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Original article

Evaluation of Nephroprotective and Antidiabetic Effects of *Gundelia tournefortii* Aqueous Extract on Diabetic Nephropathy in Male Mice

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Abstract

Background and objectives: Due to the rapid growth of global interest in use of ethno medicinal plants, their effects and safety assessment have become substantial. *Gundelia tournefortii* has been used as antioxidant, anti-inflammatory, antipyretic, anti-fungal, and antibacterial agent. In the present study, nephrprotective and antidiabetic properties of *Gundelia tournefortii* aqueous extract (GTAE) on diabetic mice has been assessed. **Methods:** Seventy mice were used and diabetes was induced by administration of 150 mg/kg of alloxan monohydrate intraperitoneally in 60 mature male mice and they were randomly divided into 6 groups. Also one group (10 mice) was considered as the negative control group which received normal saline. The treatment groups received glibenclamide 10 mg/kg (G10) and 5, 10, 20 and 40 mg/kg of GTAE through gavage for 20 days. Also, one group was considered as the non-diabetic control. On the last day, levels of blood glucose, urea and creatinine were measured in serum. After tissue processing, 5 µm sections of the kidneys were prepared and were stained by hematoxylin and eosin and used for stereological analysis. **Results:** GTAE at all doses and G10 significantly ($p \leq 0.05$) reduced the raised levels of blood glucose, creatinine and urea as compared to the untreated diabetic mice. Multiple doses of GTAE and G10 significantly ($p \leq 0.05$) decreased the volume and length of renal structures, compared to the diabetic untreated group. **Conclusion:** According to the obtained results, GTAE groups can regulate the levels of biochemical parameters and inhibit kidney damages in alloxan induced diabetic mice. It appears that GTAE can be suggested for treatment of diabetes as an anti-diabetic supplement or drug.

Keywords: alloxan monohydrate; aqueous extract; diabetes; *Gundelia tournefortii*; nephropathy

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Introduction

Diabetes mellitus is a syndrome characterized by disordered metabolism and abnormally high blood sugar resulting from either low insulin level or insulin resistance at body cells. Also this disease is the most important reason for renal failure and legal blindness and one of the major risk factors of cardiovascular diseases. Increase in sedentary lifestyle, consumption of energy-rich diets, and obesity are some of the factors resulting the rise in the number of diabetics [1]. While about 2.5 to 7 % of the world's population has been diagnosed with diabetes mellitus, it is still expected to increase in the future. Diabetes patients are five times more likely to develop

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severe chronic leg ischemia leading to foot ulceration and often amputation than non-diabetic patients [2].

Kidney is one of the organs that are affected in diabetes. 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 [3].

Renal hypertrophy and glomerular hyperfiltration are two known complications which occur in the initial stages of diabetes mellitus [4]. Some studies have revealed that in early diabetes, glomerular hyperfiltration and renal hypertrophy could be reversed by insulin treatment [5,6]; whereas, in chronic diabetes, glomerular hyperfiltration could be ameliorated by severe control of blood glucose level, but renal hypertrophy is irreversible [7].

The enormous costs of modern medicines indicate that other strategies are needed for better management of diabetes and its related problems [8]. Medicinal plants are amply used in folk medicine in different parts of the world and are considered as pivotal in human health care [9-12]. Some plants have high content of alkaloids, flavonoids, naphthaquinone, saponins, tannins, and triterpenes and can decrease the rate of diabetes [13,14]. The World Health Organization (WHO) has suggested that there should be further studies on anti-diabetic effects of ethno medicinal plants [15].

One of the most important herbal medicines, which is widely consumed in Iranian traditional medicine for treatment of diabetes is *Gundelia tournefortii* (GT) from Asteraceae family [16]. It is a thistle plant that grows in some countries including Iran, Azerbaijan, Armenia, Iraq and Anatolia [16]. *Gundelia tournefortii* 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 have been traditionally used in treating parasitic, viral, fungal, and bacterial diseases [17,18]. As far as we know, there is very little data about nephroprotective and antidiabetic effects of GT aqueous extract (GTAE) collected from Kermanshah province, west of Iran. Hence, the aim of the present study was assessing the effects of GTAE aqueous extract on diabetic mice.

Material and Methods

Ethical considerations

The study was approved by Local Research Ethics Committee of Razi University, Kermanshah, Iran with the ethical code of 397-3-002.

