soxhlet apparatus and extracted successively with, n-Hexan, dichloromethane and methanol. All the extracts 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: A total of 90 male wistar rats were used; eight weeks old (weighting 150-200 g) obtained from the laboratory animal center of Veterinary faculty of Urmia university and acclimatized with free access to food and water for at least 1 week in an air-conditioned room (25°C with 55% humidity) under an 12 h light 12 h dark cycle prior to the experiments.

Induction of experimental diabetes: Diabetes was induced by a single intraperitoneal injection (i.p.) of a freshly prepared streptozotocin (STZ) solution (50 mg kg^{-1} in acetate normal saline) to overnight-fasted rats (Elias *et al.*, 1994). Control rats received only the normal saline. Diabetes was identified by polydispia, polyuria and by measuring 48 h after injection of STZ. Animals, which did not develop more than 250 mg dL glucose level, were rejected.

Experimental groups: The diabetic animals were classified into 15 groups (1-15) 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 extracts, respectively.

Groups (8, 9, 10 and 11) received (50, 100, 250 and 500 mg kg^{-1} bw) dichloromethane extract, respectively.

Groups (4, 5, 6 and 7) received (50, 100, 250 and 500 mg kg^{-1} bw) methanolic extract and group(12, 13, 14 and 15) received (50, 100, 250 and 500 mg kg^{-1} bw) n-Hexan extract, respectively. The extracts were redissolved in 0.03 mL of physiological NaCl- solution and administered orally by a canule (gavage (I. g.)).

Collection of blood and determination of blood glucose: Blood sample were taken from the tail vein before oral administration of the extracts or the vehicle and at time 0 thereafter. The glucose concentrations was confirmed by Accurate GC and Accu-check compact equipments (Roche).

Statistical analysis: All the values of body weight and blood sugar were expressed as mean_standard error of mean±(SEM) and analyzed for ANOVA tukey test. Differences between groups were considered significant at $p<0.001$ levels.

RESULTS

Ethnobotany: The result of our 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 *Equisetum arvense* for the treatment of diabetes type 1.

Activity in diabetic rats: 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; Andrae-Cetto and Wiedenfeld, 2004). In our 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. The dichloromethane (50 and 250 mg kg^{-1}) and the n-hexan (50 mg kg^{-1}) extracts led to a significant decrease in plasma glucose level compared with the diabetic control group. The dichloromethane (100 and 500 mg kg^{-1} bw) and the n-Hexan (100, 250 and 500 mg kg^{-1} bw) extracts had no effect on plasma glucose levels changed by STZ.

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).

Animal weights: The mean weight of diabetic animals was significantly lower than that of non-diabetic animals (Fig. 1) however; 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. Diabetic animals consuming the n-hexan extract or the dichloromethane extract also weight 16-28 % less than the treated diabetic group with the methanolic extract ($p<0.0001$) (Table 2 and 3); the weights of the extracts-treated diabetic animals were statistically identical (Fig. 1).

Table 1: Effect of oral administration of methanolic extract of *Equisetum arvense* on plasma glucose concentration in diabetic rats

Treatments	Blood glucose levels (mg dL^{-1})±SE
Time	T_0
Control (+) no diabetic (vehicle)	93.00±3.511[a]
Control (_) diabetic (vehicle)	419.67±47.24[b]
Glibenclamide (5 mg kg^{-1})	193.00±5.06[a]
Equisetum arvense methanolic extract 50 mg kg^{-1}	128.40±51.77[a]
Equisetum arvense methanolic 100 mg kg^{-1}	188.00±34.78[a]
Equisetum arvense methanolic 250 mg kg^{-1}	138.33±42.30[a]
Equisetum arvense methanolic 500 mg kg^{-1}	160.60±42.88[a]

The values represent the means±SEM as compared with diabetic control group. Different numbers indicate statistical difference against the control group, all significances at least $p<0.0001$

Table 2: Effect of oral administration of n-hexan extract of Equisetum *arvense* on plasma glucose concentration in diabetic rats

Treatments	Blood glucose levels (mg dL^{-1})±SE
Time	T_0
Control(+) no diabetic(vehicle)	93.00±3.511[a]
Control (_) diabetic (vehicle)	419.67±47.24[c]
Glibenclamide (5 mg kg^{-1})	193.00±5.06[ab]
Equisetum arvense n hexan 50 mg kg^{-1}	243.00±18.82[ab]
Equisetum arvense n hexan extract 100 mg kg^{-1}	344.33±39.92[bc]
Equisetum arvense n-hexanextract 250 mg kg^{-1}	282.33±49.15[bc]
Equisetum arvense n -hexan extract 500 mg kg^{-1}	295.33±43.84[bc]

The values represent the means±SEM as compared with control time intervals. Different numbers indicate statistical difference against the control group, all significances at least $p<0.0001$

Table 3: Effect of oral administration of dichloromethane extract of *Equisetum arvense* on plasma glucose concentration in diabetic rats

