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ORIGINAL ARTICLE

Antidiabetic Effect of *Anethum graveolans* L. in Alloxan-induced Diabetic Male Rats

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ABSTRACT

Dill (Anethum graveolans L.) aerial parts a renewed interest in medicinal plants because generally they do not elicit any side effects. In present study, effect of the hydro-alcoholic extract of the Anethum graveolans L. aerial parts on serum glucose, total cholesterol, triglyceride, LDL-c, HDL-c, urea, uric acid, creatinine, alkaline phosphatase (AP), alanine aminotransaminase (ALT) and aspartate aminotransaminase (AST) enzymes levels were investigated in alloxan-induced diabetic rats. The animals were made diabetic using by alloxan (90 mg/kg, i.p.). The hydroethanolic extract at doses 50, 100, 200 and 300 mg/kg, i.p. were administered for 14 days, intraperitoneally. Blood samples were obtained from heart after 14 days. The group of control diabetic rats was administered saline as vehicle. Serum glucose, total cholesterol, triglyceride, LDL-c, HDL-c, urea, uric acid, creatinine, alkaline phosphatase (AP), alanine aminotransaminase (ALT) and aspartate aminotransaminase (AST) enzymes levels were measured by kit. Liver sections were prepared by using a rotary microtome and stained with haematoxylin and eosin dye, which was mounted in a neutral deparaffinated xylene medium for microscopic observations. The results showed the hydro-ethanolic extract of Anethum graveolans L. decreased serum glucose, total cholesterol, triglyceride, LDL-c, urea, uric acid, creatinine, alkaline phosphatase (AP), alanine aminotransaminase (ALT) and aspartate aminotransaminase (AST) enzymes levels, while increased HDL-c level in alloxan-induced diabetic rats compared to saline control diabetic rats. Also, the administration of the dill extract (50, 100, 200 and 300 mg/kg body wt) significantly decreased histophatological damage in liver tissue in diabetic rats compared with control rats. The present data indicated that hydro-ethanolic extract of Anethum graveolans has anti-diabetic effect on diabetic animals. So, this plant should be considered in future therapeutic researches.

***Keywords**: dill, Anethum graveolans, diabetes, rat, alloxan.*

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INTRODUCTION

Diabetes mellitus, one of the most common endocrine metabolic disorders, has a significant impact on the health, quality of life, and life expectancy of patients as well as on the health care system [1]. With the number of cases expected to increase rapidly in the years to come, diabetes is a growing health challenge worldwide. With the active encouragement of the WHO, an attempt is being made to collect traditional medical information used for the treatment of diabetes for study in modern laboratories in order to scientifically evaluate therapeutic efficacies [2].

In recent years much research is focused on the development of herbal medicines which offer exemplary source for drug discovery [3]. Despite remarkable advances made in the management of diabetes by the use of synthetic drugs, there has been a renewed interest in medicinal plants because generally they do

not elicit any side effects. Dill (*Anethum graveolans* L.) aerial parts have a renewed interest in medicinal plants because generally they do not elicit any side effects.

Dill is one such herbal plant known as "Shevid or Shebet" in Iran and its botanical name is *Anethum graveolans* L. belonging to family Apiaceae. *A. graveolens* is an annual or biennial herb. It grows up to 90–120 cm tall and has slender branched stems, finely divided leaves, small umbels (2–9 cm diameter) of yellow flowers, and long spindle-shaped roots. In general, dill leaves (dill weeds) and seeds (small fragrant fruits) are used as seasoning. The leaves could be used in eggs, meats, salads, rice, sea foods and soups; the seeds could be used in bread, and flavouring pickles and soups. Dill essential oil, extracted from both leaves and seeds, could also be used in chewing gums, candies and pickles [4,5]. The plant is native in Southwest Asia and is cultivated in Europe, Iran and the United States [5]. Literature demonstrates that dill leaf consumption could lower the risk of cancer [6] and reduce the level of cholesterolaemia [7]. Various pharmacological actions of *A. graveolens* such as antimicrobial, antispasmodic, antihypercholesteromic, and anti-inflammatory have been reported [8]. There is, however, no thorough report on the antidiabetic effect of dill aerial part. So, in the present study, antidiabetic effect of *A. graveolans* aerial parts is evaluated in alloxan-induced diabetic male rats.

