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ORIGINAL ARTICLE

Antidiabetic Effect of *Punica granatum* L. Hydro-Ethanolic Extract in Streptozotocin-Induced Diabetic Rats

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ABSTRACT

The antidiabetic effect of the hydro-ethanolic extract of the Punica granatum L. flower was investigated in streptozotocin-induced diabetic rats. The animals were made diabetic using by streptozotocin (70 mg/kg, i.p.). The hydro-ethanolic extract at doses 100, 150, 200 and 300 mg/kg, i.p. were administered for 18 days, intraperitoneally. The control group was administered saline. After 18 days, the animals were anaesthetized by diethyl ether. Blood samples were obtained from heart after 18 days. Serum glucose, cholesterol, triglycerides, LDL, HDL, urea, uric acid, creatinine, alanine amino transferase (ALT) and aspartat amino transferase (AST) enzyme levels were determined by kit. The results showed that the hydro-ethanolic extract of Punica granatum L. flowers significantly reduced the serum glucose, cholesterol, triglycerides, LDL, urea, uric acid, creatinine, alanine amino transferase and aspartat amino transferase enzymes levels, while increase serum HDL level in streptozotocin-induced diabetic rats in comparison to control diabetic rats. The present data indicates that the extract of Punica granatum flowers has antidiabetic effect. So, this plant should be considered as an appropriate candidate in future therapeutic research.

Keywords: *Punica granatum, Flower, Diabetes, Rat*

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INTRODUCTION

Diabetes is a chronic metabolic disorder that continues to present a major worldwide health problem. Despite the efficiency of insulin treatment and other chemical therapies to control many features of diabetes, there are common incidents of diabetic complications such as vascular dysfunctions, nephropathy, neuropathy and retinopathy [1]. Present number of diabetics worldwide is 150 million and this is likely to increase to 300 million or more by the year 2025 [2]. Reasons for this rise include increase in sedentary lifestyle, consumption of energy rich diet, obesity, higher life span, etc [3]. Regions with greatest potential are Asia and Africa, where diabetes mellitus rates could rise to 2–3-folds than the present rates [4]. Many herbal medicines have been recommended for the treatment of diabetes [5]. Plant drugs are frequently considered to be less toxic and more free from side effects than synthetic ones [6]. *Punica granatum* L. (Punicaceae) is a shrub or small tree and considered to be a native of Iran and Afghanistan. It is also found growing wild in the warm valleys and outer hills of the Himalayas, and is cultivated throughout India [7]. 'Gulnar' (flower of *P. granatum* L.) has been known for a long time in Unani literature as an astringent, haemostatic, and as a remedy for diabetes [8]. The root bark as well as the stem bark of the plant is astringent and also used as anthelmintic specifically against tapeworms. The rind is valued as an astringent in diarrhoea and dysentery. The juice of the leaves and young fruits and the decoction of the bark are used in dysentery. The powdered flower buds are useful in bronchitis. The seeds are considered to be stomachic and the pulp cardiac and stomachic. The green leaves are made into a paste and applied in conjunctivitis [7,9]. The biological activities, viz. antibacterial [10], antifungal [11], anthelmintic [12] and antifertility [13], of the various extracts of different parts of this plant have also been reported. The extracts of root of *P. granatum* and rind of this plant [14] have been reported to exert some sugar lowering action in animals. Since only the flowering part of the plant has been recommended

in Unani literature as a remedy for the treatment of diabetes, it was, therefore, considered worthwhile to investigate the antidiabetic effect of the flowers of *P. granatum* in streptozotocin-induced diabetic rats.