Plant extraction

GT at maturity was collected from around Kermanshah, Kermanshah province, Iran during March 2017. Aerial parts of the plant were shade dried for one week. Dried aerial parts were then ground and about 450 g of the obtained powder was extracted with 3600 mL of distilled water for 5 h at 40 °C with continuous shaking. The extract was left for 24 h at room temperature, then it was filtered through watman paper no. 2 and freeze dried

Animals

Seventy male Balb/c mice weighing 38-40 g were procured from laboratory animal center of Kermanshah University of Medical Sciences, Kermanshah, Iran. The animals were housed in an air-conditioned room (22±2 °C) with 12 h light/dark cycle and had free access to standard pellet diet and water.

Experimental design

Diabetes was induced by a single intraperitoneal (IP) administration of alloxan monohydrate (150 mg/kg.bw). Fasting blood glucose (FBG) level was assessed everyday by Easy Gluco glucometer (Ames, Korea). A blood glucose level higher than 250 mg/dL was considered diabetic.

Distilled water was used for dissolve the aqueous extract of plant. The mice were divided into seven following groups (n=10): (1) Control (Non diabetic) group (C) which received 200 µL normal saline orally; (2) untreated-diabetic group (UD) which received 200 µl normal saline orally; (3) treated diabetic mice which received 10 mg/kg glibenclamide for 20 days (G10); (4) treated diabetic mice which received 5 mg/kg of the aqueous extract of GTAE for 20 days (GTAE5); (5) treated diabetic mice which received 10 mg/kg of the aqueous extract of GTAE for 20 days (GTAE10); (6) treated diabetic mice which received 20 mg/kg of the aqueous extract of GTAE for 20 days (GTAE20); (7) treated diabetic mice which received 40 mg/kg of the aqueous extract of GTAE for 20 days (GTAE40).

Blood sampling and determination of biochemical parameters

Blood samples were obtained in 0, 4, 7, 10, 13,

16, 20 days of the experiment from the tail vein in routine tubes to assess the blood glucose level by Easy Gluco glucometer (Ames, Korea). At the end of the 20th day of treatment, the animals of all groups were euthanized by xylazine (5 mg/kg) and ketamine HCl (40 mg/kg). For separation of serum, the samples were centrifuged at 10,000 rpm for 15 min. Levels of creatinine and urea were evaluated in the serum [19,20].

Stereological study

Volume density

After dissection, the right kidney was removed and then weighed. The kidney was fixed in 10% neutral buffered formalin solution for one week. Immersion method was then used to determine the primary volume of the kidney. For estimation of final volume of the organs, the amount of tissue shrinkage must be specified [21,22]. Isotropic uniform random (IUR) sections must be obtained for estimating tissue shrinkage and tubular length [22,23]. Theses sections were achieved using orientator method. Totally, 7-10 slabs were obtained from each kidney through orientator method. A circular piece was sampled from a kidney slab and the area of this piece was calculated. The slabs and circular piece were processed, sectioned (5 µm thicknesses) and stained by hematoxylin and eosin method. The area of the circular piece was calculated again and tissue shrinkage was estimated as [24]:

$$\text{Volumeshrinkage}=1-(AA/AB)^{1.5}$$

Where AA and AB are the area of the circular piece after and before tissue processing. The total volume of the organ was then estimated using:

$$V_{final}=V_{primary}\times(1-\text{Volume shrinkage})$$

Tissue sections were examined using a videomicroscopy system composed of a microscope (Olympus CX2, Japan) connected to a video camera (Dinocapture ver.5, dino-lit.com 30.5 mm), a P4 PC and the stereological parameters were estimated. The fractional volume of the renal structures was estimated using a point probe (with an area of 100 cm^2 and containing 25 points) and following formula:

$$V_v=P_{structure}/P_{reference}$$

$P_{structure}$= sum of points hitting to the interested structures

$P_{reference}$ = sum of points hitting to the reference space.

Length density

The length density of the tubules and vessels was estimated using an unbiased counting probe (740×740 µm). The tubule structures were considered if they were lying completely or partly inside the counting probe and did not touch the down and left lines. Otherwise, they were not considered. The length density was estimated as:

$$L_v=2\times\sum Q/a(\text{frame})\times\sum\text{frame}$$

$\sum Q$= sum of the tubules counted, a (frame) = probe area, 547600 μm^2, $\sum$frame = total number of the counted frames.