MATERIALS AND METHODS

Fresh *Anethum graveolans* L. aerial parts were collected from Varamin area (July, 2013). Voucher specimens (Herbarium No. GUE 2572) were authenticated by Assoc. Prof. Ali Mazooji of the Department of Biology, Faculty of Biology, Islamic Azad University, and were deposited in the herbarium of the Faculty of Biology, Islamic Azad University (Varamin, Iran). The plant material was dried under shade and powdered using Ultra-Torax. The powder (60 g) was extracted with 300 ml aqueous 80% ethanol in a Soxhlet apparatus for 72 hours. The extract was filtered and concentrated to dryness under reduced pressure in a rotary evaporator at 40–50^{0}C yielding 12.3% (w/w) plant extract. The extract yield was 16%. The obtained dill alcoholic extract was stored at −20°C until usage. Plant extract was suspended in saline (doses 50, 100, 200 and 300 mg/kg body weight) prior to intrepritoneal administration to the experimental animals. Animals in the control group received only the 0.5 ml saline as vehicle.

Male Wistar rats initially weighing 200 to 250 g purchased from the Pasteur Institute (Karaj, Iran) were used in the experiments. The animals were housed in groups of 5 per cage with free access to standard laboratory chow (35% carbohydrates, 25% proteins, 7% lipids, and 3% vitamins) and tap water. The diet was purchased from Pars-Dam food service, Tehran, Iran. The animal room was maintained at 22°C ± 2°C with timed lighting on from 7 AM to 19 PM and relative air humidity of 40% to 60%. Eight animals were used for each group of study. Each animal was used once only. The study was conducted in accordance with ethical procedures and policies approved by the Animal Care and Use Committee of Islamic Azad University, Tehran, Iran. Male adult Wistar rats were injected with alloxan (90 mg/kg, i.p.). Seven days after injection, the rats with fasting blood glucose higher than 180 mg/dl were used for the experiments. The food and water were removed from cages 12 h before testing.

At the end of experiment (14 days), rats were fasted overnight, and blood samples were drawn from heart under light ether anaesthesia. The animals were removed after blood collection and liver sampling. Fasting serum glucose, cholesterol, triglycerides, LDL-C, HDL-C, urea, uric acid, creatinine, alkaline phasphatase, alanine aminotransferase, aspartate aminotransferase levels were determined by kit (Parsazmoon Company, Iran).

For qualitative analysis of liver histology, the tissue samples were fixed for 48 h in 10% formalin-saline and dehydrated by passing successfully in different mixtures of ethyl alcohol–water, cleaned in xylene and embedded in paraffin. Sections of the tissue were prepared by using a rotary microtome and stained with haematoxylin and eosin dye, which was mounted in a neutral deparaffinated xylene medium for microscopic observations. Histopathological damage included granuloma formation (picture 1A), interface hepatitis (picture 1B), portal inflammation (picture 1C) and confluent necrosis (picture 1D). Each damage is given one score and final score of each specimen is sum of damage's scores.

Statistical analyses were carried out by SPSS 10 (SPSS, Chicago, Ill) program for windows. Data were expressed as mean ± SEM. Statistical analysis was performed using one-way analysis of variance followed by Tukey post hoc test. The criterion for statistical significance was $p<0.05$.

RESULTS AND DISCUSION

Diabetes is currently growing at a fast rate throughout the world and is the 16th leading cause of global mortality [9]. Present number of diabetics worldwide is 150 million, and this is likely to increase to 300 million or more by the year 2025 [10]. With the active encouragement of the WHO, an attempt is being made to collect traditional medical information used for the treatment of diabetes for study in modern

laboratories in order to scientifically evaluate therapeutic efficacies [2]. In recent years much research is focused on the development of herbal medicines which offer exemplary source for drug discovery. Despite remarkable advances made in the management of diabetes by the use of synthetic drugs, there has been a renewed interest in medicinal plants because generally they do not elicit any side effects.

In Iranian folk medicine, some traditional and edible plants have been utilized to decrease symptoms of diabetes. Among these, dill is a perennial tree whose fruits and leaves can be used in many ways. *Anethum graveolens* L. (dill) (Apiaceae) is one of the most popular culinary herbs in the world. There have been records on use of this plant for medicinal and edible purposes dating back to the Greek and Egyptian civilizations [11]. Various pharmacological actions of *A. graveolens* such as antimicrobial, antispasmodic, antidiabetic, antihypercholesteromic, and anti-inflammatory have been reported [8]. There is no report about antidiabetic effect of dill, yet. So, in this study, we evaluated antidiabetic effect of dill aerial parts in diabetic rats.

