Hematoprotective and Nephroprotective Properties of Ethanolic Extract of *Anthemis odontostephana* Boiss in Streptozotocin-induced Diabetic Mice

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Abstract

Background: Certainly, the trend in the treatment of diabetes by ethnomedicinal plants which have fewer side effects than chemical drugs has enhanced. *Anthemis odontostephana* Boiss has been recently known as an antioxidant, antiflammatory, and antimicrobial agent.

Objective: hematoprotective and nephroprotective properties of *Anthemis odontostephana* Boiss ethanolic extract (AOEE) on diabetic mice has been evaluated in the present study.

Methods: In this study, 70 mice were used. Diabetes was experimentally induced by intraperitoneal injection of STZ (60 mg/kg) in 60 mice. After three days, they were divided randomly into 7 groups. Group I and II served as non-diabetic and untreated diabetic controls, respectively. Group III received 30 mg/kg glibenclamide orally. Groups IV, V, VI and VII were given 10, 30, 90 and 270 mg/kg, respectively of AOEE for 20 days orally. At 20th day, the mice were dissected and blood and Kidney samples of them collected for hematological and pathological parameters analysis.

Results: Daily treatment of diabetic mice with several doses of AOEE especially AOEE270 significantly declined FBG levels and improved RBC, platelet and WBC parameters in comparison of untreated diabetic control. Also kidney of the treated diabetic mice with AOEE especially AOEE270 indicated significant improvement of the renal tissue compared to those of the untreated diabetic mice.

Conclusion: According to the obtained results, all doses of AOEE especially AOEE270 can improve hematological parameters and FBG changes and inhibits from kidney damages in streptozotocin-induced diabetic mice. It appears that AOEE can be utilized for treatment of diabetic as an antidiabetic drug.

Keywords: *Anthemis odontostephana* Boiss, Ethanolic extract, Hematoprotective and nephroprotective properties

Introduction

Kidney is one of the organs that are affected in several disease such as diabetic. However, the exact pathogenesis of poor nephropathy in diabetic patients is not clearly understood, the decrease of proximal and distal cell capacity and also the oxidative and inflammatory changes are the main causes [1]. Renal hypertrophy and glomerular hyperfiltration are two known complications occur in the initial stages of diabetes [2]. Some studies had revealed that in early diabetes, glomerular hyperfiltration and renal hypertrophy could be reversed by insulin treatment [3, 4]. Whereas, in chronic diabetes, glomerular hyperfiltration could be ameliorate by severe control of blood glucose level, but renal hypertrophy is irreversible [5]. Although renal hypertrophy and glomerular hyperfiltration play a pivotal role in increasing of diabetic nephropathy, the relationship between them is still unclear [2].

Hematological parameters in diabetes mellitus are often disturbed. These parameters include erythrocyte deformability, erythrocyte aggregation, hematocrit and plasma proteins [6]. This resultant disturbance may be a risk factor for the progression of retinal failure in diabetic retinopathy and renal failure in diabetic nephropathy [6].

The enormous costs of modern medicines such as insulin, pramlintide, acarbose, miglitol, metformin and glibenclamide indicate that other strategies are need for better management of diabetes and its related problems [7]. Some plants have the high content of alkaloids, flavonoids, naphthaquinone, saponins, tannins, and triterpenes, so they can increase the quality and rate of diabetes [7]. The World Health Organization (WHO) suggested that there should be further studies on antidiabetic effects of ethno medicinal plants [8].

In Iranian traditional medicine, herbal medicines have been the basis of treatment and cure for different disease such as diabetic [9-14]. A list of medicinal plants in Iran that are consumed for their antidiabetic property including *Stevia rebaudiana*, *Trigonella foenum graecum*, *Vaccinum arctostaphylos*, *Thea sinensis*, *Silybum morianum*, *Silybum marianum*, *Satureja khuzistanica*, *Securigera Securidaca*, *Opuntia streptacantha*, *Plantago ovate*, *Ipomoea betatas*, *Ocimum sanctum*, *Cuminum cyminum*, *Ginkgo biloba*, *Citrullus colocynthis*, *Allium sativum* and *Citrullus colocynthis* [15-17].

One of the most important herbal medicines, which is widely consumed in Iranian traditional medicine for treatment of diabetic is *Anthemis odontostephana* Boiss (AO) from Compositae family, Anthemis genus [18]. AO is one of the edible plants which have generated a lot of interest throughout human history as a medicinal plant. Several extracts of this plant are traditionally used in treating gastric ulcer, and viral, parasitic, fungal, and bacterial diseases [19]. As far as we know, there is a very little data about antidiabetic effects of *Anthemis odontostephana* Boiss ethanolic extract (AOEE) collected from Kermanshah province, west of Iran. Hence, the aim of the present study was evaluation of effects of AOEE on diabetic in male mice.