MATERIAL AND METHODS

Pomegranate flowers (*Punica granatum* L.) were purchased from a west of Tehran in June 2013, identified by department of botany of Science and Research Branch, Islamic Azad University. The plant was cleaned, shed dried at 25°C, and the dried leaves of the plant were ground with a blender, and the powder was kept in nylon bags in a deep freezer until the time of experiments. Dried and ground flowers (about 100 g) were submitted to extraction with 300 ml ethanol (80%) in a soxhlet apparatus for 48 h. After extraction, the solvent was filtered and then evaporated by rotavapor. The obtained hydro-alcoholic extract was stored at -20 °C until being used. In this study, male Wistar rats weighing 200–250 g were housed in clean cages with temperature (22–24 1C), 12-h light/12-h dark cycle and relative air humidity 40–60%. Rats had continuous access to food and to tap water. Permission for the study was obtained from the Pastour institute, Tehran, IRAN. The animals were made diabetic with streptozotocin injection (70 mg/kg, i.p.). Five days after injection, the rats with fasting blood glucose higher than 180 mg/dl were used for the experiments. Eight rats were used in each group. Each animal was used once only in all of experiments. The food and water were removed from cages 12 h before testing. Flower extract was suspended in distilled water and administered interperitoneally at doses of 100, 150, 200 and 300 mg/kg body wt. The volume of administrated extract was 0.5 ml for each animal. In the present experiment, 48 rats (40 diabetic, 8 normal rats) were used. The rats were divided into six groups. Group 1: Normal control rats were administrated 0.5 ml of saline, interperitoneally. Group 2: Diabetic control rats were administrated 0.5 ml of saline, interperitoneally. Groups 3–6: Diabetic rats were administrated garlic alcoholic extract (100, 150, 200 and 300 mg/kg body wt.) daily for 18 days, interperitoneally. After 18 days of treatment, blood samples were drawn from heart. Serum glucose, total cholesterol, triglycerides, urea, uric acid, creatinine, LDL, HDL, aspartate amino transferase (AST) and alanine amino transferase (ALT) levels were determined by kit (Parsazmoon, Iran). All the data were expressed as mean ± S.E.M. Statistical analysis was carried out using one-way ANOVA followed by Tukey post hoc test. The criterion for statistical significance was $p<0.05$.

RESULTS AND DISCUSION

There were significant elevations in serum glucose, triglycerides, cholesterol, LDL, Urea, uric acid, creatinine, ALT, AST levels, while significant attenuation in serum HDL level in the diabetic rats in comparison with control group. The present results showed that treatment of pomegranate flower extract decreased serum glucose (fig. 1), triglycerides, total cholesterol (fig. 2), LDL (fig. 3), urea, uric acid, creatinine (fig. 4), ALT and AST (fig. 5) levels, while increased serum HDL level (fig. 3) in treated diabetic rats in compared to control diabetic rats.

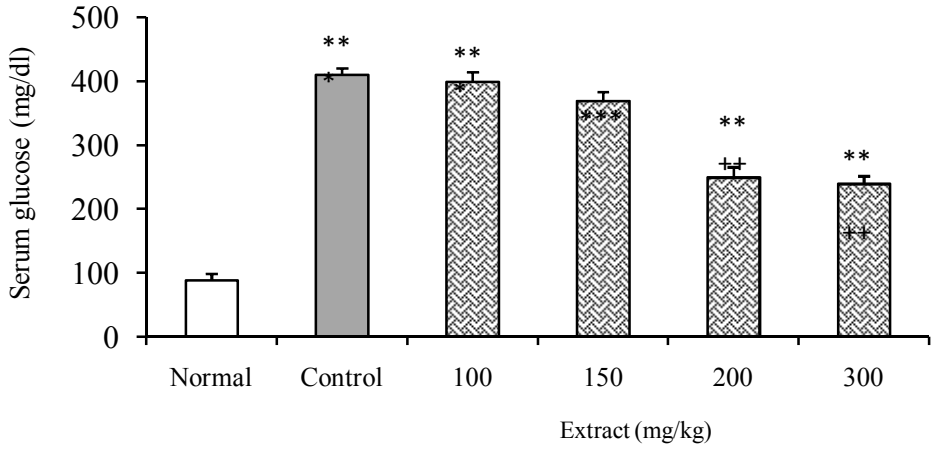


Fig. 1. Effect of i. p. administration of pomegranate hydro-ethanolic extract at doses of 100, 150, 200 and 300 mg/kg body wt. on serum glucose levels in diabetic rats. Each column represents mean±S.E.M. for 8 rats. Control group administrated with saline as vehicle. ** $p<0.01$, *** $p<0.001$ different from normal group. ++ $p<0.01$ different from control diabetic group.