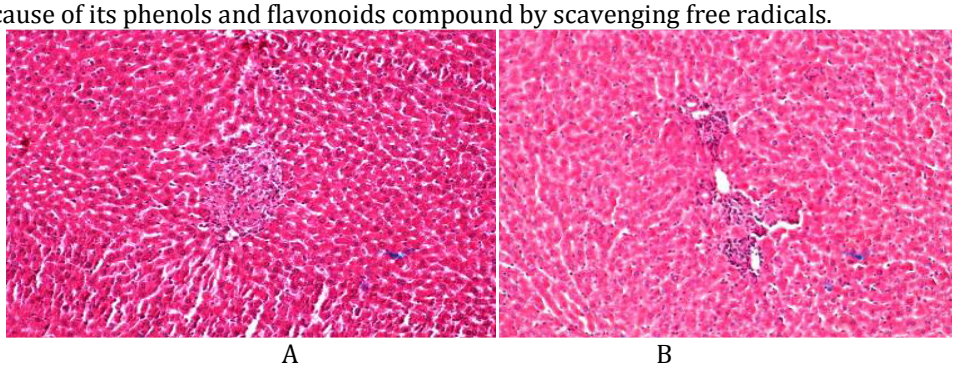
The present results showed administration of dill hydro-ethanolic extract decreased serum glucose (fig. 1), triglycerides, total cholesterol (fig. 2), LDL-C (fig. 3), urea, uric acid, creatinine (fig. 4) and AP, ALT and AST levels (fig. 5), while elevated serum HDL-C level (fig. 3) in alloxan- induced diabetic rats compared to diabetic control group, significantly. Also, treatment of plant extract improved liver histophatological damages in treated diabetic rats in comparison to control diabetic rats, significantly (fig. 6). It is the first report about antidiabetic effect of A*nethum graveolens*.

In diabetes, hyperglycaemia generates reactive oxygen species (ROS), which in turn cause lipid peroxidation and membrane damage and these free radicals play an important role in the production of secondary complications in diabetes mellitus (kidney, eye, blood vessel, and nerve damage) [12]. Antioxidants have been shown to prevent the destruction of beta cells [13] by inhibiting the peroxidation chain reaction and thus they may provide protection against the development of diabetes [14].

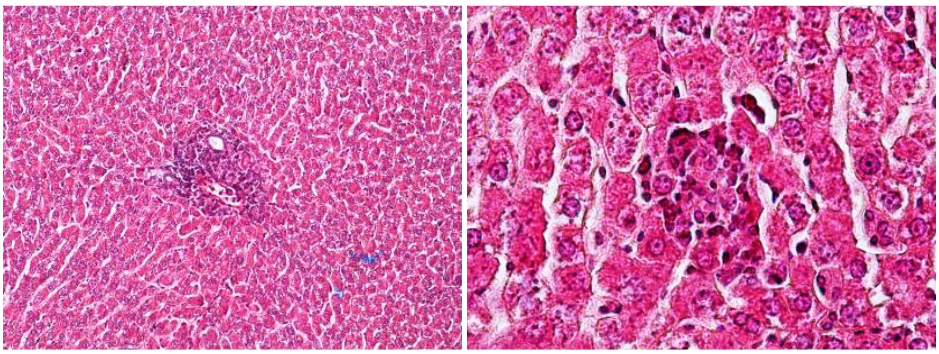
Plants contain natural antioxidants (tannins, flavonoids, vitamins C and E, etc.) that can preserve β-cell function and prevent diabetes induced ROS formation [15]. Moreover, dill leaf, seed and their essential oil could provide good antioxidant activities [16]. Many reports indicate that dill flowers have remarkable antioxidant activity [17]. The antioxidant properties of ethanolic extract from the dill flowers and its various fractions (n-hexane, ethyl acetate and ethanol) have 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging, Trolox equivalent antioxidant capacity (TEAC), reducing power, and b-carotene bleaching efficacies. Antioxidant components in these extracts were also determined [18].

