

Antidiabetic Activity of Aqueous Seed Extract of *Securigera securidaca* in Streptozotocin Induced Diabetic Rats

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Article Info

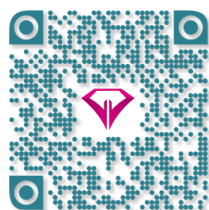
 10.30699/jambs.27.123.16

Received: 2019/01/22;

Accepted: 2019/02/29;

Published Online: 1 Jul 2019;

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ABSTRACT

Background & Objective: *Securigera securidaca* is a medicinal plant used in Persian folk medicine to decrease blood sugar. Nevertheless, there is some controversy about its effects. The current study investigated the hypoglycemic activity of the aqueous seed extract of *Securigera securidaca*.

Materials & Methods: Sixty mature male Charles-River rats were categorized and divided randomly into 10 groups with two timeframes (7 and 14 days). To induce diabetes, rats were injected intraperitoneally with 7 mg/0.5 ml streptozotocin in normal saline per 100 g body weight. The aqueous seed extract of *Securigera securidaca* was given orally to rats daily (80 mg/0.5 ml in distilled water per 100 g of rat body weight). The blood sugar of rats was measured on the 7th and 14th days. Blood lipid indices and ALT, AST, and ALP plasma levels were measured after 14 days of treatment.

Results: The findings showed that the *Securigera securidaca* aqueous seed extract had a significant effect on the blood sugar of rats after 7 and 14 days compared with the diabetic group. After 14 days, there was a significant difference in the weight of treated rats between the *Securigera securidaca* aqueous seed extract and diabetic groups. The *Securigera securidaca* aqueous seed extract had no significant effect on the blood lipid profiles of the treated rats. The AST enzyme levels were significantly higher in rats exposed to the seed extract than in the diabetic group. REM sleep significantly ($p < 0.05$) after the stress condition has been administered.

Conclusion: Based on the results, *Securigera securidaca* seed extract had a significant effect in reducing the blood sugar of diabetic rats with no significant changes in lipid profiles. More studies are needed to explore in more detail the mechanism of this hypoglycemic effect.

Keywords: Diabetes, Rat, *Securigera securidaca*, Streptozotocin



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Introduction

Diabetes mellitus (DM) is a metabolic and multifactorial disorder characterized by a chronic increase in blood sugar, or hyperglycemia, due to insulin insufficiency, its malfunction or both (1-3). About 8.3% of the world's population is affected by this disease (4, 5). The treatment of DM is based on insulin and oral anti-diabetic drugs. Even with the prolonged use of insulin, various problems arise, such as insulin resistance, anorexia, brain atrophy, and fatty liver (6). The long-term use of oral hypoglycemic agents is also associated with unwanted side effects, including hypoglycemia and weight gain (7). Therefore, research must be done on a newer anti-diabetic agent that has better therapeutic effects and fewer side effects.

Securigera securidaca (L.) Degen & Dörfel. from the *Fabaceae* family (subfamily: Papilionaceae), with the Persian names "Gandeh Talkheh" and "Adasolmolk", is a medicinal plant used in Persian and Egyptian folk

medicine to control diabetes (8, 9). *Securigera securidaca* (SS) is distributed in West Asia (especially Iran), Europe, and Africa. The plant is herbaceous, has no cork, and has small, needle-shaped, brown-colored fruits containing only a few seeds, which have flavonoids, steroids, saponins, and tannins (9-12). The antidiabetic activity of SS seeds has been the subject of several studies. The water extract of SS was used in folk medicine as an antidiabetic preparation, but to date, many experimental antidiabetic studies on SS seeds have been carried out with other extracts, such as hydro-alcoholic extract (9, 13, 14). Hosseinzadeh et al. showed that the seed extracts (both aqueous and ethanolic) were not effective in reducing blood glucose in alloxan-induced diabetic mice (10). Constituents in the aqueous extract differ from the compositions in the extracts provided with other solvents. Furthermore, there is some controversy among previous studies on the antidiabetic effects of this plant. Thus, the current

study investigated the antidiabetic activity of *SS* (water extract as used in folk medicine) in streptozotocin-induced diabetic rats to determine its usefulness as an antidiabetic preparation.

