# Investigating the Effect of Hydroalcoholic Extract of Eryngos on Plasma Concentration of Blood Glucose, Blood Cells and Pancreatic Tissue in Diabetic Rats

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# Abstract

**BACKGROUND:** Diabetes mellitus is one of the most common metabolic disorders with a severe impact on the quality of life.

**OBJECTIVES:** The objective of the present study was to investigate the impact of the hydroalcoholic extract of Eryngos on blood glucose, blood cells, and pancreatic tissue.

**METHODS:** Thirty-five male rats were prepared. After diabetes induction by streptozotocin, they were randomly divided into 5 groups, including; non-diabetic control, diabetic control, diabetic treated with hydroalcoholic extract of Eryngos at doses of 300 and 500 mg/kg administered intraperitoneally, and metformin at a dose of 500 mg/kg. At the end of the study, glucose level was measured, and blood cells and pancreatic tissue were examined.

**RESULTS:** Hydroalcoholic extract of Eryngos caused a significant reduction in blood glucose. Given the adverse effects of diabetes on the number of WBC, Eryngos extract at a dose of 300 mg/kg had a protective effect on the number of WBC. It decreased their number significantly compared with the diabetic control group. This effect was the same for both diabetic and non-diabetic groups. Histopathological results also indicated that Eryngos extract significantly increased the number of Langerhans and beta cells.

**CONCLUSIONS:** The results suggest that the hydroalcoholic extract of Eryngos may be effective in the treatment of diabetes. Moreover, based on the biochemical and histological results, it can be concluded that the hypoglycemic effect of the extract is probably due to the restoration and repair of the islets of Langerhans.

KEYWORDS: Diabetes, Eryngos, Glucose, Pancreas, Rat

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# Introduction

Diabetes is a common and high-cost chronic disease associated with several complications and a significant rate of mortality worldwide. The disease is also a known inflammatory process with insulin resistance or reduced secretion. Inflammation is involved in the initial induction and strengthening of the immune attack against pancreatic beta cells and stabilization and maintenance of inflammation of the pancreatic islets in later stages (Asgari et al., 2013). The global prevalence of type 1 diabetes among people aged 30 years and younger is about 3%. The prevalence of this type of diabetes is increasing by 2.1% per year, especially in children aged below 5 years. Among micro-and macro-vascular complications, diabetic cardiovascular disease is the most important complication and one of the causes of mortality (Einarson et al., 2018). A national report on the risk factors of non-communicable diseases in Iran released in 2005 indicated that the prevalence of diabetes among Iranian adults in the age range of 25 to 65 years was 2 million people. According to the World Health Organization (WHO), the increasing trend will lead to the suffering of 2.5 million diabetic Iranians by 2025 (Darya et al., 2019).

Diabetes mellitus is the most important cause of blindness in adults, non-traumatic amputation, and end-stage renal failure, leading to dialysis and kidney transplantation. Due to disturbed balance in fat metabolism in the liver cell in diabetic people, liver disorders are also common. One of these diseases is a fatty liver disease associated with the accumulation of fat in the liver cells. Additionally, this disease can turn into acute liver disease such as fibrosis or liver cirrhosis (Sayyadi *et al.*, 2016).

Eryngos (with the scientific name of Eryngium) is a group of flowering plants of the *Apiaceae* family that includes about 250 species. Iranian Eryngos is used for different diseases treatment. Its roots and fruits are used in traditional medicine. Its effects include cleansing the liver from toxins, activating the pancreas and lowering blood glucose and treating diabetes, gastrointestinal tract, and stomach pain, treating inflammation, bladder, and kidney stones. Eryngos contain antidiabetic polyphenols, which are mainly flavonol glycosides, which have antioxidant properties (Umeno *et al.*, 2016). Considering the lack of study in this area, the present study was conducted to investigate the effect of this plant extract on the pancreatic tissue and blood glucose levels in diabetic rats.

# **Materials and Methods**

### Animals

In the present experimental study, 35 male Wistar rats weighing 180-220 gr were purchased from the Animal Breeding Center of Shahrekord Azad University Laboratory. The animals were kept at 21  $\pm$ 2°C and 50% humidity with ad libitum access to the water and food. The 12/12-h light-dark cycles were maintained for 24 h a day. The animals were kept in the animal room of Shahrekord Azad University. This study was approved by the Ethics Committee of the University of Shahrekord.

