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# Hypoglycemic Effect of Hydroalcoholic Extract and Hexane Fraction of Persian Shallot (*Allium Hirtifolium Boiss*) Extract in Streptozotocin-Induced Diabetic Rats

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**A B S T R A C T**

The present study was aimed to evaluate the hypoglycemic and potential insulinotropic effects of hydroalcoholic extract and hexane fraction of Persian shallot on streptozotocin (STZ) induced diabetic rats. Forty nine male Wistar rats were divided into seven groups. Diabetic control and normal control received normal saline; diabetic groups received hydroalcoholic extract (500 mg/kg), hexane fraction (7 mg/kg) of Persian shallot or glibenclamide (5 m/kg) for 14 days. To measure the levels of blood glucose and insulin, blood samples of animals were collected at first, 7th and 14th days of experiment. After treatment, decreases of blood glucose levels of two treated groups were 78.73 and 100.44 %, respectively compared to glibenclamide treated group. The most effective fraction to reduce blood glucose was hexane fraction. On the other hand, hydroalcoholic extract and hexane fraction did not induce any significant effects on serum insulin level. Based on the results, it is concluded that Persian shallot exhibit promising hypoglycaemic activity in streptozotocin induced diabetic rats. Hypoglycaemic effect of shallot could represent a protective mechanism against the development of hyperglycaemia characteristics of diabetes mellitus.

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## Introduction

In 2014 the global prevalence of diabetes was estimated to be 9% among adults aged 18+ years [1]. Diabetes mellitus is a metabolic disease that is characterized by a relative or absolute lack of insulin, resulting in hyperglycemia and can lead to a variety of complications including nephropathy, retinopathy and increased risk of cardiovascular diseases [2]. There are two main types of diabetes 1 and 2. Type 1 diabetes is an autoimmune disease leading to the destruction of the insulin-producing pancreatic beta cells [3, 4]. On the other hand type 2 diabetes is a metabolic disorder characterized by a defect in insulin secretion and/or resistance of tissues to insulin uptake [4].

Recently, many medicinal plants have been used to treat metabolic diseases like diabetes [5, 6]. Several bioactive components in plant-based have been shown significant anti-diabetic activity [7]. Allium species like Shallot (*A. hirtifolium*) has attracted specific consideration of modern medicine because of its widespread health use in the world [8]. Persian shallot (*Allium hirtifolium boiss*) is a nutritive plant belongs to liliacea family [8]. Shallot is rich in flavonoids such as quercetin, a potent antioxidant [8]. Furthermore, this compound was reported to be protected against oxidative stress [9] and also able to prevent nitric oxide increase in streptozotocin (STZ) induced diabetic rats [10]. Biochemical analysis of Persian shallot extract has approved its hypoglycemic and hepatoprotective effects [11, 12]. In addition, Persian shallot extract is a stronger hypoglycemic agent compared to garlic extract and this plant could be a useful supplemental remedy in diabetes [13]. Although the previous study was investigated hypoglycemic effect of hydroalcoholic extract of Persian shallot but there is no data concerning the hypoglycemic and insulinotropic effects of hexane fraction of Persian shallot in an experimentally induced type 1 diabetes model. Therefore, the purpose of this study was to experimentally assess the anti-diabetic effect of hydroalcoholic extract and hexane fraction of Persian Shallot (*A. hirtifolium*) in normal and STZ induced diabetic rats and to compare their effects with glibenclamide as a standard reference after two weeks of oral administration

## Materials and methods

### *Plant material*

Persian Shallot bulbs (*A. hirtifolium*) were purchased from the local vegetable market in Kermanshah, Iran. The plant material was identified in Department of Agriculture College of Razi University, Kermanshah, Iran. The bulbs were cleaned, shed dried at 25 °C, and the dried material was ground with a blender. The fine powder was kept in nylon bags in a freezer (-20 °C) until the time of experiments.