Material and methods

Plant collection and ethanolic extract preparation

Flowers of the AO at maturity were collected from around of Kermanshah city during

September 2017. The plant was identified at the herbarium of research center of Faculty of Agriculture, Razi University, Kermanshah, Iran. Flowers were shade dried for one week. Dried aerial part of the plants were ground and about 300 g of the obtained powder was extracted with 900 mL of ethanol for 2 hours at 40 ◦C with continuous shaking. The extract was left for 24 hours at room temperature, then it was filtered through watman paper no. 2. In rotary evaporator (Panchun Scientific Co., Kaohsiung, Taiwan), the extract was concentrated, then lyophilized [20-22].

Experimental design

Seventy male Balb/c mice weighing between 39-40 g were procured from laboratory animal center of Kermanshah University of Medical Sciences. The animals were housed in an air-conditioned room (22±2 °C) with 12 h light/dark cycle and has free access to standard pellet diet and water. All the procedures were performed in accordance with the Institutional Animal Ethics Committee. Diabetes was induced by a single intraperitoneal (IP) administration of Streptozotocin (60 mg/kg.bw) (Sigma, St. Luis, MO, USA). Fasting blood glucose (FBG) level was assessed everyday by Easy Gluco glucometer (Ames, Korea). A blood glucose level upper than 250 mg/dL was considered diabetic [16, 17].

The mice were divided into seven following groups (n=10): (I) Control group (C): which received normal saline orally. (II) Untreated-diabetic group (III). (3) Treated diabetic mice which received 30 mg/kg glibenclamide for 20 days (G). (IV) Treated diabetic mice which received 10 mg/kg of the AOEE for 20 days (AOEE 10). (V) Treated diabetic mice which received 30 mg/kg of the AOEE for 20 days (AOEE 30). (VI) Treated diabetic mice which received 90 mg/kg of the AOEE for 20 days (AOEE 90). (VII) Treated diabetic mice which received 270 mg/kg of the AOEE for 20 days (AOEE 270) [16, 17, 23].

Blood samples were obtained in 0, 4, 7, 10, 13, 16 and 20 days from tail vein in routine tubes to assess the fasting blood glucose level by Easy Gluco glucometer (Ames, Korea). Twenty three days after diabetes induction and at the end of the 20-day treatment, the animals of all groups were euthanized by xylazine (5 mg/kg) and ketamine HCl (40 mg/kg) [16, 17].

Determination of biochemical and hematological parameters

Immediately after euthanizing, blood samples were drawn from animals' heart and inserted in serum (for determination of creatinine and urea) and plasma (for determination of hematological parameters) bottles. Blood samples collected in EDTA bottles were analyzed for hematological parameters using a hematology analyzer (Mindray Auto Hematology Analyzer, BC-5200, USA) following the manufacturer's instructions. The parameters analyzed include red blood cell count (RBC), hemoglobin (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelets and white blood cell count (WBC) and the differentials [23].

Histopathological evaluation

Appropriate tissue samples were collected from the kidney at the end of the 20-day

treatment and were then fixed in 10 % neutral buffered formalin, embedded in paraffin, sectioned at 5 µm thickness, and stained with hematoxylin-eosin staining for light microscopic examination. The sections were qualitatively (morphologic) evaluated [16].

Statistical analysis

Statistical comparison between group means were done through one-way ANOVA followed by Duncan's post-hoc test. $P \leq 0.05$ was considered as significant.

Results

Effect of AOEE on fasting blood glucose level

There was no significant change in blood glucose level of normal control mice throughout the study. The blood glucose levels of untreated diabetic mice increased to approximately 560% ($P \leq 0.05$) of the control mice in a time-dependent manner. However, treatment of STZ- diabetic mice with the AOEE in all doses could significantly ($P \leq 0.05$) reduced the blood glucose levels similar to the glibenclamide - treated at the end of the experiment. Also the difference between all dose of AOEE was significant ($P \leq 0.05$) at 4, 7, 10, 13, 16 and 20 days. The AOEE has most effect on days 20 of the experiment (Figure 1).

Effect of AOEE on creatinine and urea

The effect of the AOEE on the creatinine and urea in the diabetic mice has been shown in figure 2. The levels of these parameters were significantly ($P \leq 0.05$) increased in untreated diabetic mice. Treatment with AOEE in all doses significantly ($P \leq 0.05$) decreased levels of creatinine and urea in comparison of untreated diabetic mice. There was no significant differences in creatinine concentration among AOEE10, AOEE30, AOEE90, AOEE270, glibenclamide and control groups ($P \leq 0.05$).

Effect of AOEE on red blood cell (RBC) parameters

The levels of RBC, MCV, Hb, MCH and MCHC and percent of PCV were significantly ($P \leq 0.05$) decreased in untreated diabetic mice. Treatment with all doses of AOEE significantly ($P \leq 0.05$) increased levels of these parameters in comparison of untreated diabetic mice. There was no significant difference in PCV, MCV, MCH and MCHC levels ($P \leq 0.05$) between AOEE270 and control groups. Also, Hb level significantly ($P \leq 0.05$) increased at all doses of AOEE, similar to glibenclamide and control groups (Figure 3-7).