Materials and Methods

Plant material

Securigera securidaca seeds were collected in May 2016 from Abhar city (Zanjan province, Iran) and confirmed scientifically. The voucher specimen of the plant with the number E1-275-116 is deposited in the Faculty of Pharmacy of the Department of Pharmacognosy, Mazandaran University of Medical Sciences, Sari, Iran. The seeds were air dried and then milled using mechanical grinders.

Preparation of extract

Seeds of *Securigera securidaca* were dried in an incubator for two days at 40 °C. Seeds were powdered (mesh 200) by using electrical grinder. Extraction was performed with 50 g of the powder in 500 ml of distilled water for 24 hours by using a soxhlet extractor. The solvent was evaporated by rotary evaporator and then the extract was dried by freeze dryer.

Animals

Sixty normoglycemic (with fasting blood glucose levels of 85 ± 5 mg/dl) mature male Charles-River rats (150-160 g) were acquired from the animal house of Mazandaran University of Medical Science and housed in separate plexiglass cages (six rats per cage). The rats were kept in cages under standard laboratory conditions (temperature, 22 ± 2 °C; relative humidity, 45-55%; 12/12 h light – dark cycle) and were allowed *ad libitum* access to normal laboratory diet and tap water. All experimental protocols of the study conformed with international guidelines for the care and use of laboratory animals. All procedures were approved by the Ethics Committee of Mazandaran University of Medical Sciences prior to the study Ir.mazums.rec. 1395.17 00.

Induction of diabetes mellitus

Diabetes mellitus was induced by a single intraperitoneal (IP) injection of 7 mg/0.5 ml/100 g of streptozotocin (Upjohn, USA) (STZ) dissolved in 0.9% fresh cold normal saline in 12 h-fasted rats. After the injection, they had free access to food and water. Blood sampling for glucose level determination was taken from the tail veins of the rats. The STZ-injected animals exhibited hyperglycemia within 3 days. Rats with hyperglycemia (blood glucose level higher than 250 mg/dl) after 2 weeks were collected for experiments (1, 2, 15).

Experimental design

Sixty diabetic rats were divided equally into 10 groups as follows:

I. Control group: 6 normal rats received IP injections of 0.5 ml normal saline per 100 g body weight for 7 days.

II. Control diabetic group (STZ): 6 normal rats received a single IP injection of streptozotocin at a dose of 7 mg/0.5 ml/100 g body weight.

III. Diabetic + insulin group (STZ+Ins): 6 diabetic rats received one intramuscular injection of insulin at a dose of 5 unit/kg body weight per day for 7 days.

IV. Diabetic + Glyburide group (STZ+Gly): 6 diabetic rats were force-fed by gastric tube with Glyburide (Profarmaco, Italy) at a dose of 10 mg/kg body weight per day for 7 days.

V. Diabetic + *Securigera securidaca* extract (STZ+EXT) group: 6 diabetic rats were force-fed by gastric tube with *Securigera securidaca* aqueous extract at a dose of 80 mg/0.5ml/100g body weight per day for 7 days.

VI. Control group: 6 normal rats received intramuscular injections of normal saline at a dose of 0.5 ml/100g body weight for 14 days.

VII. Control diabetic group (STZ, this group was different from group II): 6 normal rats received a single IP injection of streptozotocin at a dose of 7 mg/0.5ml/100g body weight.

VIII. Diabetic + insulin group (STZ+Ins): 6 diabetic rats received one intramuscular injection of insulin at a dose of 5 unit/kg body weight per day for 14 days.

IX. Diabetic + Glyburide group (STZ+Gly): 6 diabetic rats were force-fed by gastric tube with Glyburide (Profarmaco, Italy) at a dose of 10 mg/kg body weight per day for 14 days.

X. Diabetic + *Securigera securidaca* extract (STZ+EXT) group: 6 diabetic rats were force-fed by gastric tube with *Securigera securidaca* aqueous extract at a dose of 80 mg/0.5ml/100g body weight per day for 14 days.

The choice of the 80 mg/0.5ml/100g dose of *Securigera securidaca* extract was derived from some dose response studies. Before giving the extract, primary blood glucose levels were measured in all groups. The fasting blood glucose levels of all groups were measured after 7 and 14 days. Animals were anesthetized with ether; then blood samples were obtained from the tail vein, and the results were expressed in mg/dl. To measure the serum lipid profiles and major liver enzymes, blood samples were taken from all groups after 14 days of treatment and sent to the laboratory for evaluation using the photometric method. Quantitative analyses were done on total cholesterol, triglycerides, HDL, and LDL levels; AST (aspartate aminotransferase), ALT (alanine transaminase), and ALP (alkaline phosphatase) enzyme levels were also determined.