The present data indicated that the pomegranate hydro-ethanolic flower extract significantly improved serum parameters in treated streptozotocin-induced diabetic rats. In agreement with the present results, it is reported the hypoglycaemic effect of pomegranate flowers extract [14,15,16], attributed mainly to its insulin releasing activity. Also, this effect could possibly be due to increased peripheral glucose utilization. Inhibition of the proximal tubular reabsorption mechanism for glucose in the kidney, if any, can also contribute towards blood lowering effect. It is reported that an infusion of the *P. granatum* inhibited the intestinal absorption of glucose in rats [17]. Thus a possibility exists that retardation of intestinal glucose absorption may also be partly responsible for inhibition of hyperglycaemia in glucose-fed rats [15].

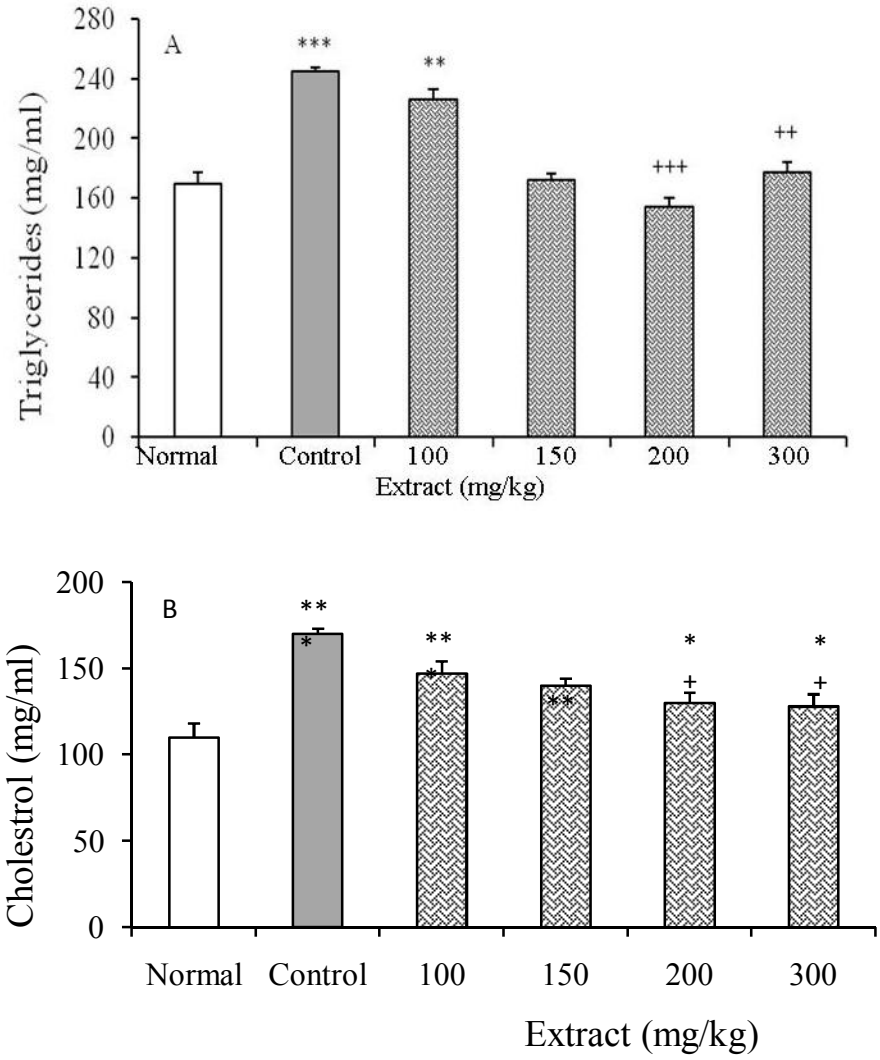


Fig. 2. Effect of i. p. administration of pomegranate hydro-ethanolic extract at doses of 100, 150, 200 and 300 mg/kg body wt. on serum triglycerides (A) and cholesterol (B) levels in diabetic rats. Each column represents mean±S.E.M. for 8 rats. Control group administrated with saline as vehicle. * p<0.05, ** p<0.01, *** p<0.001 different from normal group. + 0<0.05, ++ p<0.01, +++ p<0.001 different from control diabetic group.