Effect of AOEE on platelet number

The effect of the AOEE on the platelet number in the diabetic mice has been shown in figure 8. The number of platelet was significantly ($P \leq 0.05$) increased in untreated diabetic mice. Treatment with AOEE in all doses significantly ($P \leq 0.05$) decreased platelet number in comparison of untreated diabetic mice. No significant difference ($P \leq 0.05$) was found between glibenclamide group and several groups of AOEE.

Effect of AOEE on white blood cell (WBC) parameters

The number of WBC and percents of eosinophils and basophils were significantly ($P \leq 0.05$) increased in untreated diabetic mice.

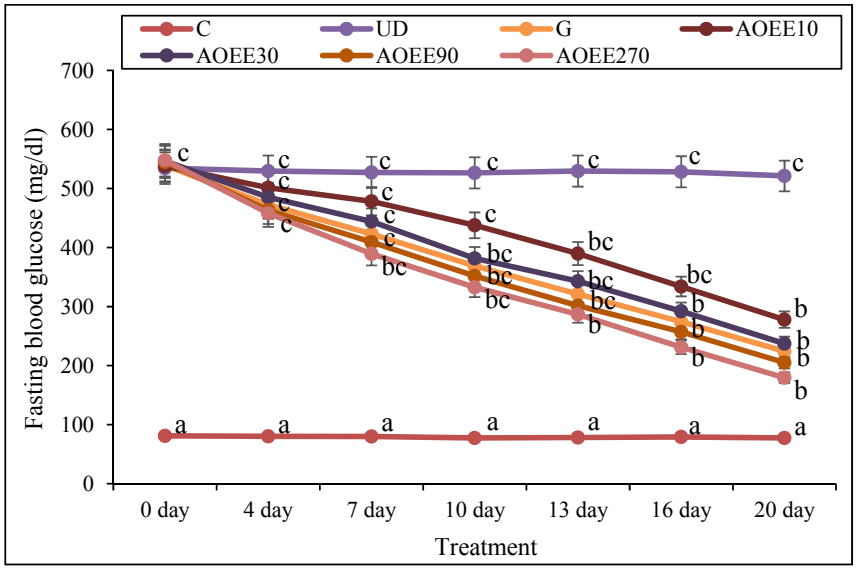

Figure 1- Blood glucose levels on different days in all of the experimental groups.

C (control), UD (untreated diabetic), G (glibenclamide treated), AOEE10 (treated diabetics with 10 mg/kg of *Anthemis odontostephana* Boiss ethanolic extract), AOEE30 (treated diabetics with 30 mg/kg of *Anthemis odontostephana* Boiss ethanolic extract), AOEE90 (treated diabetics with 90 mg/kg of *Anthemis odontostephana* Boiss ethanolic extract) and AOEE270 (treated diabetics with 270 mg/kg of *Anthemis odontostephana* Boiss ethanolic extract).
Non-identical letters indicate a significant difference between the groups (P ≤ 0.05).

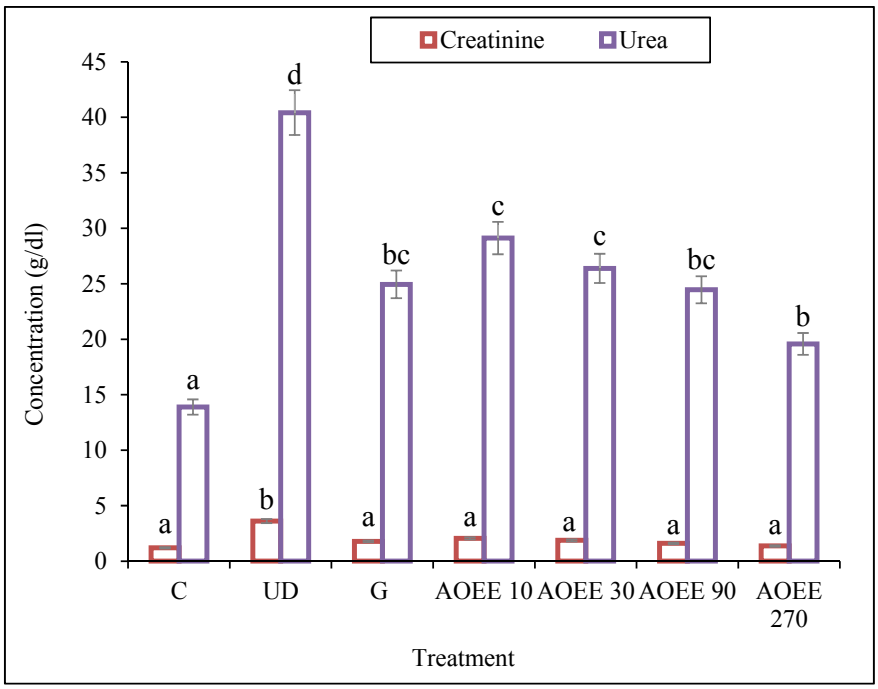

Figure 2- Creatinine and urea levels in all of the experimental groups.

