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Original Article

Effects of methanolic and butanolic fractions of *Allium elburzense Wendelbo bulbs* on blood glucose level of normal and STZ-induced diabetic rats

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Abstract

Allium elburzense (*A. elborzense*, Alliaceae), a plant rich in saponins, is an edible vegetable in northern Iran with a folk background use as antidiabetic which has not yet been examined for this indication. To evaluate the antidiabetic potential of *A. elburzense*, its hydroalcoholic (HdAE) and butanolic extracts (BuE) were examined. The acute (1, 2, 3, 4, 8 h) and sub-acute (11 days) effects of oral (p.o.) and intraperitoneal (i.p.) administration of HdAE and BuE of *A. elburzense* bulbs in different doses were evaluated on blood glucose levels of normal and streptozotocin (STZ, 55 mg/kg body weight)-induced diabetic rats. Glibenclamide (1 mg/kg b.w.) was used as reference drug. Sub-acute treatment with HdAE for 11 days reduced significantly blood glucose levels in diabetic rats (at least $P<0.05$), while BuE was effective only following i.p. administration ($P<0.01$). Acute administration did not reduce blood glucose level in normal and diabetic animals. It is concluded that HdAE of *A. elburzense* exhibited a significant antihyperglycemic activity following chronic administration. These results provide evidence for potential use of *A. elburzense* in diabetes mellitus considering the fact that this plant is endemic to a location of Iran where diabetes is a high prevalence disorder.

Keywords: *Allium elburzenses*; Diabetes mellitus; Antidiabetic; Plant extract; Streptozotocin

INTRODUCTION

Diabetes mellitus is the most common endocrine disorder that is expected to affect more than 285 million people worldwide in 2010 (6.6% of world's adult population) (1,2). Various complications develop as a consequence of the metabolic derangements in diabetes such as macrovascular disease and microangiopathy resulted in nephropathy, retinopathy and chronic heart disease (3); these in addition to economic loads and inconveniences of drug administration especially in insulin-dependent patients make this disease suffering (4).

Seventy percent of the current cases of diabetes occur in low and middle income countries. With an estimated 50.8 million people living with diabetes, India has the world's largest diabetes population, followed by China with 43.2 million (1,2). Classic medications have shown insufficiency to treat diabetes in a high population and low income countries. Therefore, traditional remedies always have been under focus in this field. The regional prevalence of diabetes in Middle East is 7.7% and survey in Iran showed the prevalence to be 6.1%. (1,2). Several herbs including *Allium* plants have been used to treat diabetes (5,6).

Allium species are very important edible plants because of worldwide use and bearing valuable pharmacologically active and chemically interesting constituents. The most

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occurring compounds in *Allium* genus are steroidal saponin/sapogenins, flavonoids and sulfuric compounds (7-10). Through history, this genus has been used for cardiovascular diseases, hypertension, hypercholesterolemia and diabetes (9,11-13). In Iranian traditional medicine, *Allium* plants have been considered as anti-hyperglycemic especially to prevent diabetes in people who are prone to this disorder by daily intake (6). Antidiabetic effects of this genus have been attributed to its sulfuric, saponin glycosides and phenolic contents (9,14). Besides, flavonoids are reported to promote insulin release and increase calcium uptake from isolated Langerhans β-cells (15).

Allium elburzense Wendelbo (*A. elburzense,* Alliaceae) is an endemic plant to Elburz Mountains area of northern Iran. Its aerial parts are usually used in cooking mixed rice. This species has been used in Iranian traditional and folk medicine as antirheumatic, aphrodisiac, anthelminthic and antidiabetic (6). Several sapogenins and new saponins with antispasmodic effects have been previously isolated from this plant (16,17). In addition, antidiabetic effect of a saponin-rich fraction of Fenugreek seed and some Chinese herbs have also been reported (18,19).

Hence, the aim of the current study was to investigate the anti-diabetic activities of the saponin-containing fraction extracted by butanol (BuE) and hydroalcoholic extract (HdAE) of this plant. Elburzensosides (R: H, Glc or triGlc; R': H or OH), a saponin steroid have been previously (16) isolated from *A. elburzense* with the rare moiety of OH at C-5 (Fig. 1).

MATERIALS AND METHODS

Chemicals

Streptozotocin (STZ) was purchased from Pharmacia & Upjohn Co. (Kalamazoo, MI, USA). Diagnostic kit for glucose determination was obtained from Pars Azmoon (Karaj, Iran). Glibenclamide was from Chemidaru Co. (Tehran, Iran). Analytical grade solvents were obtained from Merck, Darmschtod (Germany).