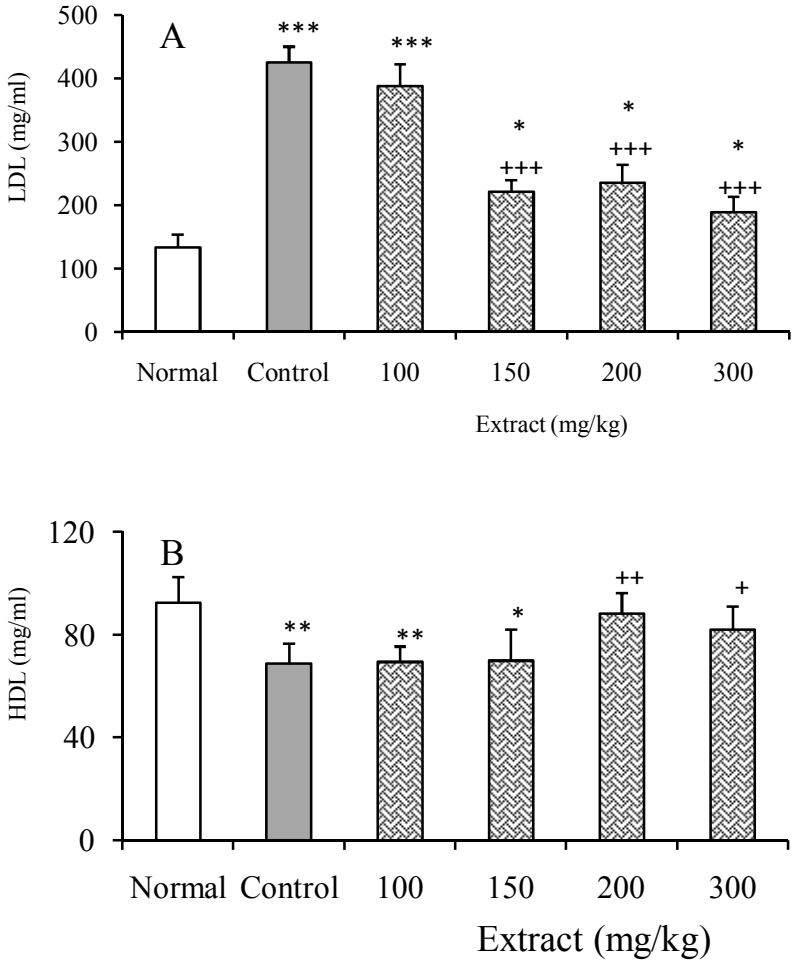
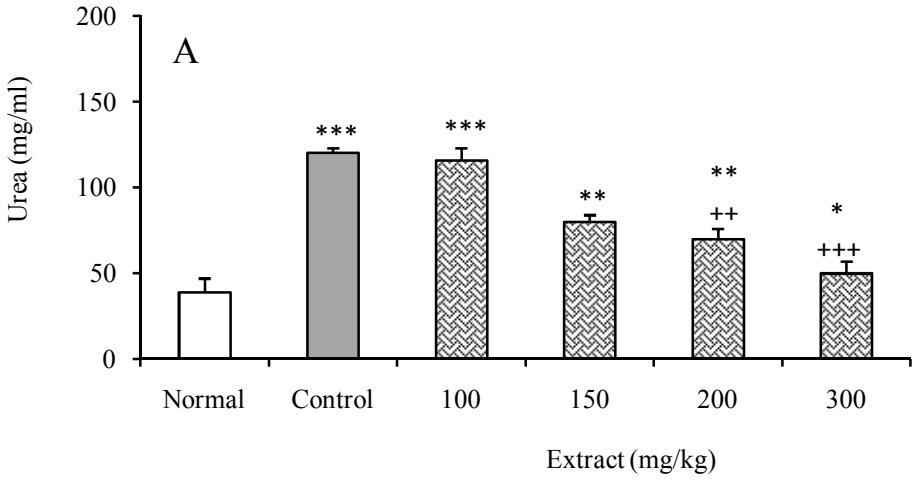


Fig. 3. Effect of i. p. administration of pomegranate hydro-ethanolic extract at doses of 100, 150, 200 and 300 mg/kg body wt. on serum LDL (A) and HDL (B) levels in diabetic rats. Each column represents mean±S.E.M. for 8 rats. Control group administrated with saline as vehicle. * p<0.05, ** p<0.01, *** p<0.001 different from normal group. + 0<0.05, ++ p<0.01, +++ p<0.001 different from control diabetic group.