C (control), UD (untreated diabetic), G (glibenclamide treated), AOEE10 (treated diabetics with 10 mg/kg of *Anthemis odontostephana* Boiss ethanolic extract), AOEE30 (treated diabetics with 30 mg/kg of *Anthemis odontostephana* Boiss ethanolic extract), AOEE90 (treated diabetics with 90 mg/kg of *Anthemis odontostephana* Boiss ethanolic extract) and AOEE270 (treated diabetics with 270 mg/kg of *Anthemis odontostephana* Boiss ethanolic extract).
Non-identical letters indicate a significant difference between the groups (P ≤ 0.05).

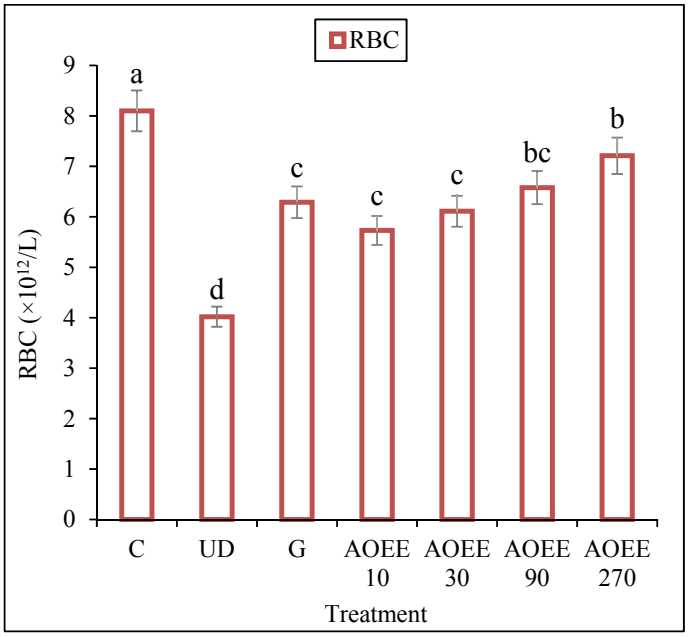

Figure 3- RBC numbers in all of the experimental groups.

C (control), UD (untreated diabetic), G (glibenclamide treated), AOEE10 (treated diabetics with 10 mg/kg of *Anthemis odontostephana* Boiss ethanolic extract), AOEE30 (treated diabetics with 30 mg/kg of *Anthemis odontostephana* Boiss ethanolic extract), AOEE90 (treated diabetics with 90 mg/kg of *Anthemis odontostephana* Boiss ethanolic extract), AOEE270 (treated diabetics with 270 mg/kg of *Anthemis odontostephana* Boiss ethanolic extract) and RBC (Red blood cell). Non-identical letters indicate a significant difference between the groups (P ≤ 0.05).

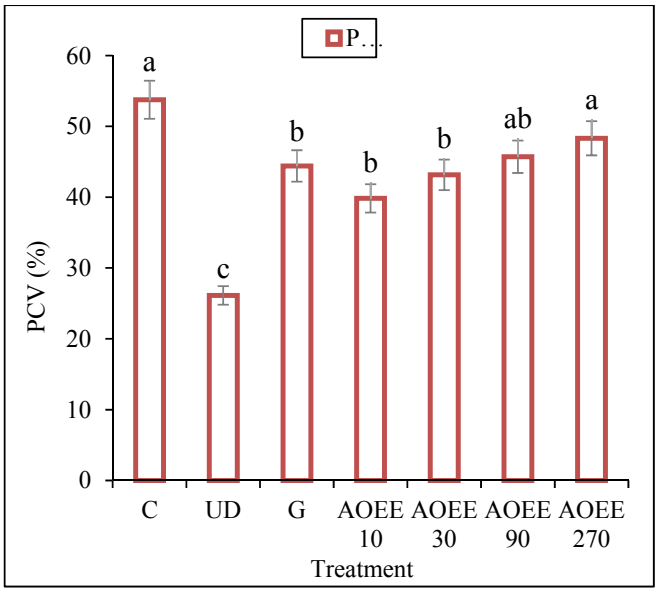

Figure 4- PCV percent in all of the experimental groups.

C (control), UD (untreated diabetic), G (glibenclamide treated), AOEE10 (treated diabetics with 10 mg/kg of *Anthemis odontostephana* Boiss ethanolic extract), AOEE30 (treated diabetics with 30 mg/kg of *Anthemis odontostephana* Boiss ethanolic extract), AOEE90 (treated diabetics with 90 mg/kg of *Anthemis odontostephana* Boiss ethanolic extract), AOEE270 (treated diabetics with 270 mg/kg of *Anthemis odontostephana* Boiss ethanolic extract) and PCV (Packed cell volume). Non-identical letters indicate a significant difference between the groups (P ≤ 0.05).