### **Extract Preparation**

Plant tissue was pulverized using an electric grinder. About 500 gr of the plant powder was measured, and its total ethanolic extract was prepared by repeated massaging method (72 3 3 hr) with 70% ethanol in distilled water. The extract was concentrated using vacuum distillation, and the solvent was removed by incubating in a vacuum oven at 40°C. The extract was stored in a dark container in a closed and cool place away from light and moisture (4°C).

The animals were divided into 5 groups: Group 1 (non-diabetic control group): Healthy rats that received the equivalent volume of physiological saline injection intraperitoneally and daily; Group 2 (diabetic control): Diabetic rats that were treated with physiological serum intraperitoneally and daily; Groups 3 and 4 (treatment groups): Diabetic rats that received Eryngos extract in physiological saline solution at 300 and 500 mg/kg of body weight and intraperitoneally (Eslami *et al.*, 2011); and Group 5 (metformin group): Diabetic rats that received metformin at 500 mg/kg dose daily by gavage.

Diabetes induction was conducted using Streptozocin (STZ) prepared from Sigma (USA). STZ was dissolved in 5.1 M citrate buffer, pH 0.6, and injected intraperitoneally at a dose of 60 mg/kg (Banda *et al.*, 2018). It is of note that STZ can cause fatal hyperglycemia due to its toxicity to the beta cells of the pancreatic islets that normally regulate blood glucose levels by insulin secretion (Banda *et al.*, 2018).

Six hours later, the rats were supported with a 10% glucose solution for 24 h to prevent hyperglycemia and insulin shock (Ebrahimzadeh *et al.*, 2010). Blood samples were taken from the tail of the rats 6-7 days after injection with anesthesia (ether) using a lancet. If the blood glucose level was between 200-300 mg/dL, treatment was continued for one month.

### **Blood Sampling and Biochemical Tests**

Sixteen h before blood sampling, the animals were kept under fasting and given only water to stabilize their blood glucose. At the end of week 4, the rats were killed without pain (xylazine and Ketamine) (Motaghi *et al.*, 2019), and blood samples were taken from their hearts. After separating the blood serum, the serum glucose concentration was measured using an autoanalyzer device (Germany-Convergys).

Finally, the pancreas was separated from the animal body for the histological studies, kept at 10% formalin solution, and sent to the laboratory for hematoxylin-eosin (H&E) staining and examined histologically. The results were expressed as mean  $\pm$  standard deviation (SD) for each group, and the means of five groups were compared using one-way analysis of variance (ANOVA). The inter-group and intra-group comparisons were performed using Tukey post-hoc test. The significance level was considered at 5%.

### Results

The mean glucose was significantly higher in the diabetic control group compared to the non-diabetic control group (P<0.05), indicating the perfect induction of diabetes. After using antidiabetic drugs, the mean serum glucose was significantly reduced (P<0.05) compared to the diabetic control group, confirming the properties of the studied drugs. Between the drugs groups, Eryngos was the most effective drug at a dose of 300 mg/kg and the mean serum glucose decreased by 408 units compared to the diabetic control group, which was a significant decrement (P=0.000). Its effectiveness decreased significantly after increasing the dose of Eryngos from 300 to 500 mg (P=0.005), while the mean blood glucose decreased by only 317 units. Although

increasing the dose of Eryngos decreased its effectiveness significantly (P=0.005), this medicinal plant was significantly more effective than metformin (P=0.019) even at a dose of 500 mg/kg.

Metformin had the lowest effect on the mean blood glucose and decreased glucose levels by 225 units. However, this difference was also significant (P=0.000), indicating its effectiveness in the treatment of diabetes. It should be noted that none of these drugs lowered the mean glucose to the non-diabetic control group level and the difference between all the studied drugs groups and the non-diabetic control group was statistically significant (P<0.007) (Figure 1).