Statistical analysis

Data is shown as mean \pm SEM and was analyzed in GraphPad Prism 6 software. Statistical analyses of data were performed using ANOVA followed by Dunnett's multiple comparison test. The difference between the means was considered significant at a probability level of $p < 0.05$.

Results

The effect of *Securigera securidaca* extract on the weight of rats

The results revealed a significant difference in weight among the different groups and the control group after 7 days ($p \leq 0.0001$); the weight of the rats in the different groups was lower than that of rats in the control group (Fig. 1). The results also showed a significant difference in weight among different groups and the diabetic group; after 14 days, the weight of the rats in all groups except the diabetic group was significantly increased. The mean weight of the rats in the extract group was not significantly increased after 7 days (157.7 ± 1.65) compared with the mean weight of the diabetic rats (155.5 ± 2.22), but after 14 days, the mean weight of the extract group (162.21 ± 1.70) was significantly higher than that of the diabetic group (149.7 ± 2.16) ($p \leq 0.001$).

Effect of *Securigera securidaca* extract on blood glucose level in rats

The results further indicated that there is a significant difference between the blood glucose levels of the studied groups and that of the diabetic group after 7 and 14 days ($p \leq 0.0001$). As shown in Figure 2, the STZ+EXT group with a mean blood glucose of 96.17 ± 3.26 compared with the diabetic group with a mean of 351.8 ± 10.69 showed that the extract had a significant effect on blood glucose levels in rats after 7 days ($p \leq 0.0001$). After 14 days, there was a significant difference between the mean blood glucose level in diabetic rats treated with the extract (90.67 ± 2.28) and the diabetic group (344.7 ± 11.3) ($p \leq 0.0001$).

Effects of *Securigera securidaca* extract on blood lipid profiles of rats

The results showed that the control group (127.3 ± 10.75) and the diabetic + insulin group (103.2 ± 7.99) had the highest and lowest cholesterol levels, respectively. The results of one-way ANOVA showed that there was no significant difference in blood cholesterol levels among the different groups. Therefore, *Securigera securidaca* extract had no significant effect on blood cholesterol levels in diabetic rats (Fig. 3).

The results indicated that there is a significant difference in the amount of triglycerides between the control group and the other groups ($p \leq 0.0001$). The highest triglyceride level (112.0 ± 7.22) was allocated to the diabetic group (Fig. 4). The amount of triglycerides in rats treated with *Securigera securidaca* extract (88.17 ± 10.14) was decreased in comparison with the diabetic group, but the difference between these two groups was not statistically significant (Fig. 4).

The results of one-way ANOVA showed that there is no significant difference between the levels of HDL and LDL in the different groups. Therefore, *Securigera securidaca* extract had no significant effect on HDL and LDL in rats (Fig. 5).

Effect of *Securigera securidaca* extract on the liver enzymes of the studied animals

The results showed that the diabetic + glyburide groups (291.20 ± 13.63) and the diabetic group (186.3 ± 19.43) had the highest and lowest AST levels, respectively. The results of one-way ANOVA showed that the AST levels of the control, diabetic + glyburide, and diabetic + *Securigera securidaca* extract groups were significantly different from those of the diabetic and diabetic + insulin groups. The mean AST enzyme level in rats treated with *Securigera securidaca* extract (274.85 ± 14.24) was significantly higher than that of the diabetic group (186.3 ± 19.43) ($p \leq 0.01$) (Fig. 6).

The results showed that the control group (72.17 ± 2.15) and the diabetic group (124.7 ± 3.44) had the lowest and highest ALT levels, respectively. The results of one-way ANOVA showed a significant difference in ALT levels between the control group and the other groups ($p \leq 0.0001$). In other words, the ALT level was lower in the control group than in the other groups. *Securigera securidaca* extract (121.7 ± 6.3) had no significant effect on ALT in rats compared with the diabetic group (124.7 ± 3.44) (Fig. 6).

The results showed that the lowest and highest levels of ALP were obtained in the control (874.11 ± 111.4) and diabetic groups (3501.0 ± 109.5), respectively. The ALP levels of the other groups were relatively similar. The results of one-way ANOVA showed a significant difference in the amount of ALP between the studied groups and the diabetic group. The amount of ALP obtained from the extract-treated diabetic group (2893 ± 77.21) was decreased significantly compared with that of the diabetic group (3501.0 ± 109.5) ($p \leq 0.001$). In other words, *Securigera securidaca* extract reduced the amount of ALP in the studied animals (Fig. 6).