Statistical analysis

All data were expressed as mean and standard deviation. 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 and Discussion

The effect of the GTAE on the fasting blood glucose in the diabetic mice has been demonstrated in figure 1. There was no notable change in blood glucose level of normal control mice throughout the study. The blood glucose levels of untreated diabetic mice increased to approximately 350% ($p\leq 0.05$) of the control mice in a time-dependent manner. However, treatment of alloxan monohydrate-diabetic mice with the GTAE at all doses could significantly ($p\leq 0.05$) decreased the blood glucose levels similar to the G10- treated at the end of the experiment. Also the difference between all doses of GTAE was significant at 4 and 20 days. The GTAE showed the most considerable effect on day 20 of the experiment.

The levels of these parameters creatinine and urea significantly ($p\leq 0.05$) increased in untreated diabetic mice. Treatment with GTAE in all doses significantly ($p\leq 0.05$) decreased levels of creatinine and urea in comparison with untreated diabetic mice. Administration of GTAE40 could significantly ($p\leq 0.05$) decrease creatinine and urea compared to the G10 and other doses of GTAE. The effect of the GTAE on the creatinine and urea in the diabetic mice has been shown in figure 2 in detail. The data of the mean absolute

volume of kidney and its subcomponents in treated and untreated diabetic groups have been presented in figures 3-5. The results showed that the kidney volume increased 48% ($p \leq 0.05$) in the untreated diabetic mice when compared to the control ones. Cortical and medullary volumes increased 54 and 37% ($p \leq 0.05$), respectively in this group ($p \geq 0.05$) in comparison with the control group.

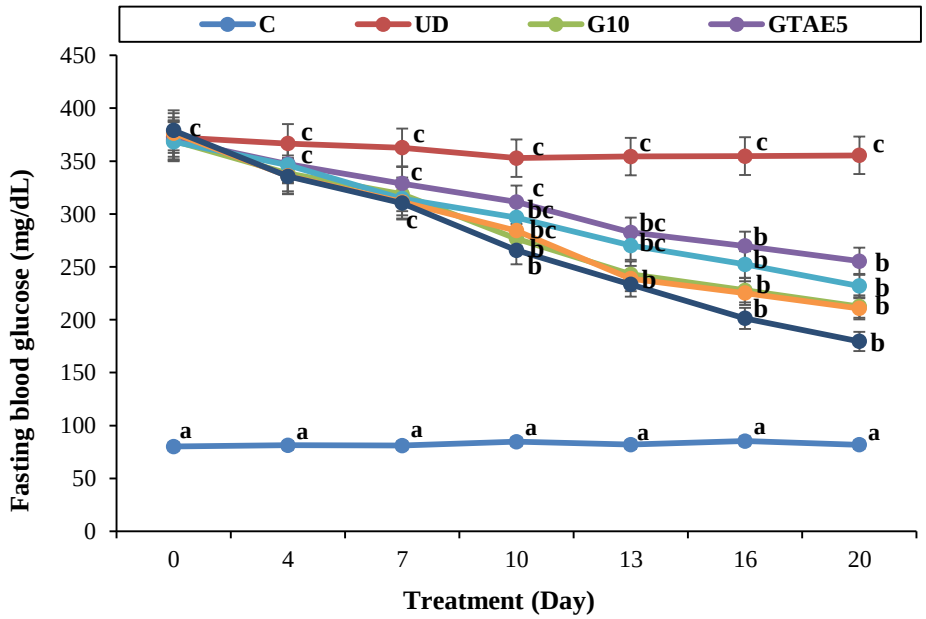

Figure 1. Blood glucose levels in different days in all of the experimental groups; C: control; UD: untreated diabetic; G10: treated diabetics with 10 mg/kg glibenclamide; GTAE 5, 10, 20 40: treated diabetics with 5, 10, 20, 40 mg/kg of *Gundelia tournefortii* aqueous extract, respectively; different letters show significant differences between the groups ($p \leq 0.05$); [a] is the best value (a>b>c)

