Journal of Traditional and Complementary Medicine 11 (2021) 53–61

Contents lists available at ScienceDirect

Journal of Traditional and Complementary Medicine

journal homepage: http://www.elsevier.com/locate/jtcme

Nephroprotective effect of *Artemisia herba alba* aqueous extract in alloxan-induced diabetic rats

Omar Sekiou, Mahieddine Boumendjel, Faiza Taibi, Lazhari Tichati, Amel Boumendjel, Mahfoud Messarah*

Laboratory of Biochemistry and Environmental Toxicology, Faculty of Sciences, University of Badji Mokhtar, BP 12 Sidi Amar, Annaba, Algeria

ARTICLE INFO

Article history:
Received 9 November 2018
Received in revised form
2 January 2020
Accepted 5 January 2020
Available online 8 January 2020

Keywords:
Artemisia herba alba
Diabetes
Free radicals
Nephroprotection
Oxidative stress

ABSTRACT

Background and aim: In the present study, we investigate the phytochemical composition and the nephroprotective effects as well as the antioxidant properties of *Artemisia herba alba* aqueous extract in alloxan-induced experimental diabetes in rats.

Experimental procedure: Wistar rats were divided into four groups of seven rats each: Group I: Normal control (NC) received saline solution at 9‰ given by intraperitoneal way; Group II: Diabetic control (DC) received alloxan (150 mg/kg b.w) intraperitoneally; Group III: Normal control (NC + AHA) received saline solution at 9‰ and treated orally by AHA aqueous extract (400 mg/kg/b.w); Group IV: Diabetic control (DC + AHA) received alloxan solution (150 mg/kg b.w) intraperitoneally and treated by aqueous extract of AHA (400 mg/kg/b.w/day) orally after one week of alloxan administration. After 30 days, blood and tissue samples were collected for biochemical and histopathological analysis, respectively. Glomerular damage markers, including creatinine, serum urea, urine creatinine and urine urea levels were estimated. Creatinine clearance was also assessed. Oxidative stress parameters were assessed in the kidney homogenate.

Results and conclusion: Alloxan-exposure resulted in significant increase in blood glucose and serum level of glomerular damage markers. The antioxidant enzyme activities were significantly downregulated associated with an increase in malondialdehyde (MDA) level over the baseline values. *Artemisia herba alba* aqueous extract supplementation significantly improved the studied parameters. In concluding, the results obtained suggests that *Artemisia herbs-alba* aqueous extract supplementation reduces alloxan-induced free radical generation, potentiates the antioxidant defense system and alleviates renal sensitivity to oxidative stress.

1. Introduction

Diabetes mellitus is a group of metabolic diseases, which are characterized by chronic hyperglycemia due to a disorder in carbohydrate metabolism regulation resulting from a defect in insulin secretion or to the defect action of this hormone.[1] Chronic hyperglycemia is associated with the development of serious long-term complications include cardiovascular disease, chronic renal failure, retinal damage.[2] Chronic renal failure represents one of the most common and critical complications that appear in diabetic patients[3] According to the Centres for Disease Control and Prevention (CDC),[4] diabetes mellitus is the leading cause of renal failure for about 40% of patients in the United States. Progressive loss of renal function in patients with diabetes is typified by increased proteinuria and low glomerular filtration barrier rate led to elevated urinary albumin defecation.[5] It is well known that chronic hyperglycemia is a pro-oxidative factor that induces the process of overproduction of reactive oxygen species (ROS) by the

Abbreviations: $AlCl_3$, Aluminum trichloride; AHA, *Artemisia herba-alba*; CAT, catalase; DC, Diabetic control; RFC, Folin-Ciocalteu; H_2O_2, hydrogen peroxide; GPx, glutathione peroxidase; GST, glutathione-S-transferase; MDA, malondialdehyde; NBT, Nitro-blue tetrazolium; ROS, reactive oxygen species; GSH, reduced glutathione; STZ, streptozotocin; SOD, superoxide dismutase; TBA, thiobarbituric acid; TCA, trichloroacetic acid; DPPH, 1,1-diphenyl-2-picrylhydrazyl; DTNB, 5,5-dithiobis (2-nitrobenzoic acid).

* Corresponding author.
E-mail address: mahfoud.messarah@univ-annaba.dz (M. Messarah).

Peer review under responsibility of The Center for Food and Biomolecules, National Taiwan University.