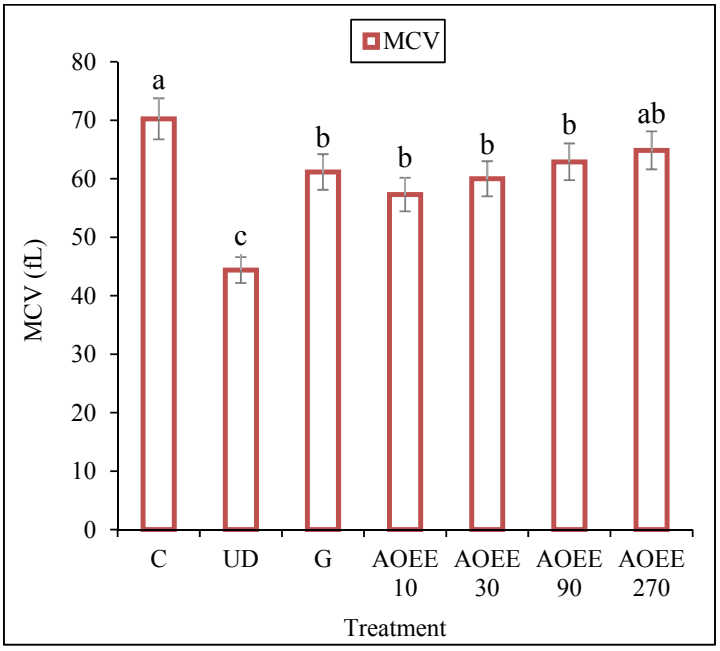

Figure 5- MCV in all of the experimental groups.

C (control), UD (untreated diabetic), G (glibenclamide treated), AOEE10 (treated diabetics with 10 mg/kg of *Anthemis odontostephana* Boiss ethanolic extract), AOEE30 (treated diabetics with 30 mg/kg of *Anthemis odontostephana* Boiss ethanolic extract), AOEE90 (treated diabetics with 90 mg/kg of *Anthemis odontostephana* Boiss ethanolic extract), AOEE270 (treated diabetics with 270 mg/kg of *Anthemis odontostephana* Boiss ethanolic extract) and MCV (Mean corpuscular volume). Non-identical letters indicate a significant difference between the groups (P ≤ 0.05).

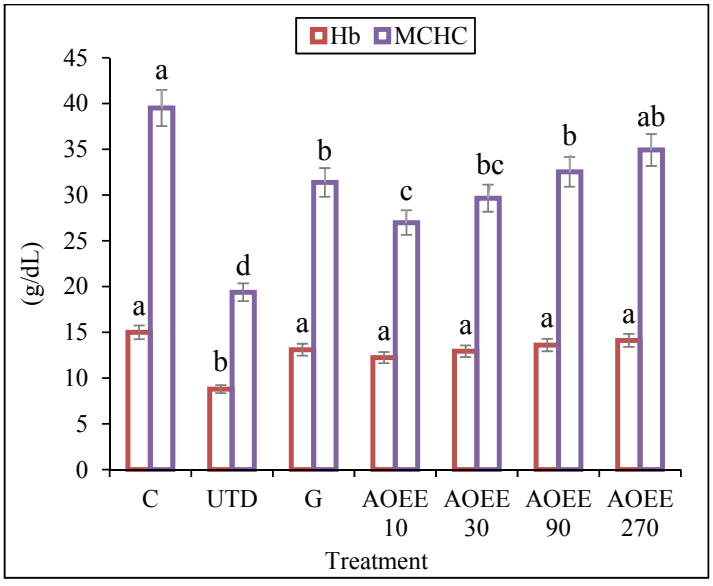

Figure 6- The levels of Hb and MCHC (g/dL) in all of the experimental groups.

C (control), UD (untreated diabetic), G (glibenclamide treated), AOEE10 (treated diabetics with 10 mg/kg of *Anthemis odontostephana* Boiss ethanolic extract), AOEE30 (treated diabetics with 30 mg/kg of *Anthemis odontostephana* Boiss ethanolic extract), AOEE90 (treated diabetics with 90 mg/kg of *Anthemis odontostephana* Boiss ethanolic extract), AOEE270 (treated diabetics with 270 mg/kg of *Anthemis odontostephana* Boiss ethanolic extract), Hb (Hemoglobin) and MCHC (Mean corpuscular hemoglobin concentration).

Non-identical letters indicate a significant difference between the groups (P ≤ 0.05).