### *Preparation of hydroalcoholic extract and fractions*

Preparation of hydroalcoholic extract and fractions were performed using successive fractionation using different polar solvents [14, 15]. The hydroalcoholic extract was prepared by mixing one weight of shallot powder with 12 volumes (3×4 volumes) of 50% (v/v) ethanol by stirring for 48 h. The extract was filtered and centrifuged at 12000 g for 20 min at 4 °C and evaporated under reduced pressure to dryness. For solvent fractionation, the powder was resuspended in distilled water (50 mg $ml^{-1}$), and partitioned with n-hexane (Hex), ethyl acetate (EA) and n-butanol (BuOH), leaving a residual aqueous fraction (Aq). Each fraction was evaporated under reduced pressure to yield Hex, EA, BuOH and Aq fractions, respectively.

### *Animals*

Male Wistar rats weighing 200–250 g (Pasture Institute Tehran, Iran) were housed in clean cages with temperature (22–24 °C), 12-h light/12-h dark cycle and relative air humidity 40–60%. Rats had continuous access to food and to tap water. All experiments were done according to Animal Care and Use Protocol of Kermanshah University of Medical Sciences.

### *Induction of experimental diabetes*

Diabetes was induced in adult Wistar rats with a single intraperitoneal injection of streptozotocin (STZ) in 0.1M cold normal saline (pH 4.5) at a dose of 60 mg $kg^{-1}$ body weight [16]. Five days after injection, the rats with fasting blood glucose levels higher than 200 mg $dl^{-1}$ were considered diabetic.

### *Experimental design*

Animal were divided into seven groups (total No: 49), and treated as follows: Group 1: Normal control rats received daily 1ml of normal saline by gavage for 14 days. Groups 2–3: Normal rats received daily hydroalcoholic extract and hexane fraction of Persian shallot (500 and 7 mg $kg^{-1}$ body wt. respectively) by gavage for 14 days. Group 4: Diabetic control rats received daily 1ml of normal saline by gavage for 14 days. Groups 5–6: Diabetic rats received daily hydroalcoholic extract and hexane fraction of Persian shallot (500 and 7 mg $kg^{-1}$ body wt.) by gavage for 14 days. Group 7: Diabetic rats received daily glibenclamide (freshly prepared, 5 mg $kg^{-1}$ body wt.) in aqueous solution by gavage for 14 days. Blood samples were taken from retro-orbital plexus under light ether anesthesia using heparinized microhematocrit tubes immediately at the first day, 7 and 14 days after the oral administration of hydroalcoholic extract and hexane fraction of Persian shallot. Before each blood draw, the animals were fasted for at least 12 hours. It is needed to note that the optimum dose of hydroalcoholic extract and its fractions were obtained through separate experiments with different doses of each fraction.

### *Biochemical Analyses*

Plasma insulin concentrations were measured by rat insulin sensitive ELISA kit (Chrystal Chemical, USA rat 90060) and fasting blood glucose was assayed by glucose oxidase (GOD) kit (glucose Liquicolor kit, Germany).

### *Statistical analysis*

All the data were expressed as means ± standard error of means (SEM) and statistical difference between the means of the various groups were analyzed using one way analysis of variance (ANOVA) followed by Tukey'smultiple test. Differences were considered significant if $P < 0.05$.