Plant material

A. elburzense bulbs were purchased from a local market in Tehran (Iran) in April 2007, and the botanical identity of the plant material was confirmed by Dr. Iraj Mehregan (Science and Research Branch, Islamic Azad University, Tehran, Iran) by comparison to the voucher specimen already deposited in the Herbarium Division of School of Pharmacy and Pharmaceutical Sciences, Isfahan, Iran (Ref. No. 1145).

Preparation of hydroalcoholic extract

Dried ground bulbs (100 g) were extracted with MeOH 70% (1 L × 4) at room temperature to get crude extract after removing MeOH *in vacuo*, and H2O via freeze drying for 48 h (8.5 g).

Preparation of butanolic extract

Dried ground bulbs (100 g) were sequentially extracted with hexane, chloroform, chloroform: MeOH (9:1) and MeOH. The latter was suspended in H_2O and extracted with BuOH to get rid of saccharides and amino acids. Dried filtered BuOH extract was used as BuE (2.25 g).

Phytochemical analysis

Qualitative phytochemical screening was performed on the HdAE to detect the presence of secondary metabolites such as anthraquinones, tannins, saponins, alkaloids and cardiac glycosides using standard phytochemical methods (20). NMR analysis (with deuteriated methanol) was also carried out on the BuE to confirm the presence of saponins which have already been reported on the plant (16).

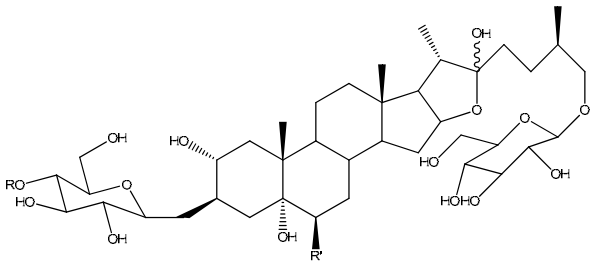

Fig. 1. Elburzensosides (R=H, Glc or triGlc; R'= H or OH). The saponin steroids have been previously isolated from *A. elburzense* with the rare moiety of OH at C-5 (16).

Animals

Male albino Wistar rats (weighing 200-250 g) bred in the animal house of the School of Pharmacy and Pharmmaceutical Sciences of Isfahan University of Medical Sciences were used. They were housed in polypropylene colony cages (4 rats per cage) at ambient temperature (23 ± 2°C) and 12/12 h light/dark cycles. They were fed *ad libitum* with normal laboratory chow.

Before testing for blood glucose levels, rats were fasted overnight (at least 12 h) with free access to water. All experimental procedures involving animals were approved by the Animal Research Ethics Committee of Isfahan University of Medical Sciences, Isfahan, Iran. Animals were assigned randomly into groups of at least six rats.

Drug administration

A. elburzense extracts were suspended in normal saline and test doses of 1 ml were orally administered by feeding tube. For parenteral injection, test extracts were reconstituted in normal saline + tween20 (0.5%) and applied intraperitonealy (i.p.). STZ was dissolved in 0.1 M citrate buffer with pH of 4.5.

Assessment of acute hypoglycemic effect of HdAE and BuE in normal rats

Rats were randomly divided into ten groups comprised of 6 animals in each group: control group treated with normal saline (5 ml/kg), standard group treated with oral (p.o.) glibenclamide (1 mg/kg) twice a day (bid), normal rats received different doses of HdAE oraly (100-600 mg/kg) or i.p. (300 mg/kg) bid. BuE was also administered orally (5-50 mg/kg) or i.p. (25 mg/kg).

Assessment of sub-acute hypoglycemic effect of HdAE and BuE in normal rats

Blood glucose levels of normal rats treated with different extracts at different doses, were determined on the day11 after induction of the disease to assess the sub-acute effect of the extract. Control group receiving vehicle and reference group receiving glibenclamide were used as pointed out earlier.

Induction of diabetes

Diabetes was induced by a single i.p. injection of STZ (55 mg/kg, i.p.) to the rats. They had free access to food and water *ad libitum*, and after three days, their fasted blood glucose levels (mg/dl) were measured. Only diabetic rats with a fasting blood glucose level of at least 250 mg/dl were included in the experiments. Furthermore, diabetes was verified considering polydipsia and polyuria.