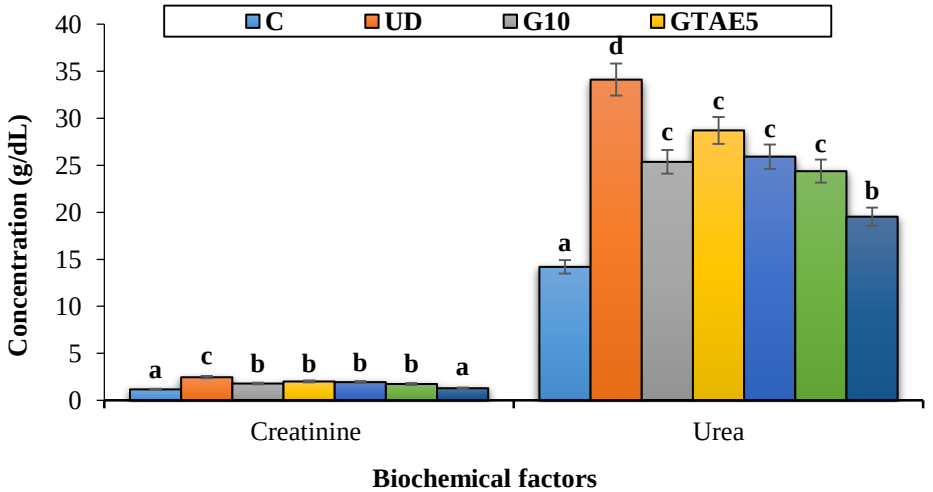

Figure 2. Creatinine and urea levels in all of the experimental groups; C: control; U : untreated diabetic; G10: treated diabetics with 10 mg/kg glibenclamide; GTAE 5, 10, 20 40: treated diabetics with 5, 10, 20, 40 mg/kg of *Gundelia tournefortii* aqueous extract, respectively; different letters show significant differences between the groups ($p \leq 0.05$); [a] is the best value (a>b>c)

Administration of GTAE40 could significantly ($p \leq 0.05$) improve the kidney and cortical volume compared to G10 and other doses of GTAE. Furthermore, the difference between G10 and GTAE5, GTAE10 and GTAE20 doses treated groups wasn't significant ($p \leq 0.05$). No significant difference was observed in medullary volume between G10 and all doses of GTAE ($p \leq 0.05$) (figure 3).

The volume of proximal convoluted tubule, distal

convoluted tubule, collecting duct, loop of Henle, vessels and interstitial tissue increased significantly ($p \leq 0.05$) in untreated diabetic mice compared to the control ones (figures 4,5). Administration of GTAE at all doses to the diabetic mice could significantly ($p \leq 0.05$) decrease the volume of the above structures in comparison with the untreated diabetic group. Administration of GTAE40 significantly ($p \leq 0.05$) decreased the volume of proximal convoluted tubule, collecting duct and interstitial tissue compared to G10 and other doses of GTAE. No significant difference was oberved in distal convoluted tubule, loop of Henle and vessels volume between G10 and all doses of GTAE ($p \leq 0.05$) (figures 4,5).