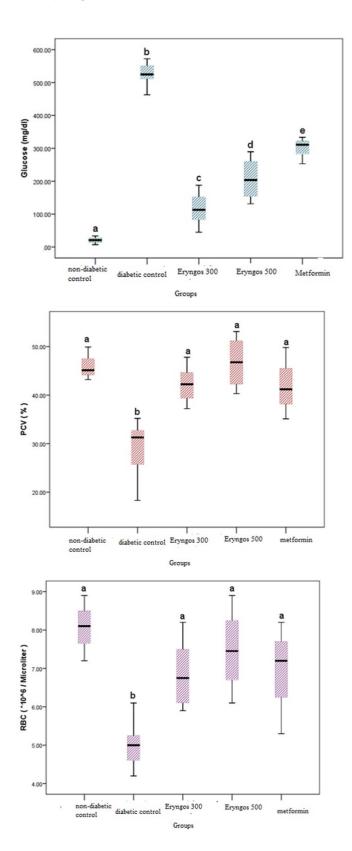
In the diabetic control group, the mean hematocrit decreased significantly (P=0.000) compared to the non-diabetic control group, indicating the adverse effects of diabetes on the hematocrit. The used drugs had a protective effect on hematocrit. As a result, all three drugs groups improved the level of hematocrit significantly (P<0.002) compared to the diabetic control group, and the mean hematocrit increased significantly in the drugs groups (P<0.002), which was equal to the non-diabetic control group. No statistically significant difference was found among the tested drugs in terms of their effect on hematocrit (P>0.05) (Figure 2).

In the diabetic control group, the mean number of red blood cells (RBCs) decreased significantly (P=0.000) compared to the non-diabetic control group, suggesting the adverse effects of diabetes on the number of RBCs. The used drugs had a protective effect on the number of RBCs so that all three drugs groups showed significant improvement in the number of RBCs (P<0.007) compared to the diabetic control group. Also, the mean number of RBCs increased significantly in the drugs groups (P < 0.007), equal to the non-diabetic control group. There was no significant difference between the tested drugs in terms of their effect on the number of RBCs (P>0.05) (Figure 3). The used drugs also had a protective effect on the level of hemoglobin, so that all three drugs improved the mean hemoglobin significantly (P<0.001) compared to the diabetic control group. As can be seen, the mean hemoglobin level increased significantly in the drugs groups (P<0.-001), and it was equal to the non-diabetic control group. There was no significant difference among

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the drugs in terms of their effect on hemoglobin (P>0.05) (Figure 4).



**Figure 1.** Mean serum glucose in mg/dL; statistically significant values (P < 0.05) are shown by different letters.

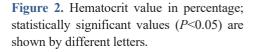
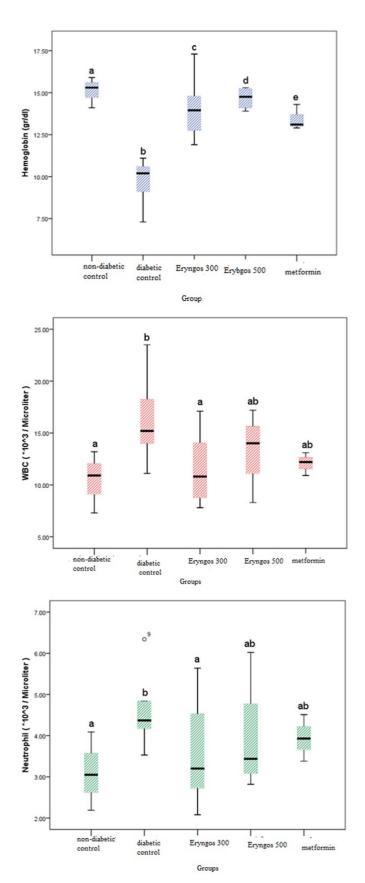


Figure 3. In the diabetic control group, the mean number of red blood cells decreased significantly (P=0.000) compared to the non-diabetic control group, which indicates the adverse effects of diabetes on the number of red blood cells.



**Figure 4.** In the diabetic control group, the mean hemoglobin decreased significantly (P=0.000) compared to the non-diabetic control group, indicating the adverse effects of diabetes on hemoglobin.

**Figure 5.** In the diabetic control group, the mean number of neutrophils increased significantly (P=0.043), compared to the non-diabetic control group, indicating the adverse effects of diabetes on the number of neutrophils.