Phenolic compounds widely exist in plants are bioactive substances. It is well known that they are highly effective antioxidants. Plant phenols comprise a great diversity of compounds, such as flavonoids (anthocyanins, flavanols, flavonols, flavones, amongst others), proanthocyanidins also known as condensed tannins (the oligomeric and polymeric flavan- 3-ols). Flavonoids have the basic skeleton of diphenylpropanes (C6–C3–C6) with various oxidation level of the central pyran ring [19]; they could provide strong antioxidant activities associated with their capacity to scavenge free radical and terminate radical chain reactions [20]. The proanthocyanidins, a group of biologically active polyphenolic bioflavonoids have beneficial effects in radical scavenging and other relevant redox active properties [21]. Ascorbic acid and tocopherols are the important antioxidants (vitamins) in organisms to quench free radicals also widely existing in plants [22]. Ethanolic extract of dill flower had higher antioxidant activity than corresponding extracts of dill leaf and seed. Through correlation analysis of phytochemical contents and antioxidant capacities for the original dill flower extract and its soluble fractions, the representative antioxidant components could be regarded as phenols, flavonoids and proanthocyanidins. Chlorogenic acid, myricetin, and 3, 3', 4', 5, 7-pentahydoxyflavan (4→8)-3, 3', 4', 5, 7-pentahydoxyflavan were the major phenolic acid, flavonoid, and proanthocyanidin, respectively, in the dill flower extract [23].

It is concluded that extract of *Anethum graveolens* has antidiabetic effect in diabetic animals and its effect may be because of its phenols and flavonoids compound by scavenging free radicals.



A B



C D

Picture 1 – Histopathological sections included granuloma formation (A, ×100), interface hepatitis (B, ×100), portal inflammation (C, ×100) and confluent necrosis (D×400). Samples were stained with Hematoxylin-Eosin.

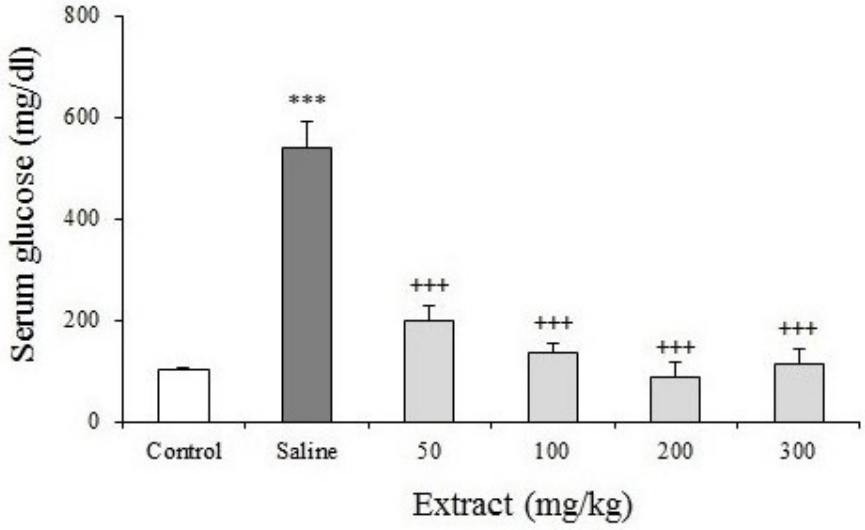
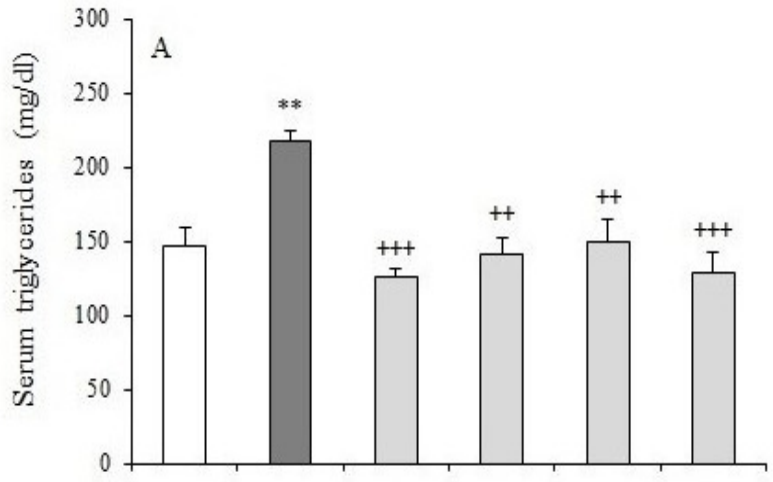


Fig. 1. Effect of i. p. administration of dill alcoholic extract at doses of 50, 100, 200 and 300 mg/kg body wt on serum glucose level in diabetic rats. Each column represents mean ± SEM for 8 rats. Control group was administrated saline as vehicle. ***P<0.001 different from control group; +++P<0.001 different from saline group.