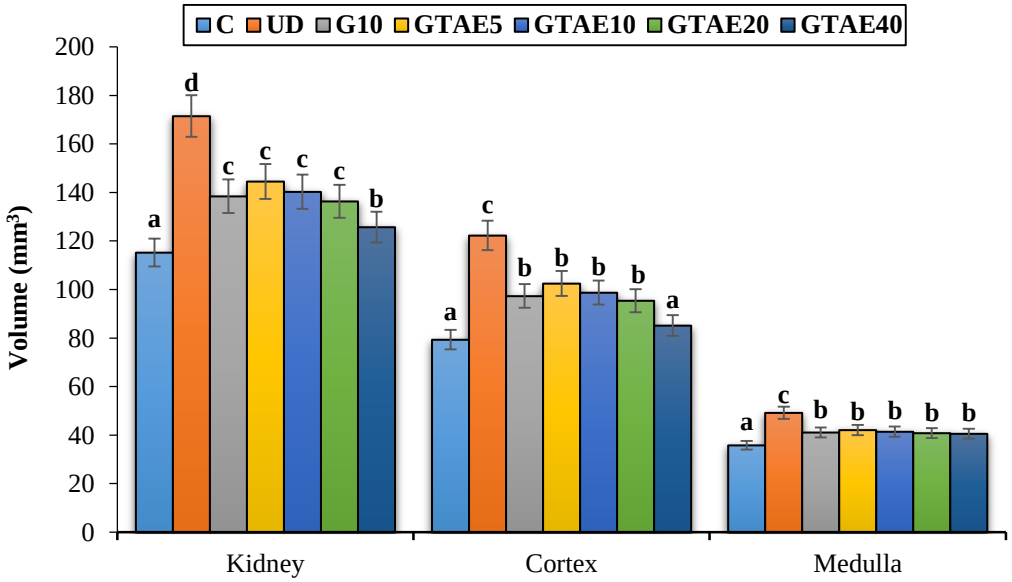

Figure 3. Absolute volume of the kidney (mm^3), and absolute volume (mm^3) of cortex and medulla in all of the experimental groups; C: control; UD: untreated diabetic; G10: treated diabetics with 10 mg/kg glibenclamide; GTAE 5, 10, 20 40: treated diabetics with 5, 10, 20, 40 mg/kg of *Gundelia tournefortii* aqueous extract, respectively; different letters show significant differences between the groups ($p \leq 0.05$); [a] is the best value (a>b>c)

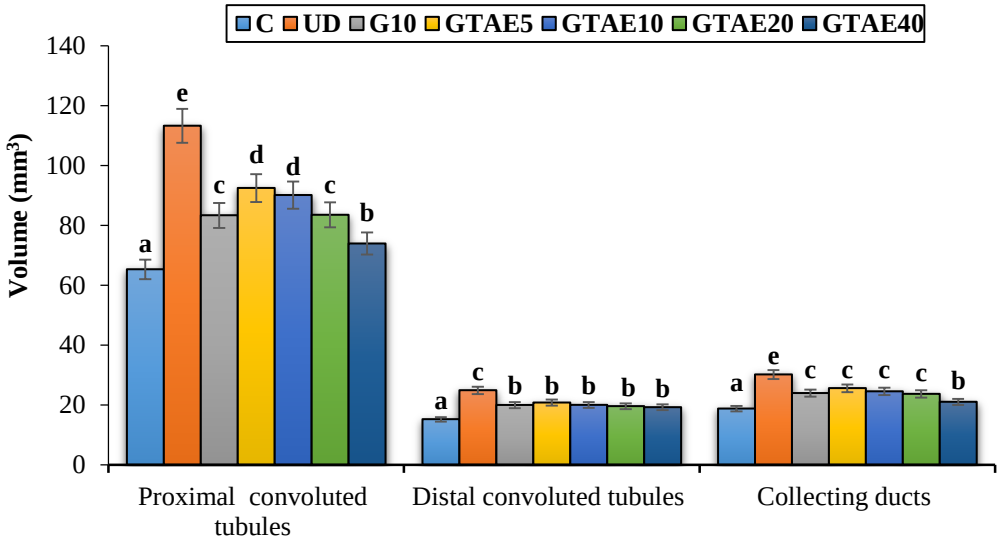

Figure 4. Absolute volume (mm^3) of proximal and distal convoluted tubules, collecting ducts in all of the experimental groups; C: control; UD: untreated diabetic; G10: treated diabetics with 10 mg/kg glibenclamide; GTAE 5, 10, 20 40: treated diabetics with 5, 10, 20, 40 mg/kg of *Gundelia tournefortii* aqueous extract, respectively; different letters show significant differences between the groups ($p \leq 0.05$); [a] is the best value (a>b>c)

The length of the proximal convoluted tubule, distal convoluted tubule, collecting duct, loop of Henle and vessels significantly ($p \leq 0.05$) increased in untreated diabetic mice compared to the control ones (figure 6). GTAE40 significantly ($p \leq 0.05$) decreased the length of the proximal convoluted tubule, distal convoluted tubule, collecting duct, loop of Henle and vessels compared to other treated groups ($p \leq 0.05$). No significant difference was observed in collecting duct volume between GTAE20 and GTAE40 ($p \leq 0.05$) (figure 6). The effect of the GTAE on the length of the renal cell structures in the diabetic mice has been shown in figure 6 in detail.