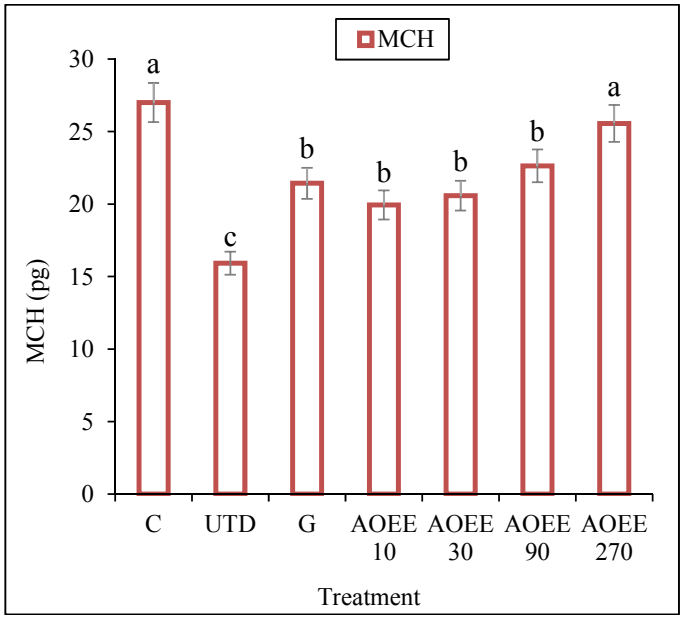

Figure 7- The levels of MCH (pg) in all of the experimental groups.
C (control), UD (untreated diabetic), G (glibenclamide treated), AOEE10 (treated diabetics with 10 mg/kg of *Anthemis odontostephana* Boiss ethanolic extract), AOEE30 (treated diabetics with 30 mg/kg of *Anthemis odontostephana* Boiss ethanolic extract), AOEE90 (treated diabetics with 90 mg/kg of *Anthemis odontostephana* Boiss ethanolic extract), AOEE270 (treated diabetics with 270 mg/kg of *Anthemis odontostephana* Boiss ethanolic extract) and MCH (Mean corpuscular hemoglobin).
Non-identical letters indicate a significant difference between the groups (P ≤ 0.05).

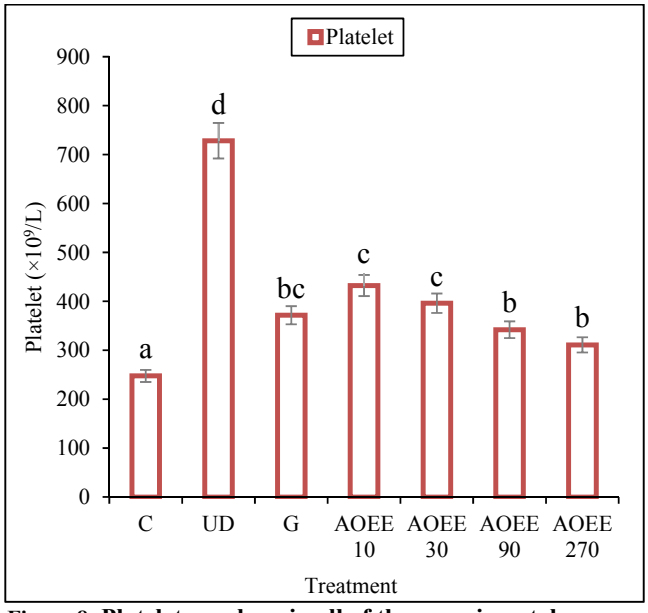

Figure 8- Platelet numbers in all of the experimental groups.
C (control), UD (untreated diabetic), G (glibenclamide treated), AOEE10 (treated diabetics with 10 mg/kg of *Anthemis odontostephana* Boiss ethanolic extract), AOEE30 (treated diabetics with 30 mg/kg of *Anthemis odontostephana* Boiss ethanolic extract), AOEE90 (treated diabetics with 90 mg/kg of *Anthemis odontostephana* Boiss ethanolic extract) and AOEE270 (treated diabetics with 270 mg/kg of *Anthemis odontostephana* Boiss ethanolic extract).
Non-identical letters indicate a significant difference between the groups (P ≤ 0.05).

Also, lymphocytes and monocytes percents significantly ($P \leq 0.05$) reduced in untreated diabetic mice. Treatment with AOEE in all doses significantly ($P \leq 0.05$) decreased WBC number and eosinophils and basophils percents and increased lymphocytes and monocytes percents in comparison of untreated diabetic mice. There was no significant difference in WBC number ($P \leq 0.05$) between AOEE270 and control groups. Also no significant difference ($P \leq 0.05$) were found at percents of lymphocytes, monocytes, eosinophils and basophils among AOEE10, AOEE30, AOEE90, AOEE270, glibenclamide and control groups. Changes wasn't significant ($P \leq 0.05$) in the neutrophils of all of the experimental groups (Figure 9, 10).

Histopathological findings

The kidneys of the normal control mice had normal structure and the proximal and the distal convoluted tubules, renal corpuscles, glomerulus and glomerular capsule had normal architecture. Microscopic examination of the kidneys of the treated diabetic mice with AOEE in all doses don't show tubular necrosis with necrotic changes in the glomerular epithelium and diffused interstitial and glomerular hemorrhages.