**Figure 6.** The number of neutrophils  $(\mu L/10^3)$ ; statistically significant values (*P*<0.05) are shown by different letters.

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In terms of white blood cells (WBC), the diabetic control group showed a significant increment in the mean number of WBCs (P=0.023) compared to the non-diabetic control group, indicating the adverse effects of diabetes on the number of WBC. Among the drugs used, only Eryngos at a dose of 300 mg/kg had a protective effect on the number of WBC. Thus, it significantly reduced the number of WBC (P=0.014) compared to the diabetic control group, and it was equal to the non-diabetic control group (Figure 5).

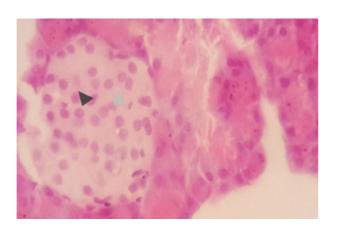
Among the drugs used, only Eryngos at a dose of 300 mg/kg showed a protective effect on the number of neutrophils. Thus, it decreased the number of neutrophils significantly (P=0.037) compared to the diabetic control group, and it was equal to the non-diabetic control group (Figure 6).

In the diabetic control group, the mean number of lymphocytes increased significantly (P=0.025) compared to the non-diabetic control group, indicating the adverse effects of diabetes on the number of lymphocytes. Among the drugs used, only Eryngos at a dose of 300 mg/kg showed a protective effect on the number of lymphocytes, and the number of neutrophils in the group receiving 300 mg/kg extracts was the same as the non-diabetic group.

#### **Histopathological Results**

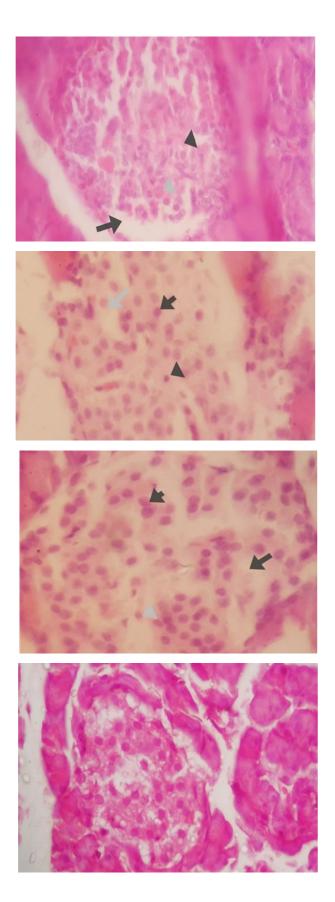
To examine the preventive effects of Eryngos extract on the incidence of diabetes-induced pancreatic lesions in rats, the samples were taken from the pancreas of the rats in 5 groups. Then, microscopic sections were prepared and stained by H&E. In the control group, the pancreas tissue was completely healthy, and no changes were observed (Figure 7). In the group of diabetes, necrosis of islets, vacuole formation, and some beta cells were observed (Figure 8). In the group of 300 mg/kg Eryngos injection, necrosis and low alpha and beta cells were observed in the tissue (Figure 9); however, in the group of 500 mg/kg Eryngos injection, the presence of alpha and beta cells and necrosis were less observed (Figure 10). In the metformin group, the number of beta cells was lower than the extract-treated groups, and the islets of Langerhans were smaller (Figure 11). According to the Duncan post-hoc test's ANOVA results, a statistically significant difference was found between the mean number of islets of Langerhans in the studied groups (P<0.001). No significant difference was found between the diabetic and metformin groups in terms of the mean number of islets of Langerhans (P=0.052).

There was no significant difference between the mean number of islets of Langerhans in metformin, 300 mg, and 500 mg treatment groups (P=0.064). Also, no significant difference was obtained between the mean number of islets of Langerhans in 300 mg treatment and the control groups (P=0.317). The ANOVA results using Duncan post-hoc test, revealed a significant difference between the mean number of beta cells in the studied groups (P<0.001). No significant difference was found between the mean number of beta cells or in the diabetic, metformin, 500 mg treatment, and 300 mg treatment groups (P=0.105), nor in the metformin, 500 mg treatment, 300 mg treatment, and control groups (P=0.182).