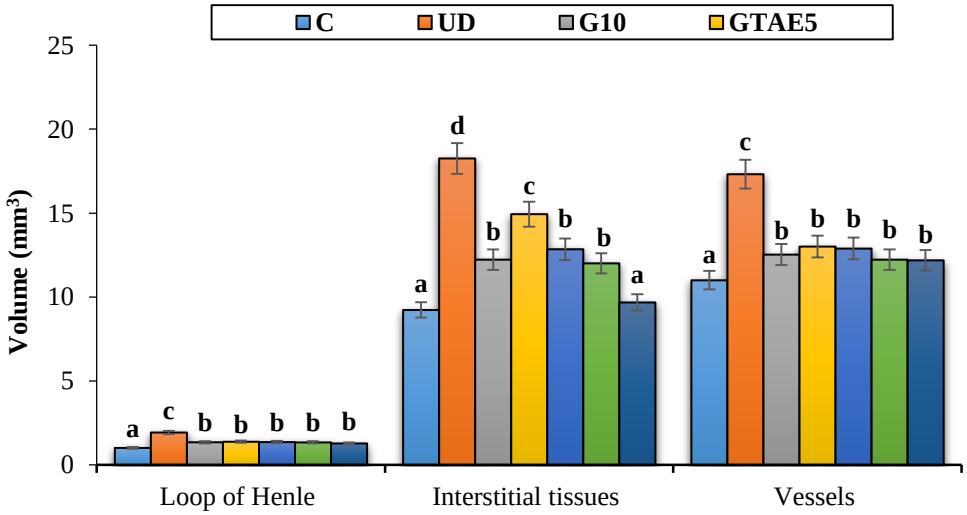

Figure 5. Absolute volume (mm^3) of loop of Henle, interstitial tissues, vessels in all of the experimental groups; C: control; UD: untreated diabetic; G10: treated diabetics with 10 mg/kg glibenclamide; GTAE 5, 10, 20 40: treated diabetics with 5, 10, 20, 40 mg/kg of *Gundelia tournefortii* aqueous extract, respectively; different letters show significant differences between the groups ($p \leq 0.05$); [a] is the best value (a>b>c)

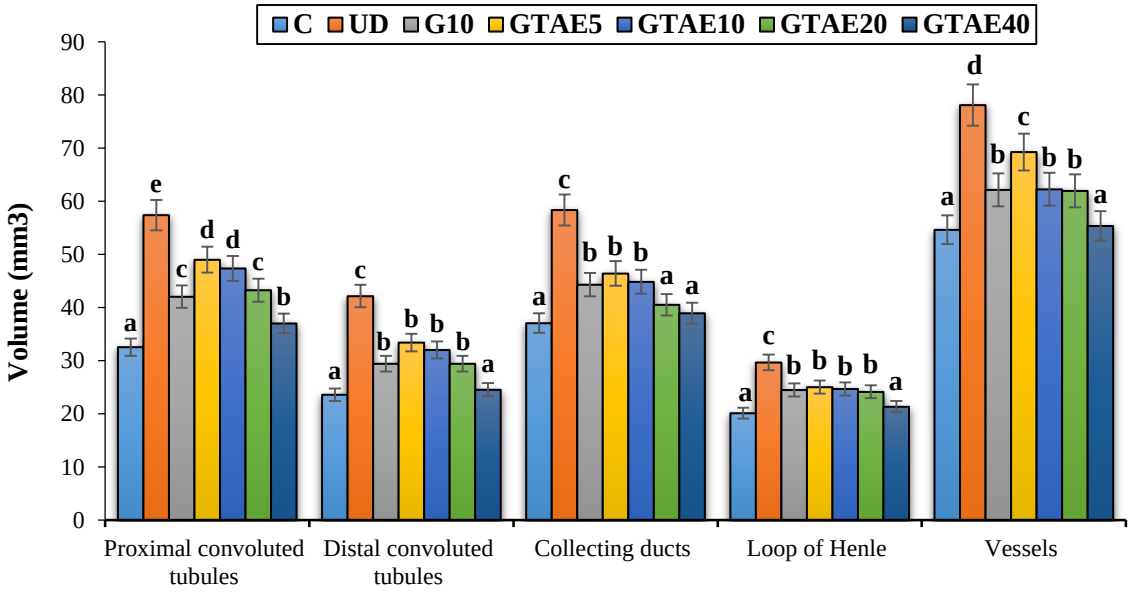

Figure 6. Absolute length (m) of the proximal and distal convoluted tubules, collecting ducts, loop of Henle and vessels in the control and experimental groups treated with GTAE. C: control; UD: untreated diabetic; G10: treated diabetics with 10 mg/kg glibenclamide; GTAE 5, 10, 20 40: treated diabetics with 5, 10, 20, 40 mg/kg of *Gundelia tournefortii* aqueous extract, respectively; different letters show significant differences between the groups ($p \leq 0.05$); [a] is the best value (a>b>c)