Assessment of acute antihyperglycemic effect of HdAE and BuE in diabetic rats

Rats were divided into ten groups (6 animals in each group): control groups received normal saline (5 ml/kg), reference groups treated with glibenclamide (1 mg/kg). HdAE and BuE were given to diabetic rats orally or intraperitoneally with similar doses as mentioned above.

Anti-hyperglycemic effect of the plant extract was assessed in diabetic rats at 0 h (before drug administration) and after p.o. or i.p. administration by measuring blood glucose levels at 1, 2, 3, 4 and 8 h after drug administration.

Assessment of sub-acute anti-hyperglycemic effect of HdAE and BuE in diabetic rats

Blood glucose levels of diabetic rats treated bid with HdAE or BuE, with different doses were determined on day 11 to assess the sub-acute effects of the extracts.

Estimation of blood glucose levels

Blood samples were taken by heparinized microhematocrit capillaries from orbital sinus plexus and were transferred into heparinized centrifuge tubes for plasma separation. Blood glucose levels were measured using glucose kits (Pars Azmoon, Iran) based on glucose oxidase method (21).

Statistical analysis

Data are presented as mean ± SEM. One-way analysis of variance (ANOVA) with Scheffe Post-hoc test was performed to assess the differences between means (SPSS software; Version 12.0) with the level of significance set at $P<0.05$.

RESULTS

Phytochemical analysis

The qualitative phytochemical screening of the HdAE revealed the presence of flavonoids, steroids, phenols, glycosides, lipids, and saponins with the absence of anthraquinones. In preliminary NMR analysis of BuE, there were characteristic signals of glycoterpenoid nature of the mixture containing singlet methyls (0.8-1.1) and anomeric protons of glycosyl part (ca. δ_H 4-5), implying previously isolated saponins (16), *i.e.* furastanols as elburzensosides (Fig. 1). These compounds are of chemical importance because of possessing an α-OH in C5 which is a rare occurrence in natural saponins (16,20).

Acute and sub-acute hypoglycemic effect of HdAE and BuE in normal rats

The effects of HdAE and BuE on fasting blood glucose levels of normal rats are presented in Figs. 2 and 3, respectively. Both HdAE and BuE induced no hypoglycemic

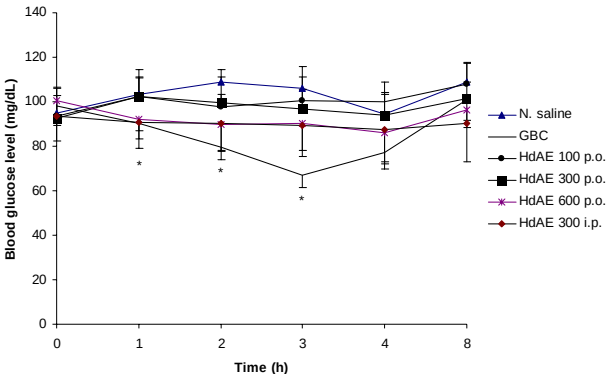

Fig. 2. Effect of Hydroalcoholic extract (HdAE) *of A. elburzense* on blood glucose levels of normall rats at different doses of 100, 300 or 600 mg/kg given p.o. or 300 mg/kg administered i.p. within 0-8 h after extract administration. Glibenclamide (GBC, 1 mg/kg) and normal saline (5 ml/kg) have been used as positive and negative controls, respectively (n=6). * P<0.05: significant difference compared to normal saline group.

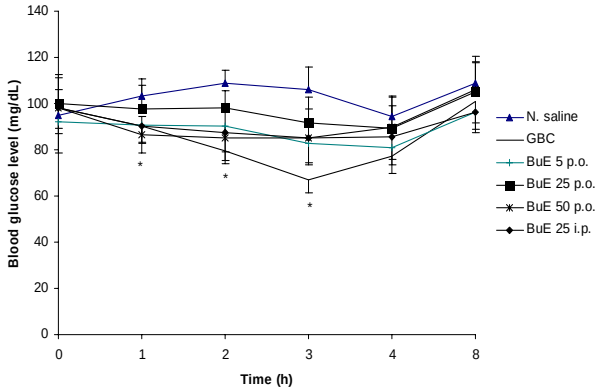

Fig. 3. Effect of *A. elburzense* butanolic extract (BuE) on blood glucose levels of normal rats at different doses of 5, 25 or 50 mg/kg given p.o. or 25 mg/kg given i.p.within 0-8 h after administration of the extract. Glibenclamide (GBC, 1 mg/kg) and normal saline (5 ml/kg) have been used as positive and negative controls, respectively (n=6). * P<0.05: significant difference compared to normal saline group.