**Figure 7.** Pancreas in the control group: Presence of alpha and beta cells (hematoxylin-eosin staining at a 40× magnification)

#### Effect of Eryngos Extract on Diabetes and Pancrease

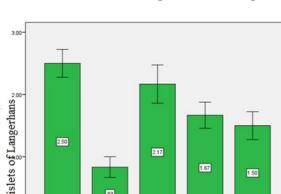


**Figure 8.** Pancreas in the diabetic group: necrosis of islets, vacuolar formation, and observation of some beta cells (hematoxylin-eosin staining at a  $40 \times$  magnification)

**Figure 9.** The pancreas of treatment 300 group, observation of alpha and beta cells and low necrosis in tissue (Hematoxylin-eosin staining at a  $40 \times$  magnification)

**Figure 10.** Pancreas in treatment 500 group: the presence of alpha and beta cells and less necrosis (hematoxylin-eosin staining at a  $40 \times$  magnification)

**Figure 11.** The metformin group has fewer beta cells in the tissue than the Eryngos extract groups. The islets of Langerhans are also smaller (Hematoxylineosin staining at a  $40 \times$  magnification)



2.17

.83

1.67

1.50

2.50

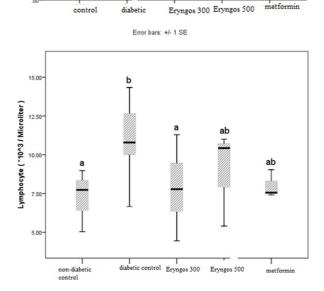


Figure 12. Comparison of the mean number of islets of Langerhans in experimental groups

Figure 13. Significantly decreased the number of lymphocytes (P=0.014) compared to the diabetic control group and it was equal to the non-diabetic control group.

## Discussion

Regarding the recent growing interest in medicinal plants, several studies have been conducted on the effect of different medicinal plants on the activity of the body systems of living organisms. In the present study, an indigenous medicinal plant called Eryngos was used to evaluate its effect on serum glucose related to the pancreatic tissue and blood cells. Eryngos with the scientific name of Eryngium caeruleum M.B belongs to the Apiaceae family. The roots and leaves of this plant are mainly used in most species. The species of this genus have diuretic, appetizing, and laxative effects and treat bloating. They also possess anti-inflammatory properties, treat skin disorders and liver diseases, and are effective in relieving kidney and genital disorders (Bown, 1995).

Research findings have demonstrated that all parts of Eryngo, including the dried leaves, roots, blossoms, and stems, have antioxidants (Behmanesh et al., 2019), reno-protective, and scavenging properties (Ebrahizadeh., 2010).

Recent studies have demonstrated that this plant chemical composition mainly consists of phenols and flavonoids. Phenolic compounds include a large group of aromatic secondary herbal metabolites. These compounds have shown varieties of biological effects both in vitro and in vivo. Flavonoids, for instance, can affect antimicrobial, antineoplastic, antioxidant, hypolipidemic, antiplatelet aggregation, anti-prostaglandins, and anti-inflammatory activities. The biochemical effects of flavonoids can act through inhibition of a number of enzymes such as aldose reductase, phosphodiesterase, Ca<sup>+2</sup>-ATPase, xanthine oxidase, lipoxygenase, and phospholipase A<sub>2</sub>, etc. Some kinds of polyphenols and flavonoids can also have a regulatory role on different hormones such as estrogens and thyroid hormone (Greuenwald et al., 1998; Narayana et al., 2001).

In the present study, a significant difference was observed between the mean number of islets of Langerhans and beta cells in the studied groups (P<0.001) (Figure 12), indicating the positive effect of the drug. Injecting 300 and 500 mg/kg of Eryngos extract for 12 days intraperitoneally in Wistar rats could significantly reduce the mean serum glucose in the treated groups (P < 0.05) compared to the diabetic control group. Metformin also had the lowest effect on the mean blood glucose among the studied drugs. Among the tested drugs, Eryngos at a dose of 300 mg/kg was the most effective on lowering blood glucose.