## Results and discussion

### *Effect of shallot extract on blood glucose levels*

The effect of oral administration of hydroalcoholic extract and hexane fraction of Persian shallot extract on blood glucose levels are presented in Fig. 1A and 1B. The normal and control diabetic groups did not show significant changes in blood glucose levels on day 14 compared to the values on day 1 ($P < 0.05$). While, group treated with glibenclamide (5 mg $kg^{-1}$ BW) and two groups treated with hydroalcoholic extract and hexane fraction of Persian shallot (500 mg $kg^{-1}$, 7 mg $kg^{-1}$ BW, respectively) showed reduced blood glucose levels by 29.94, 19.50 and 30.16%, respectively. In the single dose study, the different fractions of shallot were compared. The results of glucose tolerance of glibenclamide treated group, hydroalcoholic extract and hexane fraction of Persian shallot treated groups were 49.09, 38.65 and 49.31%, respectively, when compared to the diabetic control. The hypoglycemic efficiency of hydroalcoholic extract and hexane fraction at doses of 500 mg $kg^{-1}$, 7 mg $kg^{-1}$ BW, respectively was 78.73 and 100.44% in glibenclamide treated group. Furthermore, the results showed that the hexane fraction (7 mg $kg^{-1}$ $day^{-1}$) of Persian shallot was more potent than the hydroalcoholic extract (500 mg $kg^{-1}$ $day^{-1}$) in reducing blood glucose levels (Fig. 1A and 1B).

 

### *Effect of shallot extract on insulin levels*

The effect of oral administration of hydroalcoholic extract and hexane fraction of Persian shallot extract on serum insulin in diabetic rats is shown in Fig. 2. Diabetic groups showed statistically lower insulin levels in comparison to normal control. The diabetic group treated with glibenclamide (5 mg kg-1 BW) significantly increased serum insulin by 37.5%. However, the two diabetic groups treated with hydroalcoholic extract and hexane fraction of Persian shallot (500 mg kg-1, 7 mg kg-1 BW, respectively) did not increase serum insulin levels significantly.

**Table 1.** Effect of oral administration of hydroalcoholic (500 mg $kg^{-1}$ body wt.) extract and hexane (7 mg $kg^{-1}$ body wt.) fraction of shallot on body weight in normal and diabetic rats. The values are mean ± SEM, for 7 rats in each group.*p < 0.05, **p < 0.01, ***p < 0.001, significantly different from control diabetic group.

| weight | 1 day | 7 day | 14 day |
|---|---|---|---|
| **Group1** (Control Normal receiving normal saline) | 237.71 ± 6.6 | 241.14 ± 6.3 | 249.42 ± 6.1 |
| **Group2** (Control Normal receiving hydroalcoholic extract ) | 241.57 ± 6.7 | 244.14 ± 6.8 | 253.57± 7.02 |
| **Group3** (Control Normal receiving hexane fraction ) | 241.83 ± 4.7 | 249.5 ± 4.4 | 257.83 ± 3.9 |
| **Group4** (Control Diabetic) | 233.5714 ± 7.20 | 212.2857 ± 5.21 | 164.1429 ± 9.34 |
| **Group5** (Diabetic+ Glybenclamid) | 231.4286 ± 5.80 | 220.71 ±7.23 | **200.2857 ± 5.768 |
| **Group6** (Diabetic + hydroalcoholic) | 243.1429 ± 5.152 | 225.2857 ± 6.41 | *196.8571 ± 3.35 |
| **Group7** (Diabetic + Hexane) | 205.5714 ± 6.64 | 225.5714±6.571 | ***237.7143 ± 6.55 |

 