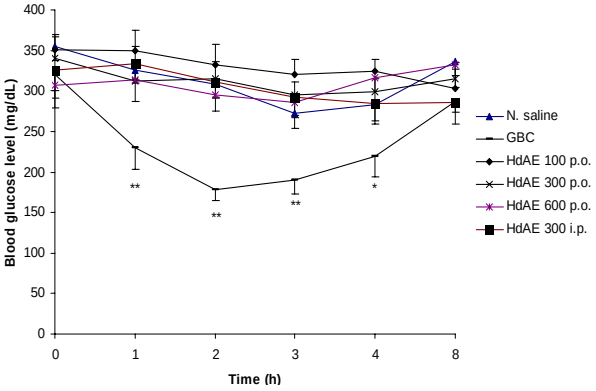

Fig. 4. Effect of Hydroalcoholic extract (HdAE) *of A. elburzense* on blood glucose levels of STZ-induced diabetic rats at different doses of 100, 300 or 600 mg/kg given p.o. or 300 mg/kg administered i.p. within 0-8 h after extract administration. Glibenclamide (GBC, 1 mg/kg) and normal saline (5 ml/kg) have been used as positive and negative controls, respectively (n=6). * P<0.05, ** P<0.01: significant difference compared to normal saline group.

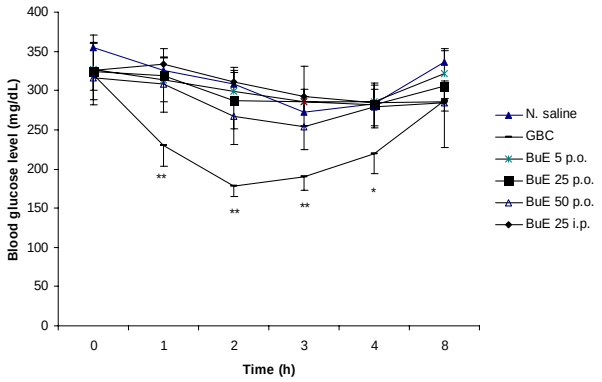

Fig. 5. Effect of *A. elburzense* butanolic extract (BuE) on blood glucose levels of STZ-induced diabetic rats at different doses of 5, 25 or 50 mg/kg given p.o. or 25 mg/kg given i.p.within 0-8 h after administration of the extract. Glibenclamide (GBC, 1 mg/kg) and normal saline (5 ml/kg) have been used as positive and negative controls, respectively (n=6). * P<0.05, ** P<0.01: significant difference compared to normal saline group.

Table 1: Sub-acute anti-hyperglycemic effect of *A. elburzense* hydroalcoholic extract (HdAE) and butanolic extract (BuE) in STZ-induced diabetic rats.

Group	Blood glucose level at day 11th (mg/dL)
Control (normal saline, 5ml/kg)	338.83 ± 18.08
Glibenclamide, 1mg/kg, p.o.	169.17 ± 20.45 **
HdAE, 100 mg/kg p.o.	271.83 ± 24.09 *
HdAE, 300 mg/kg p.o.	225.33 ± 22.31 **
HdAE, 600 mg/kg p.o.	189.17 ± 30.35 **
HdAE, 300 mg/kg i.p.	221.67 ± 20.45 **
BuE, 5 mg/kg, p.o.	297.83 ± 32.26
BuE, 25 mg/kg, p.o.	296.33 ± 37.38
BuE, 50 mg/kg, p.o.	295.67 ± 31.97
BuE, 25 mg/kg, i.p.	221.67 ± 20.45 **

Values are expressed as mean ± SEM (n=6);
p.o.= oral, i.p.= intraperitoneal, * $P<0.05$, ** $P<0.01$ compared with control group.

effect on normal animals. Giving glibenclamide to normal animals produced a significant ($P<0.05$) hypoglycemic effect until third hour, as compared with the normal control group or with time 0 (Fig. 2, 3). HdAE or BuE did not show hypoglycemic effects in normal rats after 11 days of twice daily treatment with either extracts (data not shown).