Also, the plant extract in the present study showed a completely protective effect on hematocrit. Therefore, all three drugs improved the levels of hematocrit, hemoglobin, and RBCs significantly (P<-0.002) compared to the diabetic control group. Moreover, the mean hematocrit, hemoglobin, and erythrocytes increased significantly in the drugs groups (P < 0.002), which were equal to the non-diabetic control group. Among the drugs used, only Eryngos at a dose of 300 mg/kg had a protective effect on the number of WBC. As a result, it significantly decreased the number of WBC (P=0.014) compared to the diabetic control group, and it was equal to the non-diabetic control group.

In a study conducted by Semnani et al., (2002), 70.8% of the compounds in the eryngium bungei boiss essential oil and 84.2% of the compounds in Eryngos essential oil were identified. Eryngium bungei boiss essential oil included monoterpenoids (40.5%), sesquiterpenoids (3.9%), and non-terpenoid compounds (26.4%).

Accordingly, another justification for the hematologic quantities reduction in this study could be the rats which may lead to a decrease in insulin in the animals, followed by the impaired glucose metabolism, resulting in the decreased production of erythropoietin from other sources. It has been able to reduce the hematological quantities. The amount of WBC after diabetes was significantly reduced. The results of this section were consistent with the study by Ohaeri et al., who studied the effect of garlic oil on diabetes (Ohaeri et al., 2006). It has been reported that garlic oil increases the number of WBCin diabetic animal models. According to the results of Alba-loureior et al., the function and metabolism of 448

neutrophil WBC were altered in diabetic patients (Alba-loureior et al., 2006).

Eryngos essential oil included monoterpenoids (71.0%), sesquiterpenoids (12.6%), and diterpenoids. Since the compounds of these species were identified for the first time, they were compared with the compounds isolated from the species of other genera. By comparing the components of essential oils of species of E. bungei and E. foetidum with E. caeruleum by other researchers, major differences were observed in the type and amount of the components of essential oils of these species (Wong et al., 1994).

For example, in a study conducted on essential oils of leaves of E. foetidum (1994), it was found that Dodecano-2-(E) at the level of 59.7% was the most abundant component of the essential oil. In another study conducted on this species in 1997 in Cuba, 5, 4, 2-trimethyl benzaldehyde (20.5%), hexadecanoic acid (12.1%), and carotene (9.9%) were recognized as the main components of the essential oils. Pasayeva et al. (2017) investigated the effect of Eryngos methanolic extract on MCF7 breast cancer cells and showed that a concentration of 99.5 mg/ml had cytotoxic effects (Pasayeva et al., 2017).

Umaro et al., (2018) examined the antidiabetic effects of Barringtonia Racemosa leaf extract in alloxan-induced diabetic rats. The results showed a significant difference in glucose levels on different days. Moreover, it was found that Barringtonia Racemosa reduced blood glucose levels of the diabetic rats. These results were consistent with those of the present work (Umaru et al., 2018).

Banda et al., in 2018 examined the antidiabetic effect of aqueous extract of Lannea Edulis on alloxaninduced diabetic rats and showed that aqueous extract of this plant had antidiabetic effects and lowered blood fat. These results were in line with those of the present study (Banda et al., (2018).

Eyni et al., (2016) examined the effects of hydroalcoholic extract of Rosa Canina on glucose plasma values in male diabetic rats and showed that Rosa Canina declined the levels of blood glucose. Their results were consistent with those of the present study (Ilchizadeh et al., 2015).

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Mottaghi *et al.*, (2018) examined the effect of alcoholic extract of aerial parts of *Ziiphora Tenuiorl* on blood glucose levels and fat indices of streptozotocin-induced diabetic rats. The results showed an increase in insulin level, which reduced blood glucose in type 1 diabetic rats. Their results were consistent with those of our results (Motaghi *et al.*, 2019).

It is concluded that the administration of Eryngos extracts as a therapeutic agent in diabetic patients at doses of 300 and 500 mg/kg leads to a significant reduction in blood glucose. Eryngos extract at a dose of 300 mg/kg was the most effective drug and showed the highest blood-glucose-lowering effect than Eryngos extract at a dose of 500 mg/kg and metformin.