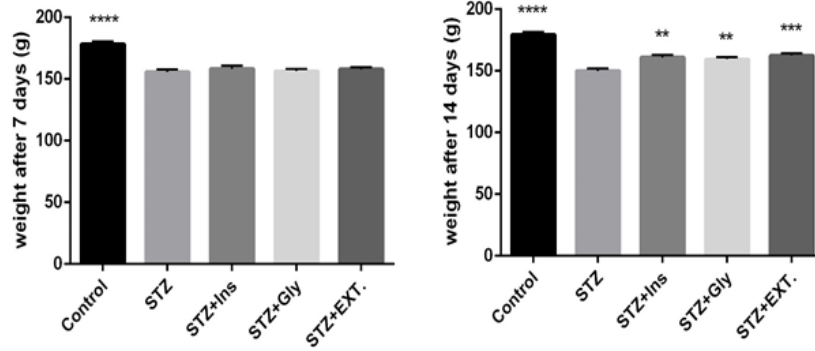


Figure 1. Comparison of rat weights in the studied groups after 7 and 14 days. Data is expressed as mean±SEM. (n=6)

**** $p \leq 0.0001$ *** $p \leq 0.001$ ** $p \leq 0.01$ * $p \leq 0.05$ compared to the STZ group

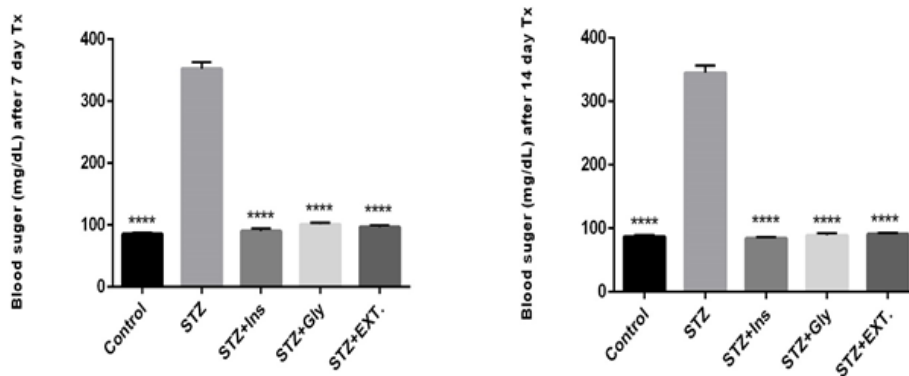


Figure 2. Comparison of blood glucose levels in the studied groups after 7 and 14 days. Data is expressed as mean±SEM. (n=6)

**** $p \leq 0.0001$ compared to the STZ group

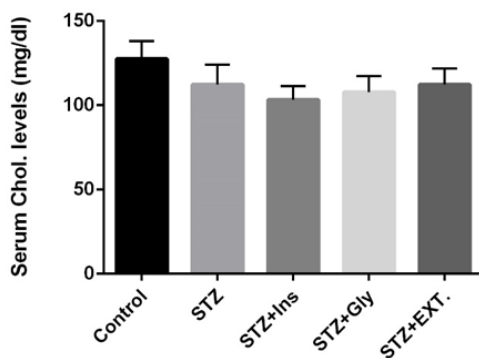


Figure 3. Comparison of serum total cholesterol levels in the studied groups after 14 days of treatment. Data is expressed as mean±SEM.

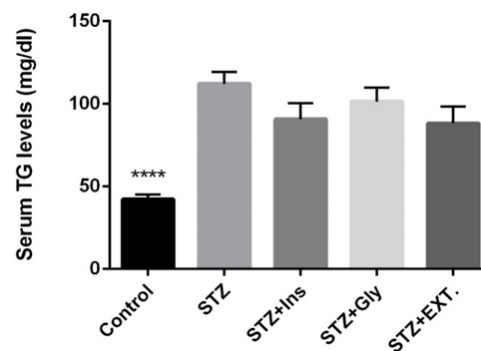


Figure 4. Comparison of serum triglyceride levels in the studied groups after 14 days of treatment. Data is expressed as mean±SEM. (n=6).

