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Original Article

Effect of *Hordeum vulgare* L. (Barley) on blood glucose levels of normal and STZ-induced diabetic rats

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Abstract

Barley (*Hordeum vulgare* L.) is the world's fourth most important cereal crop after wheat, rice and maize. It is readily available with reasonable cost, and has the highest amount of dietary fiber among the cereals which may be beneficial for metabolic syndrome. In the present study, the effect of hydroalcoholic extract of barley seeds and a protein enriched fraction on blood glucose of normal and streptozotocin (STZ)-induced diabetic rats (STZ, 55 mg/kg, i.p) were investigated. Normal and diabetic male Wistar rats were treated daily with normal saline (1 ml), barley hydroalcoholic extract (BHE) (0.1, 0.25, 0.5 g/kg), protein enriched fraction (PEF) (0.1, 0.2, 0.4 g/kg) and glibenclamide (1 and 3 mg/kg), separately and the treatment was continued for 11 days. Blood samples were taken at 0, 1, 2, 3, 9 h in the first day and the days 5 (120 h) and 11 (264 h) for measuring the blood glucose levels (BGL). Our results indicated that none of the BHE and PEF, were effective to reduce BGL in normal or diabetic rats in acute phase of treatment (1st day). Nevertheless, BHE at doses of 0.25 and 0.5 g/kg, were only effective in detracting BGL of diabetic rats after 11 days of continued daily therapy. Moreover, BHE restored body weight of diabetic rats at the end of treatment. Glibenclamide had also hypoglycemic action in normal and diabetic rats after both acute and extended treatments. These findings suggest that barley seeds hydroalcoholic extract, has a role in diabetic control in long term consumption, and this effect might be at least due to its high fiber content. More detailed studies are warranted to demonstrate its mechanism of action and identify active components.

Keywords: Barley seeds, Hydroalcoholic extract, Antihyperglycemic, Normal and diabetic rats

INTRODUCTION

Diabetes mellitus (DM) is one of the most important metabolic disorders manifested by hyperglycemia and impaired insulin secretion. Its progressive form is followed by ketosis and proteinuria, and also associated with a number of complications like retinopathy, nephropathy, neuropathy and peripheral vascular insufficiencies that can lead to limb amputation (1).

Insulin is the principle hormone that modulates uptake of glucose into most of cells from blood, and its insufficiency or the insensitivity of its receptors plays a fundamental role in DM (2). Diabetes mellitus is the most common endocrine disorder that affects more than 285 million people worldwide, and if nothing is done to control it, affected people will exceed 333 million by 2025 (1,3).

The advantage of functional foods and herbal medicines during the daily life has been demonstrated. For this purpose, several studies on functional foods and medicinal plants in addition to their active ingredients have been accomplished to confirm their usefulness in controlling the diabetes and its complications (4-6).

Cereals and their derivatives are the most important foods mainly because of the energy that they supply, due to their high carbohydrate contents. Barley (*Hordeum vulgare* L., *H. volgare*) is an important miscellaneous grain and a widely used cereal, because of its dietary

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health advantages, ready availability and low costs. Barley is mostly known for its high amount of dietary fiber such as β-glucan that may decrease the risk of coronary heart disease (7). Barley leaves have also a high antioxidant activity that might be useful in metabolic syndrome prevention or therapy, as well as diseases caused by oxidative stress damage. This property is mainly attributed to saponarin, a flavonoid with potent antioxidant activity found in young green barley leaves (8). Barley is a rich source of magnesium, a mineral that acts as a co-factor for more than 300 enzymes, including those involved in glucose metabolism and insulin secretion.

It is also a very good source of fibers and selenium and a good source of phosphorus and copper. It was found that constant consumption of whole grains decreased the risk of type II diabetes by 31%, pointing out that whole grains extend special benefits in motivating healthy blood sugar control (9). According to Nilsson and coworkers, eating whole grain barley by human can regulate blood sugar for up to 10 h after consumption (10). What seems to have been responsible for barley's effectiveness in regulating blood glucose is probably its soluble fiber content (11).

Nowadays, the use of plant protein as a part of treatment is increasing. For instance, the effect of soy protein on blood total and LDL cholesterol concentrations has been shown (12), and many angiotensin converting enzyme (ACE) inhibitory peptides have recently been discovered from enzymatic hydrolysates of different food proteins, like rice (13).