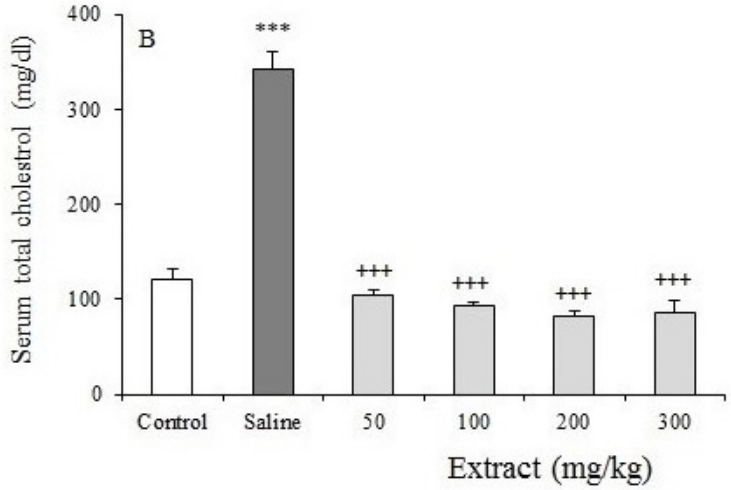


Fig. 2. Effect of i. p. administration of dill alcoholic extract at doses of 50, 100, 200 and 300 mg/kg body wt on serum triglycerides (A) and total cholesterol (B) levels in diabetic rats. Each column represents mean ± SEM for 8 rats. Control group was administrated saline as vehicle. **P<0.01; ***P<0.001 different from control group; ++p<0.01, +++p<0.001 different from saline group.

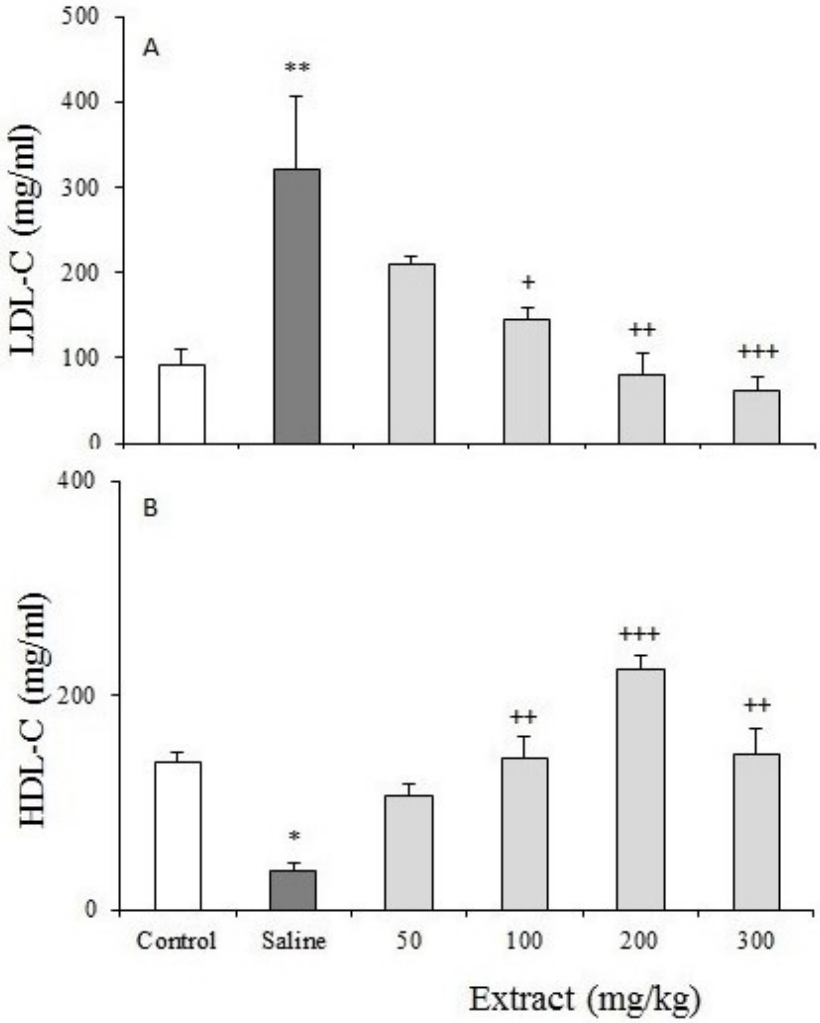


Fig. 3. Effect of i. p. administration of dill alcoholic extract at doses of 50, 100, 200 and 300 mg/kg body wt on serum LDL-C (A) and HDL-C (B) levels in diabetic rats. Each column represents mean ± SEM for 8 rats. Control group was administrated saline as vehicle. *p<0.05; **p<0.01 different from control group; +p<0.05, ++p<0.01, +++p<0.001 different from saline group.