**** $p \leq 0.0001$ compared to the STZ group

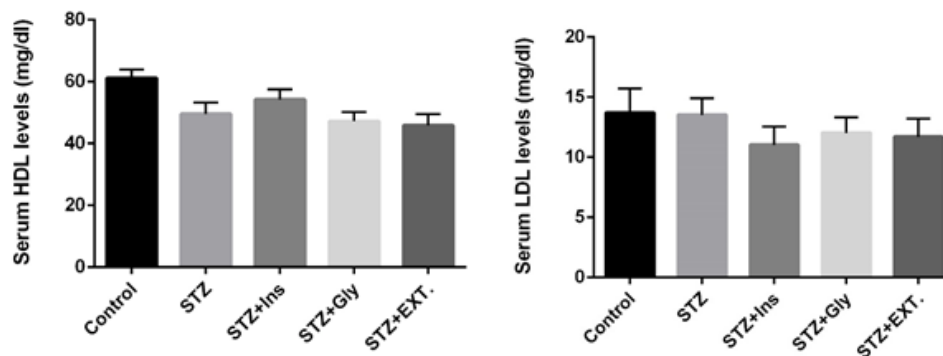


Figure 5. Comparison of HDL and LDL serum levels in the different studied groups after 14 days of treatment. Data is expressed as mean \pm SEM. (n=6)

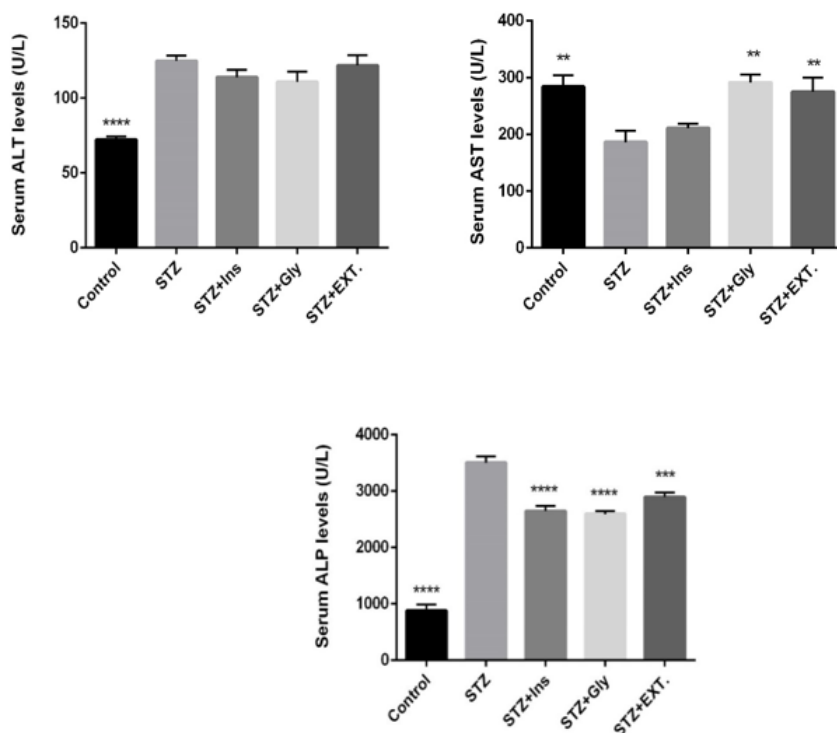


Figure 6. Comparison of serum levels of AST, ALT, and ALP enzymes in the studied groups after 14 days of treatment. Data is expressed as mean \pm SEM. (n=6)

** $p \leq 0.01$, *** $p \leq 0.001$ and **** $p \leq 0.0001$ compared to the STZ group.

Discussion

In this study, the hypoglycemic activity of *Securigera securidaca* aqueous seed extract was evaluated in streptozotocin-induced diabetic rats. Additional biochemical items, including serum lipid profiles and AST, ALT, and ALP enzyme levels, were also measured.

The results of the blood glucose analysis showed a significant difference in blood glucose levels in the extract-treated diabetic group compared with the diabetic group. Thus, it can be concluded that the aqueous extract can significantly reduce blood glucose

after 7 days. It should be noted that after 14 days, blood glucose levels in the aqueous extract group were not significantly different compared to the seven days group. This result was also found in another study (14).