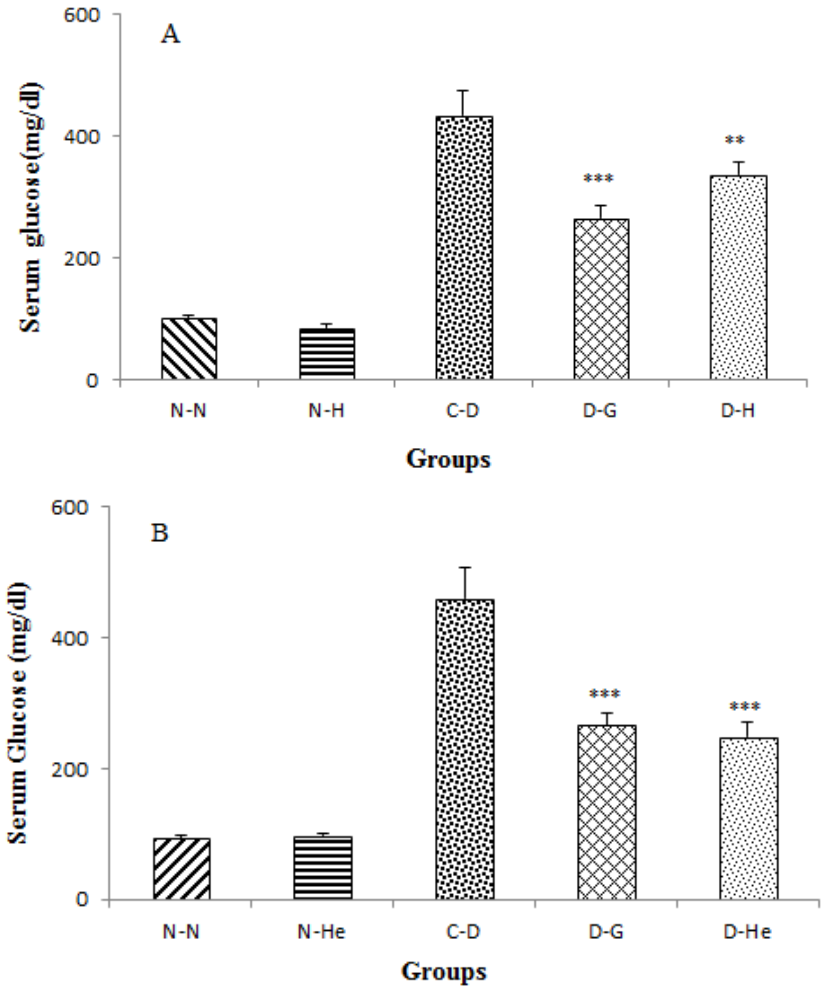


**Fig. 1.** Effect of oral administration of hydroalcoholic (500 mg $kg^{-1}$ body wt.) extract and hexane (7 mg $kg^{-1}$ body wt.) fraction of shallot on blood glucose levels (A, B) in normal and diabetic rats. Glibenclamide (5 mg $kg^{-1}$) was treated only on diabetic rats. Each column represents mean ± SEM for 7 rats .
Control group administrated with normal saline as a vehicle. *p < 0.05, **p < 0.001, ***p < 0.0001 different from control diabetic group.
N= normal, C= control, H= hydroalcoholic, He= hexane, G= glybenclamide

### *Effect of shallot extract on body weight*

The effect of oral administration of hydroalcoholic extract and hexane fraction of Persian shallot on body weight in diabetic rats are shown in Table 1. The body weight increased in the normal control rats (in 14 days period) compared to initial body weight, whereas in the diabetic control rats, there was a significant decrease ($p < 0.001$) in the body weight over the same period. Hydroalcoholic extract and hexane fraction of Persian shallot (500 mg $kg^{-1}$, 7 mg $kg^{-1}$ BW, respectively) treated diabetic rats gained significant weight ($p < 0.01$) but the increase was lower than the nondiabetic controls. Glibenclamide (5 mg $kg^{-1}$) treatment

 

significantly increased the body weight of diabetic rats ($p < 0.01$)