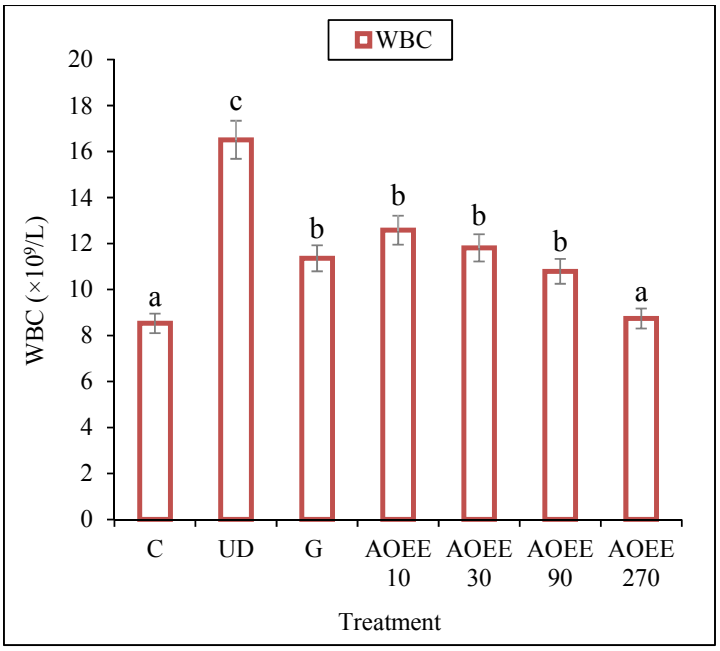

Figure 9- WBC number in all of the experimental groups.

C (control), UD (untreated diabetic), G (glibenclamide treated), AOEE10 (treated diabetics with 10 mg/kg of *Anthemis odontostephana* Boiss ethanolic extract), AOEE30 (treated diabetics with 30 mg/kg of *Anthemis odontostephana* Boiss ethanolic extract), AOEE90 (treated diabetics with 90 mg/kg of *Anthemis odontostephana* Boiss ethanolic extract), AOEE270 (treated diabetics with 270 mg/kg of *Anthemis odontostephana* Boiss ethanolic extract) and WBC (White blood cell). Non-identical letters indicate a significant difference between the groups ($P \leq 0.05$).

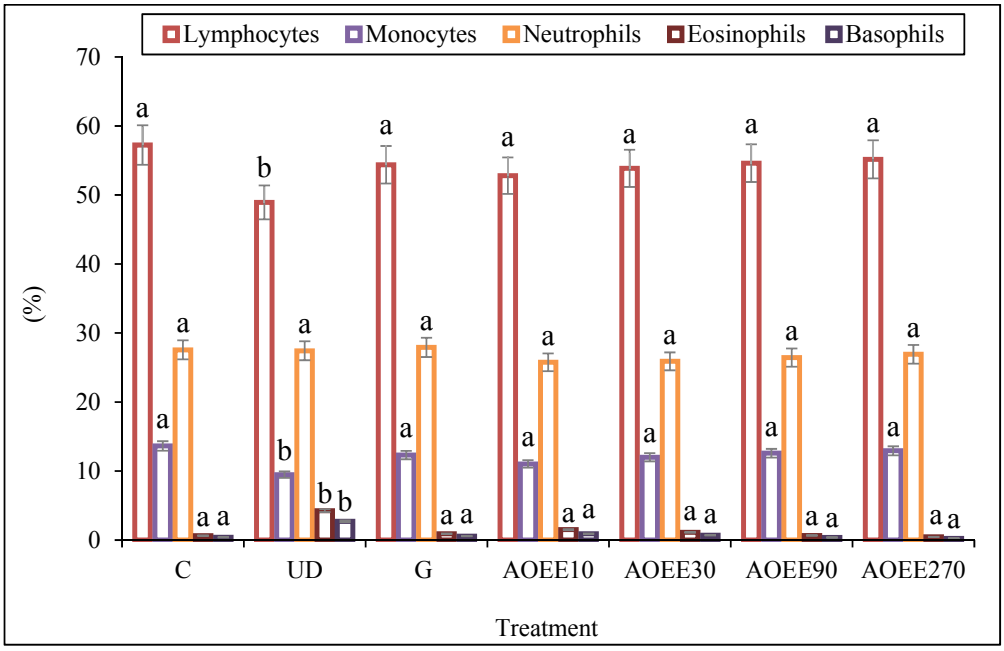

Figure 10- Lymphocyte, monocyte, neutrophil, eosinophil and basophil percent in all of the experimental groups. C (control), UD (untreated diabetic), G (glibenclamide treated), AOEE10 (treated diabetics with 10 mg/kg of *Anthemis odontostephana* Boiss ethanolic extract), AOEE30 (treated diabetics with 30 mg/kg of *Anthemis odontostephana* Boiss ethanolic extract), AOEE90 (treated diabetics with 90 mg/kg of *Anthemis odontostephana* Boiss ethanolic extract) and AOEE270 (treated diabetics with 270 mg/kg of *Anthemis odontostephana* Boiss ethanolic extract). Non-identical letters indicate a significant difference between the groups (P ≤ 0.05).