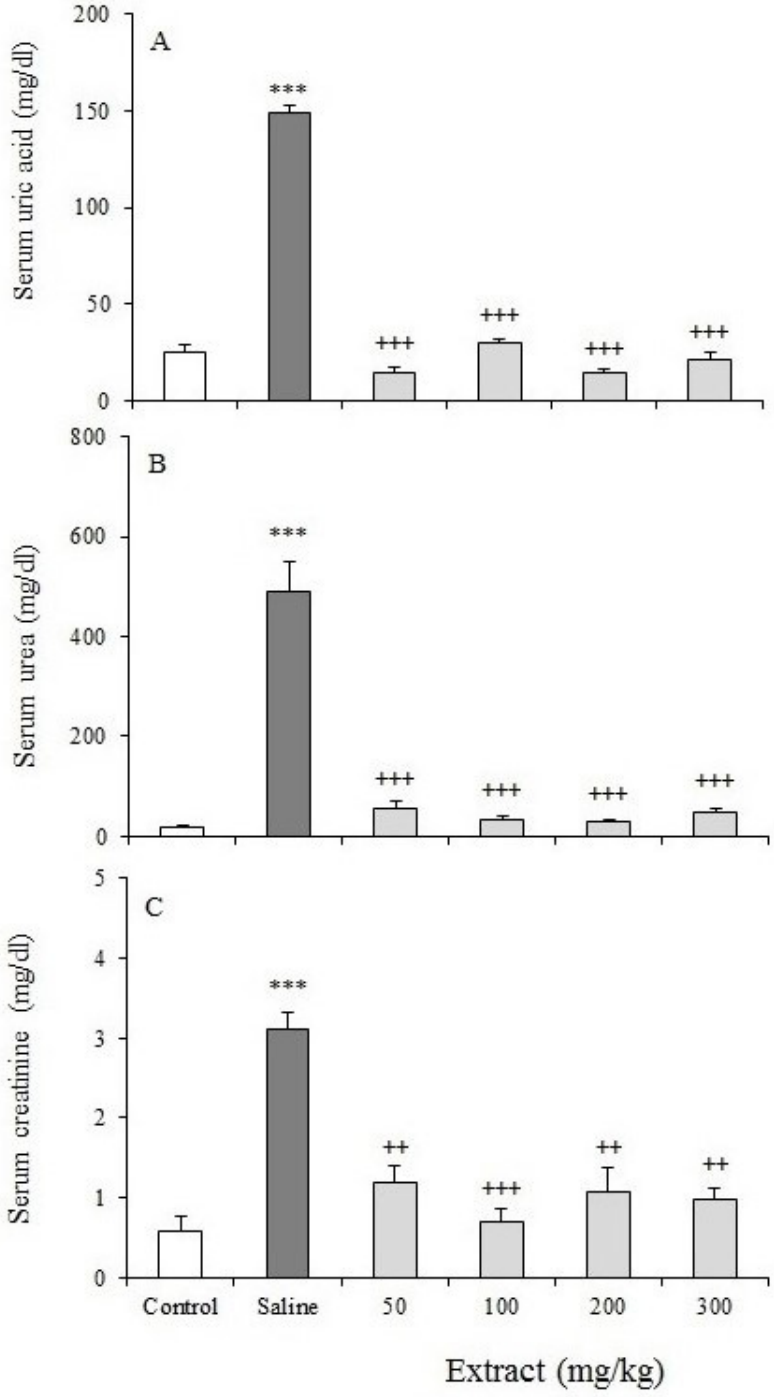


Fig. 4. Effect of i. p. administration of dill alcoholic extract at doses of 50, 100, 200 and 300 mg/kg body wt on serum uric acid (A), urea (B) and creatinine (C) levels in diabetic rats. Each column represents mean ± SEM for 8 rats. Control group was administrated saline as vehicle. ***p<0.001 different from control group; +p<0.05, ++p<0.01, +++p<0.001 different from saline group.