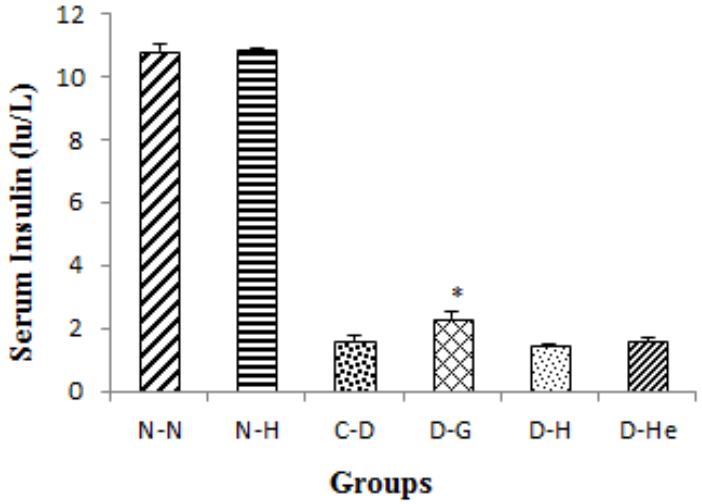


**Fig. 2.** Effect of oral administration of hydroalcoholic (500 mg $kg^{-1}$ body wt.) extract and hexane (7 mg $kg^{-1}$ body wt.) fraction of Persian shallot on serum insulin levels in normal and diabetic rats. Glibenclamide (5 mg $kg^{-1}$) was treated only on diabetic rats. Each column represents mean ± SEM for 7 rats .*$p < 0.05$, different from control diabetic group.

## Discussion

Our findings demonstrated that hydroalcoholic extract and hexane fraction of Persian shallot significantly decreased blood glucose levels, while they did not affect insulin levels significantly in treated diabetic rats compared with control diabetic group. Our findings are consistent with previous studies on the hypoglycemic effect of hydroalcoholic extract of *A. hirtifolium* [17, 18].

In spite of hypoglycemic effect of hydroalcoholic extract of Persian shallot addressed in the previous study, to our knowledge, the present study is the first one addresses the hexane fraction as responsible fraction for hypoglycemic effect of Persian shallot. Furthermore, our result showed that hexane fraction is more effective than hydroalcoholic extract of Persian shallot in reducing blood glucose levels in diabetic rats.

Several antioxidants and flavonoids present in Persian shallot (*A. hirtifolium*) extract have been reported to improve hyperglycemia in diabetes mellitus [13]. According to the report of Mahmoodi et al, hypoglycemic and beneficial effects of Persian shallot may be associated with a marked enhancement of the Glucokinase (GCK) mRNA expression in the liver [18]. On the other hand some flavonoids have insulin like properties [19, 20]. One of the richest sources of flavonoids in human diet is common shallot (A. hirtifolium) [21]. Leighton et al. reported that" shallot contains the highest level of total flavonols among the onion varieties". Furthermore, the findings of Fattorusso et al. showed that bulbs of shallot had high concentrations of quercetin, isorhamnetin, and their glycosides. Seify et al. reported that total flavonoid content in hydroalcoholic extract of Persian shallot is 41.5 ± 5.4 (mg of quercetin quiv/100 g of sample). Quercetin is an important constituent of the flavonoid family, find in high concentrations in shallot [25] and its hypoglicemic activity was also documented [26].

Moreover, Persian shallot is rich of organosulfur compounds especially Allicin (one of the important organosulfur compounds). These compounds may be responsible for some beneficial properties of this plant [24]. Several studies have shown the hypoglycaemic effect of

 

garlic, attributed mainly to allicin-type compounds [27, 28].

Finally, it is concluded that the mechanism of the hypoglycaemic effects of shallot extract remains speculative, therefore further studies are required to unravel the pathway of hypoglycaemic effect of Persian shallot and to shed more light on the hypoglycaemic constituents of this plant. It is however evident from this research that hydroalcoholic extracts and hexane fraction of Persian shallot contain hypoglycaemic agents capable of lowering blood glucose levels in stereptozotosin induced diabetic rats. Nevertheless, Persian shallot may be beneficial in management of diabetes.

## Conclusions

It is concluded that the level of total blood glucose levels which were actually increased in stereptozotosin induced diabetic rats, can be reduced by hydroalcoholic extract and hexane fraction of Persian shallot. The hypoglycaemic effect is thus protective mechanisms against the development of hyperglycaemia common in diabetes mellitus. This may provide a basis for dietary supplementation of shallot compounds in diabetics to reduce over dependence on drug.

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### Conflict of interest

Authors certify that there is no actual or potential conflict of interest in relation to this article.

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