Discussion

Diabetes mellitus is a syndrome characterized by disordered metabolism and abnormally high blood sugar resulting from either low insulin level or insulin resistance at many body cells. This disorder affects more than 285 million people worldwide. This number is expected to grow to 438 million by 2030, corresponding to 7.8% of the adult population. Diabetes is the most important reason of renal failure and legal blindness and one of the major risk factors of cardiovascular diseases [24]. Diabetes patients are five times more likely than nondiabetes patients to develop severe chronic leg ischemia, leading to foot ulceration and often, amputation [25]. Diabetes is now recognized as one of the major killer disease and a leading cause of death. Oral hypoglycemic chemical drugs have been commonly used in the management of the disease, especially type 2 diabetes, although they have serious side effects. Consequently, attention has been focused on the use of plants and herbal remedies believed to be safer and devoid of serious side effects as alternatives in the treatment of diabetes.

Effect of AOEE on fasting blood glucose

The significant low blood sugar level in diabetes mice by administration of the AO flower confirms that it could serve as a more accessible and affordable alternative to orthodox antidiabetics. In this study, AOEE in all doses apparently decreased serum glucose levels in diabetic's mice than untreated diabetic mice seems to suggest that AOEE has the

capacity to reduce the risk of other complications associated with people suffering with diabetes and hence use as a hypoglycemic agent by the Kermanshah people in west of Iran.

Effect of AOEE on renal histopathology

The histopathological findings showed that not only the AOEE in all doses used in the present study was safe and did not have adverse toxic effects on the kidneys but also reduces the effects of diabetes mellitus on the kidney by reducing blood glucose levels. Also AOEE can reduced significantly creatinine and urea levels in comparison to untreated diabetic group which indicates recovery of renal function.

Effect of AOEE on hematology parameters

Alteration in the various hematological parameters and the immune system during the course of diabetes have been reported [26]. Anemia has been noted to be a common pathophysiological feature and a complication of diabetes mellitus [27]. Diabetes associated anemia is reported to be due to the increased non-enzymatic glycosylation of RBC membrane proteins which correlates with hyperglycemia [28]. Oxidation of these membrane proteins in the presence of chronic hyperglycemia in uncontrolled diabetes mellitus increases the production of lipid peroxides that leads to hemolysis of RBC [29]. One of the pathological consequences of this membrane lipid peroxidation is reduced erythrocyte survival [30]. Although the RBC membrane lipid peroxide level in diabetic mice was not measured in this study, other RBC parameters such as Hb, PCV, MCV, MCH, and MCHC were measured so as to investigate the effect of AOEE on the anemic status of STZ-induced diabetic mice. The decrease in the RBC and its indices following treatment with diabetogenic agent in experimental diabetes is an indication of reduced and abnormal erythropoiesis. This observation is consistent with earlier report [31] but differ from the report of some others [32]. Administration of AOEE to STZ-induced diabetic mice appreciably ameliorate the levels of RBC and its indices ($P \leq 0.05$). This offers that some phytoconstituents present in the extract can stimulate the formation or secretion of erythropoietin which stimulates the stem cells in the bone marrow to produce RBC which is evidenced by the improved levels of MCH and MCHC [33].

WBC serves as a scavenger that removes foreign substances. Changes in WBC have been associated with insulin resistance and cardiovascular complications [34]. Leukocytosis is reported to be associated with insulin resistance, Type 2 diabetes mellitus, coronary artery disease, stroke and diabetes induced macro and microangiopathy [35]. Leucocytes are reported to be activated by oxidative stress, angiotensin II and pro-inflammatory cytokines [35]. The result of this study showed a significant ($P \leq 0.05$) increase in WBC of diabetic control mice which became reduced significantly ($P \leq 0.05$) on AOEE treatment. This may have been as a result of the ability of the extract to restore insulin sensitivity, reduce oxidative stress within the blood cells. This finding is in agreement with earlier reports [33, 35].

A study that platelet count was significantly higher among diabetics compared to non-diabetics and a positive correlation between platelet count and poor glycemic control exists

[36]. Raised platelet values are commonly seen in inflammatory and infectious diseases [37] and are considered as an acute phase reaction to infection or inflammation as is the case with STZ-induced diabetogenesis caused by free radicals [38]. In the present study, thrombocytosis was evident in the diabetic untreated control mice. However, treatment with AOEE in all doses significantly ($P \leq 0.05$) reduced the platelet count dose dependently. This observation may suggest the ability of the AOEE in all doses to achieve glycemic control and protect against vascular events.

Conclusion

In conclusion, the present study indicate that hematological hyperglycemia and changes could be improved by AOEE in all doses. So AOEE can be used as antidiabetic drug. It is suggest that in this connection supplementary study should be done on human.

Conflict of Interest

The authors have no conflict of interest to declare.

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