In this study, three increasing doses of barley hydroalcoholic extract (BHE) and protein enriched fraction (PEF) were examined in normal and streptozotocin (STZ)-induced diabetic rats in comparison to glibenclamide as reference drug to examine the antidiabetic potential of this raw material.

MATERIALS AND METHODS

Plant materials

Hordeum vulgare (Barley seeds, Nosrat cultivar) was prepared from Isfahan Center of Research in Agricultural Sciences and Natural Resources and confirmed by Mr. Mazroei, Botanist of Agricultural Sciences and Natural Resources Center, Isfahan, Iran. The seeds were powdered and extracted by ethanol/water (75/25) using percolation method. For preparing PEF with alkaline extraction, the powdered seeds were mixed with 0.2% NaOH solution (1:10, approximate pH 13) and stirred at room temperature for 1 h, and then left overnight (14). The mixture was then centrifuged at 3000 *g* for 15 min and the supernatant was collected. The pH of the extract was adjusted to 6 with 1 N HCl and the barley crude protein precipitated.

The precipitate was collected by centrifugation at 3000 *g* for 15 min and washed 3 times with distilled water. Finally both of the hydroalcoholic extract and protein fractions were concentrated and freeze-dried. The yields values of 5.5 and 1.4 were obtained for BHE and PEF, respectively.

Animals

Male Wistar rats, 4 months old (200-250 g) were obtained from the animal house of School of Pharmacy, Isfahan University of Medical Sciences and maintained under uniform and standard conditions of temperature and humidity and light/dark cycle (12 h/12 h) and fed with standard rat chow pellets and tap water *ad libitum*. All the experiments were performed in accordance with Ethics Committee guidelines for research on laboratory animals of Isfahan University of Medical Sciences, Isfahan, Iran.

Experimental design

The animals were randomly assigned into eight normal and eight diabetic groups, 6 rats in each. One ml of normal saline was administered orally to both normal and diabetic control groups. Glibenclamide (Tehran Chemi Co., Tehran, Iran), was administered at two doses of 1 and 3 mg/kg orally in normal and diabetic reference groups, respectively. Other groups in each normal and diabetic set were treated with three increasing doses of BHE (0.1, 0.25, 0.5 g/kg) and three increasing doses of PEF (0.1, 0.2, 0.4 g/kg). All the treatments with freshly prepared fractions as suspensions in carboxymethyl

cellulose (CMC) 1% were made orally (p.o.) by gavage and started 72 h after diabetes induction.

Diabetes induction and blood sampling

Diabetes was induced in rats by a single i.p. injection of buffered STZ (55 mg/kg) solution (0.1 M citrate, pH 4.5), after a period of overnight fasting. Diabetes was confirmed by measuring the fasting blood glucose level (BGL). Rats with BGL between 200-480 mg/dl were considered diabetic (15). Blood samples were taken at 0, 1, 2, 3, 9 h in the first day, the days 5, and 11 after treatments using micro-hematocrit capillary tubes (Hirschmann, Germany) and through choroid plexus puncture near the eyes under light ether anesthesia (16).

Body weight was also measured at the beginning and end (11th day) of the treatments. Twenty four h after the last treatment, a blood sample was taken and all the animals were then euthanized by overdose ether inhalation. BGLs (mg/dl) were measured by commercially available glucometer (Bionime®, Switzerland) (17).

Statistical analysis

The measured values represent mean ± SD. For assessment of differences between groups one-way analysis of variance (ANOVA) using SPSS 10 software followed by Tukey post hoc test was used. Body weight changes were analyzed by paired sample t-test. The results were considered significant when *P*-values were <0.05.

RESULTS

As it is shown in Table 1, weight reduction was significant in diabetic animals. Treatment of diabetic rats with BHE (0.25, 0.5 g/kg/d) restored animals' weight while other treatments were ineffective in this regard. Glibenclamide was also effective to oppose with weight reduction in diabetic rats. Table 2 represent that all normal groups (except glibenclamide), treated by barley extracts did not show hypoglycemia even after extended period of treatment.

Glibenclamide as expected was effective to diminish BGL at the first hour of the treatment and most of other sampling times. Our findings (Table 3) also indicated that BHE at doses of 0.25 and 0.5 g/kg/d were effective in reducing BGL in diabetic rats in comparison to controls after subacute (11 days) phase of the study, whereas there was no remarkable effect during the acute phase (1st day) of the treatment.