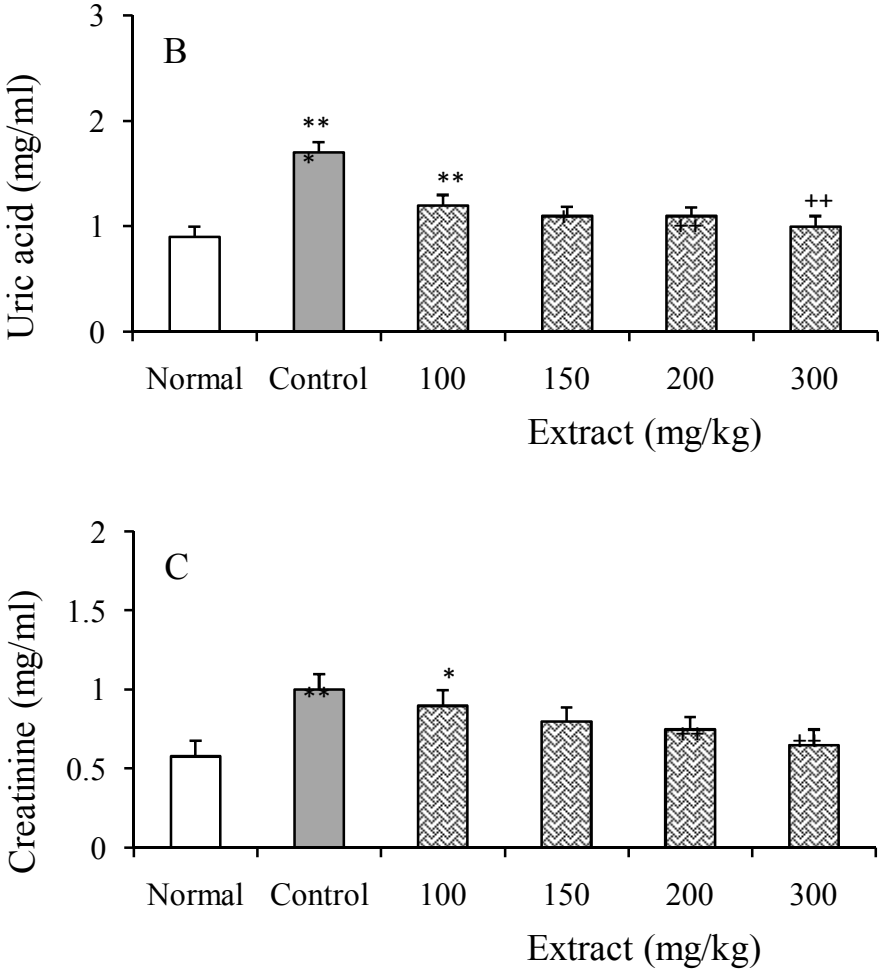


Fig. 4. Effect of i. p. administration of pomegranate hydro-ethanolic extract at doses of 100, 150, 200 and 300 mg/kg body wt. on serum urea (A), uric acid (B) and creatinine (C) levels in diabetic rats. Each column represents mean±S.E.M. for 8 rats. Control group administrated with saline as vehicle. * p<0.05, ** p<0.01, *** p<0.001 different from normal group. + 0<0.05, ++ p<0.01, +++ p<0.001 different from control diabetic group.

Dyslipidemia is one of the major cardiovascular risk factors. It has been demonstrated that insulin deficiency in diabetes mellitus leads to a variety of derangements in metabolic and regulatory processes, which in turn leads to accumulation of lipids such as total cholesterol and triglycerides in diabetic patients [18]. The present results indicated that the pomegranate flowers hydro-ethanolic extract significantly decreased serum triglycerides, total cholesterol and LDL, while increased HDL levels in treated streptozotocin-induced diabetic rats as compared with control diabetic rats. In agreement with the present results, Bagri et al., reported oral administration of pomegranate flowers aqueous extract reduced total cholesterol, triglycerides, LDL and VLDL in streptozotocin-induced diabetic rats [16,19]. Pomegranate is now gaining importance because of its potent antioxidant activity. Pomegranate fruit juice, fruit and peel extracts have been found to possess a tremendous antioxidant activity [20,21]. The present results showed that serum ALT and AST levels increased in diabetic rats. Pomegranate flowers hydro-ethanolic extract significantly decreased serum ALT and ASP enzymes streptozotocin-induced diabetic rats as compared with control diabetic rats. So, *Punica granatum* flower extract has protective role against the oxidative damage in diabetic rats. A very high content of polyphenolics was obtained in pomegranate flower extract [22]. Singh et al. [23] determined the levels of polyphenols in extracts of pomegranate peel and seeds [23]. The amount of polyphenols obtained in pomegranate flower extract lies in between that reported for extracts of peel and seeds, being lower than the former and greater than the latter. The flower extract significantly inhibited elevation in ALT and AST suggesting it to prevent liver damage [22]. The present results showed that serum urea, uric acid, creatinine levels in diabetic rats