Acute and sub-acute anti-hyperglycemic effect of HdAE and BuE in STZ-induced diabetic rats

Neither HdAE nor BuE could alter significantly the blood glucose levels of diabetic rats within the first 8 hours after the treatment compared to control group (Fig. 4, 5). Glibenclamide decreased blood glucose levels significantly from the first h ($P<0.01$) up to the forth ($P<0.05$) h after treatment. In sub-acute mode of treatment, HdAE significantly reduced blood glucose levels of diabetic rats at all test doses (100-600 mg/kg) after p.o. and i.p. (300mg/kg) administration as compared to the control group ($P<0.01$) (Table 1). BuE could significantly reduce blood glucose levels only after i.p. administration ($P<0.01$). Glibenclamide (1 mg/kg, p.o.) exerted significant anti-hyperglycemic effect at sub-acute mode of administration too ($P<0.01$).

DISCUSSION

There is an ongoing research regarding new antidiabetic drugs. Natural products especially edible plants whose safeties have been shown through the decades of consumption have the potentials to be considered as new drug leads. *A. elburzense* which is being utilized as a food additive has also been used as anti-diabetic in folk medicine (9). In the meantime it is discovered that steroid saponins which are also contained in this plant possess anti-diabetic effect (16). Therefore, this herbal medicine could be a good candidate for the treatment of diabetes mellitus (18,19).

Using STZ-induced diabetes, a common method to induce diabetes in animal model, we investigated anti-hyperglycemic effects of HdAE and BuE of *A. elburzense*. HdAE decreaseed significantly the blood glucose levels in diabetic rats, both after oral and i.p. sub-acute administration.

Our results indicated that BuE was only effective to reduce hyperglycemia after chronic administration and via parenteral route. This might be due to increased availability of active components of the extract following parenteral administration compared to oral route. Extensive first pass effect or low oral bioavailability is common for many plants bioactive materials like saponins, polyphenols and anthocyanins (22). Effectiveness of plant extracts after prolonged administration has indicated that time dependent mechanisms such as inhibition of glucose uptake from intestine, promotion of tissue glucose uptake, decreased serum glucagon, inhibition of α-amilase or α-glucosidase and an increase in hepatic glycogen content might be involved (23). For instance inhibition of glucose

absorption by saponins (24) and inhibition of α-amilase or α-glucosidase and increase in hepatic glycogen content have been already proved in *Allium species* (24,25). Other mechanisms like enhancement of insulin release or insulin like effects are not postulated as they behave in a rapid-acting manner (26). On the other hand the *A. elborzense* fractions couldn't decrease blood glucose levels in normal rats suggesting a beneficial and favorable effect for this plant as a cookery additive. Indeed hypoglycemic activity under normal conditions could be problematic (24). Lacks of hypoglycemic effect together with antihyperglycemic activity of HdAE of *A. elborzense* in diabetic rats indicate that some mechanisms which are independent of insulin release and/or insulin effect potentiataion might be involved (27). Anti-diabetic effects of saponin-rich extracts of Fenugreek seed and some Chinese herbs have already been shown (18,19). However, lack of efficacy of the BuE in the present study at least in comparison to HdAE, necessitates more research on anti-diabetic evaluation of saponins (23). In addition, some other identified constituents of *A. elburzense*, including flavonoids and sulfuric compounds (16) might be responsible for these findings. Stajner et al. demonstrated that sulfuric constituents of *Allium*s species were responsible for blood glucose lowering effect accompanied with ameliorating body weight loss in diabetic rats (14). Furthermore, in *Allium* species it has been shown that total extract sometimes renders a better effect on hyperglycemia than isolated compounds proposing a within and/or between group of compounds synergistic effect (28). In a clinical study (29) it was revealed that total extract of *Allium sativum* could alleviate hyperglycemia and improve life quality of diabetic patients through secondary candidial infection elimination. This multi-functional activity and chemical synergism has frequently been reported on plants with potential medicinal indications (30). It is notable that many *Allium* species possess other bioactivities like anti-hyperlipidemic and anti-hypertensive activities which might be beneficial for patients with different types of diabetes mellitus.

CONCLUSION

In conclusion, it was shown that *A. elburzense* bulb HdAE and BuE exert antidiabetic effect in long term use in accordance with folk beliefs. Further pharmacological, biochemical and phyto-chemical investigations are required to elucidate the mechanism of action and the active phytochemicals of *A. elburzense* which are responsible for this activity.

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