Glibenclamide as the reference drug was also effective and reduced BGL during both periods of the treatments. Results with protein fraction (PEF) showed no significant effect both after subacute and acute phases of the treatments.

Table 1. Changes in body weight of normal and STZ-induced diabetic rats treated orally with BHE and PEF.

Groups	Initial body weight (g) at day one	Final body weight (g) at day 11
Control	231 ± 20	240 ± 27
STZ + Normal Saline	217 ± 16	190 ± 18*
STZ + BHE 0.1	212 ± 12	191 ± 16*
STZ + BHE 0.25	223 ± 17	205 ± 17
STZ + BHE 0.5	218 ± 19	207 ± 23
STZ + PEF 0.1	211 ± 12	185 ± 10*
STZ + PEF 0.2	231 ± 22	192 ± 13*
STZ + PEF 0.4	231 ± 21	201 ± 10
STZ + Gliben 3	241 ± 11	248 ± 13

Data represent mean ± SD, STZ: streptozotocin (55 mg/kg), Control: normal saline (1 ml/rat), BHE: barley hydroalcoholic extract (0.1, 0.25, 0.5 g/kg/d), PEF: protein enriched fraction (0.1, 0.2, 0.4 g/kg/d), Gliben: glibenclamide (3 mg/kg/d), *P<*0.05* versus respected initial body weight (student's T paired test).

Table 2. Effect of orally administered BHE and PEF on blood glucose levels (mg/dl) of normal rats.

	Time (h)						
Groups	**0**	**1**	**2**	**3**	**9**	**120**	**264**
Control	119 ± 8	124 ± 6	118 ± 6	109 ± 6	145 ± 15	113 ± 8	125 ± 9
BHE 0.1	116 ± 10	113 ± 9	112 ± 11	120 ± 9	136 ± 13	108 ± 14	118 ± 15
BHE 0.25	111 ± 11	119 ± 8	115 ± 15	118 ± 12	133 ± 12	120 ± 9	120 ± 13
BHE 0.5	111 ± 5	114 ± 13	114 ± 15	114 ± 6	146 ± 7	117 ± 11	122 ± 12
PEF 0.1	110 ± 9	111 ± 14	107 ± 18	111 ± 9	123 ± 13	115 ± 11	107 ± 4
PEF 0.2	106 ± 8	109 ± 12	107 ± 11	113 ± 13	136 ± 7	116 ± 10	112 ± 12
PEF 0.4	119 ± 13	120 ± 8	126 ± 6	124 ± 4	130 ± 6	130 ± 17	118 ± 5
Gliben 1	118 ± 9	95 ± 8***	82 ± 7***	91 ± 6***	122 ± 8*	99 ± 5	100 ± 12**

Data represent mean ± SD, Control: normal saline (1 ml/rat), BHE: barley hydroalcoholic extract (0.1, 0.25, 0.5 g/kg/d), PEF: protein enriched fraction (0.1, 0.2, 0.4 g/kg/d), Gliben: glibenclamide (1 mg/kg/d), *P<*0.05*, **P<*0.01*, ***P<*0.001* versus control group (ANOVA with Tukey multiple comparison test).

Table 3. Effect of orally administered BHE and PEF on blood glucose levels (mg/dl) of STZ-induced diabetic rats.

	Time (h)						
Groups	**0**	**1**	**2**	**3**	**9**	**120**	**264**
Control	377 ± 31	384 ± 42	380 ± 32	354 ± 24	388 ± 28	354 ± 44	371 ± 21
BHE 0.1	396 ± 33	402 ± 45	395 ± 52	389 ± 53	392 ± 44	401 ± 54	386 ± 49
BHE 0.25	330 ± 48	441 ± 40	423 ± 27	404 ± 31	370 ± 30	318 ± 67	192 ± 50**
BHE 0.5	374 ± 32	431 ± 55	393 ± 40	370 ± 45	459 ± 74	325 ± 55	218 ± 42**
PEF 0.1	351 ± 41	414 ± 32	383 ± 33	366 ± 32	404 ± 36	348 ± 45	318 ± 44
PEF 0.2	370 ± 49	413 ± 50	368 ± 31	404 ± 40	456 ± 40	327 ± 115	382 ± 118
PEF 0.4	362 ± 65	373 ± 73	345 ± 60	319 ± 43	458 ± 29	339 ± 150	239 ± 123
Gliben 3	306 ± 29	237 ± 29**	186 ± 17***	167 ± 25***	176 ± 37***	185 ± 20**	180 ± 22**