increased, when compared with normal rats. The administration of the pomegranate extract tended to bring serum urea, uric acid, creatinine levels significantly toward normal values.

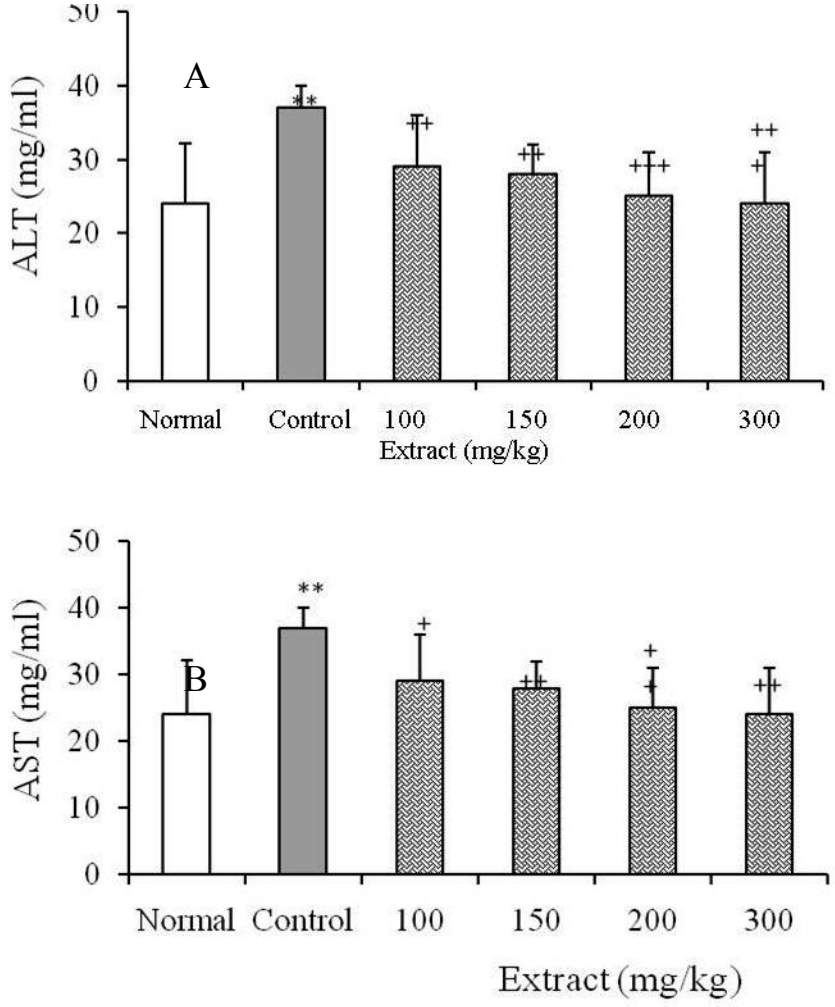


Fig. 5. Effect of i. p. administration of pomegranate hydro-ethanolic extract at doses of 100, 150, 200 and 300 mg/kg body wt. on serum ALT (A) and AST (B) levels in diabetic rats. Each column represents mean±S.E.M. for 8 rats. Control group administrated with saline as vehicle. ** $p<0.01$ different from normal group. + $0<0.05$, ++ $p<0.01$, +++ $p<0.001$ different from control diabetic group.

In conclusion, the results have shown that hydro-alcohol extract of pomegranate flowers possesses antidiabetic effect on streptozotocin-induced diabetic rats, thus the present research validates to some extent the folk use of this plant.

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