Zahedi et al. showed that the chloroform and hydroalcoholic extracts of *Securigera securidaca* reduced fasting blood glucose (7). Hosseinzadeh et al. attributed the observed hypoglycemic effect of the *Securigera securidaca* hydroalcoholic extract to its flavonoid content (10). According to Moitra et al., the reduction in blood glucose levels caused by the

Securigera securidaca extract is due to the presence of several compounds, including five dihydrobenzene derivatives (15). The findings of this study are consistent with the results of a study by another researcher (16-18).

A comparison of the different groups showed no significant difference in weight of rats among different groups except the control group after 7 days. However, after 14 days, the *Securigera securidaca* aqueous extract increased the weight of rats relative to the diabetic group. In other words, the extract had an effect on the weight gain of the rats. These results and previous findings show that *Securigera securidaca* extract can increase the weight of rats by reducing blood glucose levels in diabetics (7).

The results of the blood cholesterol analysis showed that there was no significant difference in blood cholesterol levels among the studied groups. Therefore, the extract had no significant effect on cholesterol levels in the studied rats. The values obtained for triglycerides indicated that the amount of this factor decreased in the extract group compared with the diabetic group, but the difference was not significant. Triglyceride values obtained from other groups were also within normal range. The HDL level was normal in all studied groups, though it was higher in the extract-treated group than in the diabetics, but there was no significant difference between them. The values obtained from the LDL assay in the studied groups indicated that the level of this factor is within normal range in all groups. The LDL values were found to be lower in the extract-treated group, which showed no significant reduction compared to the diabetic group.

The investigation of AST values in the studied groups showed a significant difference between the control, diabetic + glyburide, and the extract groups and the diabetic and diabetic + insulin groups. The extract significantly increased AST levels compared with diabetics after 14 days. It is suggested that the AST values should be considered more in future studies. There was no significant difference in ALT values except for the control group in which it was lower than in the others. The extract was not able to significantly reduce the ALT level in comparison with diabetics. Diabetes induction led to an increase in serum ALP values, and the extract was able to significantly reduce them.

Several studies have examined the effects of *Securigera securidaca* extract on blood lipids and liver enzymes. Azarmi et al. concluded that *Securigera securidaca* seed extract significantly reduces serum LDL and triglyceride levels in rats on a high-fat diet

(19). Plant-based flavonoids have been claimed to increase the removal of LDL from blood and to decrease plasma lipids by increasing the level of LDL receptors on the surface of the liver cells and binding them to beta-apolipoproteins (20). In a study by Fathi Azad et al., the extract of the plant also resulted in a significant reduction in serum triglycerides (21). Fallah Hosseini et al., however, found that *Securigera securidaca* seed extract did not affect the levels of cholesterol, LDL, triglycerides and AST, ALT, or ALP liver enzymes (22).

The discrepancies seen among different studies evaluating the effects of *Securigera securidaca* seed extract on blood glucose, lipid profile, and some important enzymes have different explanations, from different solvents used for extraction, to the agent used for diabetes induction, to the animal species used in the experiments for the duration of treatments. Each of these reasons could contribute to different and sometimes controversial findings.

Conclusion

Data from the present study reconfirmed that *Securigera securidaca* aqueous seed extract can significantly decrease serum glucose in diabetic rats compared with control diabetics, but there was no significant difference in blood cholesterol, triglyceride, LDL and HDL levels in the studied groups. Moreover, the hypoglycemic effects of this plant could have been more definitely expressed if more fractions had been investigated and if the experiments had been performed in at least two different animal genera. *Securigera securidaca* aqueous seed extract could increase the weight of the rat relative to the diabetic group. The extract after 14 days increased AST levels, but the ALT and ALP levels were affected by the extract. Thus, it is suggested that AST be evaluated more precisely in future studies.

Considering the results obtained in this study and the similarities and inconsistencies with other research, it is suggested that further studies embracing different plant fractions, the detection and isolation of its main constituents, the exploration of the exact mechanisms of its hypoglycemic effect, and an evaluation of its safety profile should be carried out.

Acknowledgements

The authors thank all those who helped them writing this paper.

Conflict of Interest

Authors declared no conflict of interests.

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Azadbakht M, Ahmadi S A, Ahangar N. Antidiabetic Activity of Aqueous Seed Extract of *Securigera securidaca* in Streptozotocin Induced Diabetic Rats. *J Adv Med Biomed Res.* 2019; 27 (123) :16-22

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