Treatments	Blood glucose levels (mg dL)±SE
Time	T_0
Control (+) no diabetic (vehicle)	93.00±3.511[a]
Control (_) diabetic (vehicle)	419.67±47.24[c]
Glibenclamide (5 mg kg^{-1})	193.00±5.06[ab]
Equisetum arvense dichloromethane 50 mg kg^{-1}	270.00±17.32[b]
Equisetum ravense dichloromethane 100 mg kg^{-1}	315.00±20.82[bc]
Equisetum arvense dichloromethane 250 mg kg^{-1}	281.00±14.29[b]
Equisetum arvense dichloromethane 500 mg kg^{-1}	304.67±18.56[bc]

The values represent the means±SEM as compared with control time intervals. Different numbers indicate statistical difference against the control group, all significances at least $p<0.0001$

The body weight of 5 weeks diabetic rats treated with n-Hexan? dichloromethane extracts of *Equisetum arvense* the doses of 50, 100, 250 and 500 (mg kg^{-1} bw day) and at a dose of 100 mg kg^{-1} bw of methanolic extract 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 (Fig. 1).

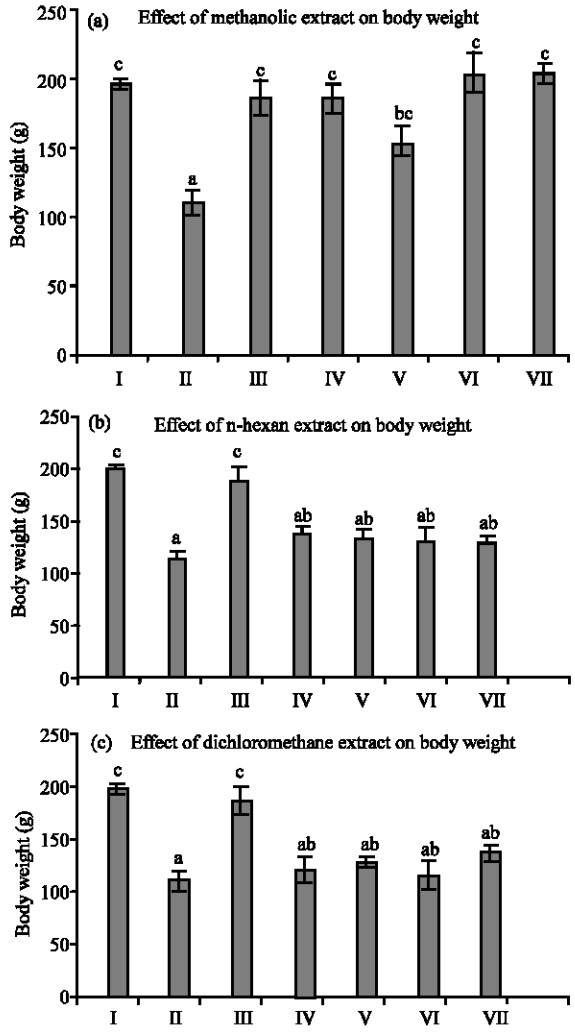

Fig. 1: Effect of different extracts of, *Equisetum arvense* (L) treatment on body weight in streptozotocin-diabetic rats. I-non diabetic control; II-diabetic-control; II-diabetic treated with 5 mg kg^{-1} bw gilbenclamide; IV-diabetic treated with 50 mg kg^{-1} bw extract; V-diabetic treated with 100 mg kg^{-1} bw extract; VI-diabetic treated with 250 mg kg^{-1} bw extract; VII-diabetic treated with 500 mg kg^{-1} bw extract. Values are mean± SEM $p<0.0001$ compared with diabetic control rats

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 (Baily and Flatt, 1986). The enormous costs of modern medicines indicate that alternative strategies are required for better management of diabetes. Traditional plant medicines are used throughout the world for a range of diabetic complications. The study of such medicines might offer a natural key to unlock a diabetologist's pharmacy for the future. In adult animals, streptozotocin selectively destroys the pancreatic insulin-secreting β-Cells leaving less active cells resulting in type1 diabetic state (Kamtchouing *et al*., 1999; Sokeng *et al*., 2005) streptozotocin, N- (methylnitrocarbamoyl)-D-glucosamine is potent methylating agent for DNA and acts as nitric oxide donor in pancreatic β-cells and thus β-cells are more sensitive to damage by nitric oxide and free radical scavenging enzymes (Lukic *et al*., 1998). Single dose administration of STZ produces diabetogenic effects in the first 24 h in rats (Gunes *et al*., 1999). 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 type2 diabetic patients. In the present investigation, the methanolic, n-Hexan and dichloromethane extracts of *Equisetum arvense* was investigated for its antidiabetic 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 (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 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 epicatechinisolated 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. Methanol 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 just methanolic extract produce a hypoglycemic effect

in rats and n-Hexan (100, 250 and 500 mg kg^{-1}) and dichloromethanolic (100 and 500 mg kg^{-1}) extract didn't show any significant affects as compared to diabetic control groups.

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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