Data represent mean ± SD, STZ: streptozotocin (55 mg/kg), Control: normal saline (1 ml/rat), BHE: barley hydroalcoholic extract (0.1, 0.25, 0.5 g/kg/d), PEF: protein enriched fraction (0.1, 0.2, 0.4 g/kg/d), Gliben: glibenclamide (3 mg/kg/d), **P<*0.01*, ***P<*0.001* versus control group (ANOVA with Tukey multiple comparison test).

DISCUSSION

The lower consumption of grains that are rich sources of dietary fiber may be associated with the increasing prevalence of chronic diseases. *H. vulgare* seeds (barley) are great sources of soluble fibers, which its effects on metabolic syndrome, lipid metabolism and bowel function has been demonstrated (18-20). In this study, we investigated the effect of two fractions of barley including total SHE and PEF on experimental diabetes. This is because of growing research on the role of some edible plants protein in improvement of metabolic syndrome (13,21).

According to the results, none of the Barley extracts (BHE and PEF) were effective to cause hypoglycemia in normal rats even after extended period of the treatment. Our outcomes are similar to those obtained by Sireesha and coworkers who investigated the hypoglycemic activity of aqueous extract of *Setaria italica* (22). They disclosed that *S. italica* seeds aqueous extract with different test doses could not induce hypoglycemia in normal rats. Ineffectiveness of BHE and PEF in normal rats suggests that tested fractions were not able to induce insulin secretion or lower BGL.

On the other hand, our findings indicated that BHE at doses of 0.25 and 0.5 g were effective to reduce BGL in diabetic rats after subacute (11 days) phase of the study, whereas there was no remarkable effect during the acute phase (1st day) of the treatment. The mechanism of insulin secretagogue like sulfonylureas and meglitinides is ruled out because there is no prompt and acute response by examined fractions. Moreover, these results propose that the probable mechanisms of used fractions could be similar to biguanids or α-glucosidase inhibitors, which result in

decreased insulin resistance and interference with carbohydrates absorption and/or metabolism. A possible mechanism for anti-hyperglycemic effect of BHE, after subacute phase of the treatment, might be due to its antioxidant capacity of minerals *e.g.* magnesium, selenium, copper and chromium which are abundant in barley seeds and act as cofactors for many enzymes including those with antioxidant activity (23).

Our results in this part of the study are also in accordance with Li and coworkers findings. They investigated long term effects of barley, rice and corn starch as high fiber diet on blood glucose tolerance and lipid metabolism in type II diabetic model GK rats. The investigators found that treatment of GK rats with barley, corn starch and rice diets for first month had no significant effect on fasting BGL. However, fasting BGL in the barley group was lower compared with that of the corn starch diet group at the second month and was lower than that of both rice and corn starch diet groups at the third month (24).

This suggests that carbohydrates rich in fiber may delay absorption of glucose resulting in a better coincidence between the timing of insulin release and peak blood glucose concentration (25).

Contrary to the Lee study which indicated antidiabetic efficacy of oral soy protein fraction in diabetic rats, our results showed no effect with protein fraction on normal and elevated BGL both after subacute and acute phase periods of the treatments (26). As it is reported previously, small proteins and essential amino acids like; L-leucine, L-arginin, and L-gluthamine are effective to induce insulin release or amplify its secretion induced by glucose (27,28). Therefore antidiabetic activity of some cereals like soy bean could be attributed to the above mentioned properties. Differences in content quality and amount of active ingredients exist in barley fractions may be responsible for these discrepancies.

Measuring the metabolic enzymes involved in glucose metabolism of liver, HbA1c, and other biochemical markers in treated rats help to figure out the accurate underlying mechanisms. Parentreal administration of PEF is also recommended for elucidation of its potential antihyperglycemic activity.

CONCLUSION

These findings demonstrate that *H. vulgare* seeds hydroalcoholic extract, in long term consumption, might have some benefits in diabetes mellitus control and management, probably through a mechanism similar to euglycemic agents like biguanids. Further investigation is needed to detect the principle active components and to elucidate the involved mechanisms.

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