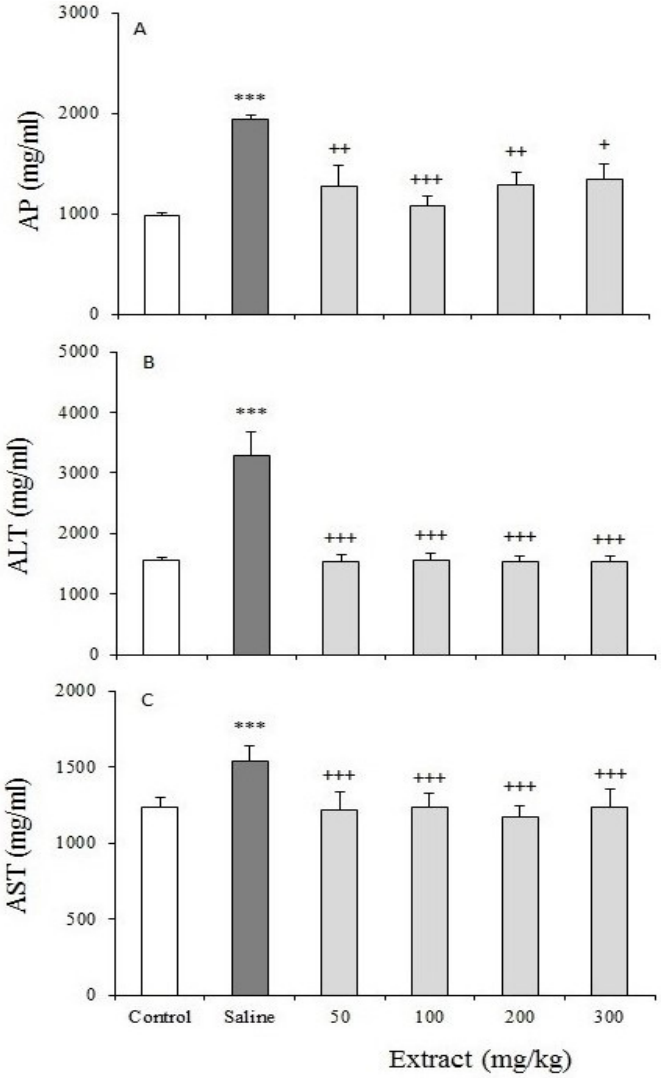


Fig. 5. Effect of i. p. administration of dill alcoholic extract at doses of 50, 100, 200 and 300 mg/kg body wt on serum AP (A), ALT (B) and AST (C) levels in diabetic rats. Each column represents mean ± SEM for 8 rats. Control group was administrated saline as vehicle. ***p<0.001 different from control group; +p<0.05, ++p<0.01, +++p<0.001 different from saline group.

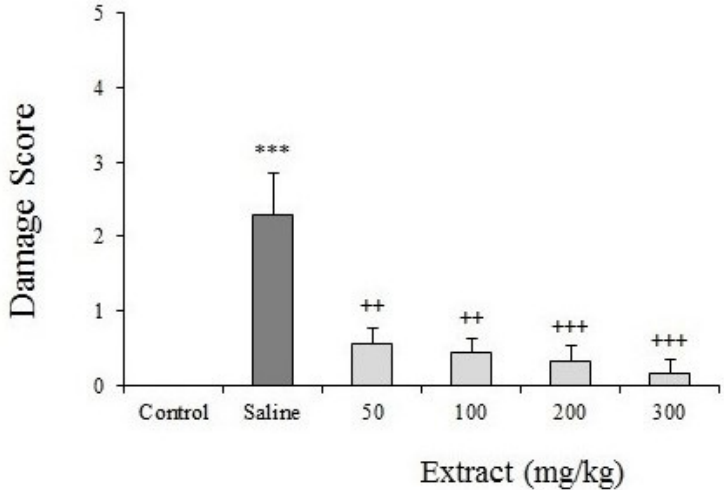


Fig. 6. Effect of i. p. administration of dill alcoholic extract at doses of 50, 100, 200 and 300 mg/kg body wt on pathohistological damage score in diabetic rats. Each column represents mean ± SEM for 8 rats.

Control group was administrated saline as vehicle. ***p<0.001 different from control group; ++p<0.01, +++p<0.001 different from saline group.

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