Iran has a rich flora that is widely distributed throughout the country, particularly in the west of the country [25-28]. Hereof a number of reports concerning the antioxidant, anti-inflammatory and antimicrobial effects of several plants have appeared in the literature, but the vast majority has yet to be explored [29-30]. In Iranian traditional medicine, herbal medicines have been the basis of treatment and cure for diabetic diseases [19,20,31]. A list of medicinal plants in Iran that have been consumed for their antidiabetic property includes *Silybum marianum*, *Allium sativum*, *Satureja khuzistanica*, *Opuntia streptacantha*, *Plantago ovate*, *Securigera securidaca*, *Trigonella foenum graecum*, *Vaccinum arctostaphylos*, *Thea sinensis*, *Ginkgo biloba*, *Citrullus colocynthis*, *Ipomoea betatas*, *Ocimum sanctum* and *Cuminum cyminum* [32].

GT as a plant in Iranian traditional medicine has demonstrated some optimal treatment effects due to its antioxidant effects both in vitro and in vivo. It has also presented protective activities against toxicity in most body organs [16].

In the present study, diabetes was induced in all mice by single intraperitoneal injection of alloxan monohydrate. Alloxan monohydrate partially annihilates the beta cells of Langerhans islets, hepatocytes, nephron, RBC resulting in inexpressive insulin secretion causing type 2 diabetes, hepatotoxicity, nephrotoxicity, hematotoxicity [33]. The results of serum glucose levels showed that all doses of GTAE on days 16 and 20 demonstrated significant difference in comparison with untreated diabetic group, but there was no significant difference between the experimental doses of GTAE and classic anti-diabetic drug, glibenclamide. In agreement with the present results, there is a study which has shown the anti-diabetic effects of *G. tournefortii* genus with decreasing of blood glucose in diabetic mice [34].

During the short term study, the administration of GTAE produced significant antihyperglycemic activity and improved the renal morphological changes at all doses especially 40 mg/kg in diabetic mice. Untreated diabetic mice showed some degree of renal hypertrophy which was mainly due to the enlargement of the cortex and its subcomponents. These changes improved significantly with GTAE. It is well established that renal hypertrophy can be treated at the beginning of the diabetes; however, belated treatment is not successful [35]. The pathogenesis of the renal hypertrophy can be attributed to the overproduction of oxygen-free radicals following hyperglycemia and inducible nitric oxide synthase (iNOS) which is expressed in response to cytokines [36-38]. Therefore, compounds with anti-oxidant properties can ameliorate these changes and inhibit the progression of diabetic nephropathy [36-38]. GT as an ethno-medicinal plant is rich in antioxidants such as polyphenolic and flavonoid [39-40]. Plant phenols and polyphenols are effective in preventing various pathological conditions due to their antioxidant properties. They have cytoprotective and hepatoprotective effects against CCl_4. High antioxidant activities of GT could be attributed to gallic acid and quercetin which can inhibit glutathione-S-transferase activity [17].

In conclusion, the results of this study suggest that *Gundelia tournefortii* may be useful for controlling blood glucose level and alleviation of diabetic complications such as nephropathy generally observed in diabetic patients.

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Author contributions

Ghobad Mohammadi and Mohammad Mahdi Zangeneh prepared the manuscript; Mohammad Mahdi Zangeneh and Akram Zangeneh performed the biochemical analysis; Khodabakhsh Rashidi designed and performed the stereological plan; Mohammad Mahdi Zangeneh and Akram Zangeneh contributed in the statistical analysis; Akram Zangeneh was involved in animal handling and treatments; Ghobad Mohammadi and Mohammad Mahdi Zangeneh prepared the plant extract;

Declaration of interest

The authors declare that there is no conflict of interest. The authors alone are responsible for the content of the paper.

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Abbreviations

Bw: body weight; GTAE: *Gundelia tournefortii* aqueous extract; FBG: fasting blood glucose