According to our results, Eryngos at a dose of 300 mg/kg combined with the chemical drug used in this study (metformin) could be a suitable alternative for the treatment of diabetes. This extract also had protective and positive effects on hematocrit, RBCs, and hemoglobin. The plant investigated in the present study had the best protective effect at a dose of 300 mg/kg on WBC, neutrophils, and lymphocytes (Figure 13). Histopathologically, the results suggest that in the diabetic group treated with Eryngos extract, the number of islets of Langerhans and beta cells increased significantly compared to the diabetic group (Figures 7-10 12).

Accordingly, another justification for the reduction of hematologic quantities in this study could be the rats that may lead to a decrease in insulin in these animals, followed by impaired glucose metabolism, resulting in the decreased production of erythropoietin from other sources. The extract evaluated in the present study was able to reduce the hematological quantities. The amount of WBC after diabetes was significantly reduced. The results of this section were consistent with the study conducted by Ohaeri and colleagues, who studied the effect of garlic oil on diabetes. It was reported that garlic oil increased the number of WBCin diabetic animals (Ohaeri *et al.*, 2006). According to the results of Alba-loureior and colleagues, the function and metabolism of neutrophil were altered on diabetic patient, which was for the impaired metabolism of glucose and glutamine, which are mostly used by neutrophils (Albaloureior *et al.*, 2006).

### Conclusion

According to our results, the use of Eryngos extract can be effective in the treatment of diabetes. Although the details of the mechanism of action of this plant extract are unknown, this effect might be attributed to the antioxidant compounds existing in the extract or the effect of the extract on pancreatic beta cells, which requires further investigation in this area.

### Acknowledgments

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# **Conflict of Interest**

The authors declare no conflict of interest.

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# بررسی اثر عصاره هیدروالکلی بوقناق برغلظت پلاسمایی قند خون، سلولهای خونی و بافت پانکراس در رتهای دیابتی

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زمینه مطالعه: مقدمه: دیابت یکی از شایعترین بیماریهای متابولیک است که میتواند کیفیت زندگی افراد را تحت تاثیر قرار دهد.

**هدف**: هدف از این مطالعه بررسی اثر عصاره هیدروالکلی بوقناق بر گلوکز خون، سلولهای خونی و بافت پانکراس است.

مواد و روشها: در این تحقیق از ۳۵ سر موش صحرایی نر استفاده شد. پس از دیابتی کردن موشها با استرپتوزوسین بهطور تصادفی در ۵ گروه ۲۷ ایی، کنترل غیردیابتی، کنترل دیابتی، دیایتی تیمارشده با عصاره هیدروالکلی بوقناق با دوز ۳۰۰ و ۵۰۰ میلی گرم بر کیلو گرم، به صورت داخل صفاقی و متفورمین با دوز ۵۰۰ میلی گرم بر کیلو گرم به صورت گاواژ تقسیمبندی شدند. در پایان این پژوهش میزان گلو کز وسنجش سلول های خونی انجام شد. همچنین بافت پانکراس برای بررسی هیستوپاتولوژی به آزمایشگاه ارسال شد.

**نتایج**: استفاده از عصاره هیدروالکلی گیاه بوقناق باعث کاهش معنیدار قند خون شد. همچنین عصارهٔ بوقناق با دوز ۳۰۰ میلی گرم بر کیلو گرم اثر حفاظتی بر تعداد گلبولهای سفید، نوتروفیلها و لنفوسیتها داشت، و باعث کاهش معنیدار تعداد آنها نسبت به گروه شاهد دیابتی شده و همتراز گروه شاهد غیردیابتی قرار گرفته بود. نتایج هیستوپاتولوژی نیز بیانگر آن بود که عصاره بوقناق به طور معنی داری باعث افزایش تعداد جزایر لانگرهانس و سلولهای بتا شد.

**نتیجهگیری نهایی:** این نتایج نشان میدهند که عصاره هیدروالکلی بوقناق ممکن است در درمان دیابت موثر باشد. همچنین با استناد به نتایج بیوشیمیایی و بافتشناسی میتوان نتیجه گرفت که، اثر هیپوگلیسمی عصاره، احتمالا به دلیل بازسازی و ترمیم جزایرلانگرهانس است.

واژههای کلیدی: بوقناق، پانکراس، دیابت، رت، گلوکز

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