https://doi.org/10.1016/j.jtcme.2020.01.001

mitochondrial electron-transport chain. ROS overproduced causing lipid peroxidation, antioxidant dysfunction, cell membrane loss and organ damage.[6–8] Hyperglycemia-induced oxidative stress is a molecular mechanism involved in the genesis of diabetic renal complications.[9] Numerous studies such as those of Beisswenger et al.[10] have shown that oxidative stress increases susceptibility to diabetic nephropathy. In addition, Witko-Sarsat et al.[11] demonstrated that advanced oxidation protein products are oxidation signs in patients with renal impairment. These findings suggest that antioxidant therapy may be a useful approach to the treatment of diabetes, promoting research on effective antioxidant molecules. To this aims, several surveys have been conducted to test medicinal plants that have hypoglycemic and antioxidant activity in various animal models.[12] Mohanasundaram et al.,[13] reported a regularize levels of antioxidant enzymes in streptozotocin-induced diabetic rats treated by a hydroethanolic extract of *Senna alata.* Patel et al.,[14] reported that pomegranate (*Punica granatum* Linn.) leaves attenuate hyperglycemia mediated hyperlipidemia and oxidative stress in streptozotocin induced diabetic rats. Nakashima et al.[15] have shown that linagliptin reduces renal oxidative stress and albuminuria in diabetic mice induced by STZ, Marques et al.[16] also have shown that Sitagliptin prevents renal inflammation and apoptosis induced by diabetes in the kidney of type 2 diabetic animals. In this study, experimental diabetes dedicated to the exploration of this disease is induced by intraperitoneal injection of alloxan. Glucose transporter ($GLUT_2$) expressed by tubular cells of the kidney, carry this glycomimetic into the cytosol, which increases the tubular cells deterioration by alloxan, as shown by Lenzen.[17] The objective of this study was to evaluate the influence of the alloxan administration on metabolic and renal changes associated with oxidative stress. In this research, the main assumption was that the renal and metabolic alterations induced by alloxan could be moderated by supplementation of *Artemisia herba-alba* AHA aqueous extract.

2. Materials and methods

2.1. Plant material and aqueous extract preparation

Fresh aerial part leaves of *Artemisia herba-alba* were collected in April 2016 from Djebel Ettarf Oum El Bouaghi/Algeria (35°45′22″ N, 7°5′13″ E). Collection of leaves was carried out according to good harvesting practices for medicinal plants recognized by OMS[18] Taxonomic identification of the plant was performed by **Azzedine Chefrour**, a professor of botany at Badji Mokhtar University Annaba, Algeria. The plant was cleaned, dried in shade, powdered then stored in airtight container. The aqueous extract of AHA was obtained as follows: 50 g of AHA powder was macerated with agitation in 500 ml of distilled water (1 g powder/10 ml distilled water) for 24 h. The aqueous extract was recovered by filtering the mixture with a filter paper (Whatman No.1). The mixture was then removed from the filtrate by evaporation (40 °C) in a furnace (memmert®), which allowed us to obtain a freeze-dried residue. The residue obtained was stored at (4 °C).

2.2. Chemical screening

2.2.1. Phenols determination

Polyphenols in plant extracts react with specific redox reagents (Folin-Ciocalteu reagent) to form a blue complex that can be quantified by visible-light spectrophotometry (JENWAY®6105) according to the method described by Donald et al.,[19] A test portion of 0.5 ml of the stock solution (1 mg/ml) prepared in a mixture of methanol/water (50:50). is mixed with 5 ml of Folin-Ciocalteu reagent (diluted 1/10 in ultra-pure water) and 4 ml of sodium carbonate Na_2CO_3 (1 M). The mixture is stirred and left for 15 min in the dark at room temperature. At the end of the reaction. The phenol content is expressed relative to a reference compound of Galic acid, a standard range of this acid was achieved [($y = 0.0046x+0.0263$ ($R^2 = 0.9929$)], then the standard curve giving the variation of the absorbance at 765 nm was plotted. The total phenol contents are expressed in mg of Galic acid equivalent per gram of dry vegetable matter (mg EAG/g MS). The phenol content is expressed relatively to a reference standard of Galic acid compound. A standard range of this acid was achieved [($y = 0.0046x+0.0263$ ($R^2 = 0.9929$)], then the standard curve giving the variation of the absorbance at 765 nm was plotted. The total phenol contents are expressed in mg of Galic acid equivalent per gram of dry vegetable matter (mg EAG/g MS).

2.2.2. Flavonoids determination

The determination of total flavonoids is based on a colorimetric test using aluminum trichloride $AlCl_3$. The total flavonoids were determined by the method described by Pourmorad et al.[20] Briefly, 0.5 ml of the extract solution (1 mg/ml) is added to 1.5 ml of methanol, 0.1 ml of 10% $AlCl_3$, 0.1 ml of sodium acetate (1 M) and 2.8 ml of ultra-pure water. The mixture was stirred vigorously and then left for 30 min in the dark. The reading of the absorbance at 430 nm by means of a UV/visible spectrophotometer (JENWAY®6105) was performed. The calibration curve of quercetin was performed under the same conditions: [$y = 0.0111x+0.0116$ ($R^2 = 0.995$)]. The total flavonoid contents are expressed in mg of quercetin equivalent per gram of dry vegetable matter (mg EAG/g MS).

2.2.3. Condensed tannin determination

Condensed tannin assay was performed by the Vanillin method described by Levin and Julkunen-tiitto21. An aliquot (50 μl) of each extract solution was mixed with 1.5 ml of 4% vanillin (prepared with methanol), then 750 μl of HCl (12 M) was added. The well-mixed solution was incubated in the dark at ambient temperature for 20 min the absorbance against blank was at 500 nm. Catechin was used to make the standard curve [$y = 0.0099x+0.0343$ ($R^2 = 0.994$)]. The condensed tannin content is expressed in mg of catechin equivalent per gram of dry vegetable matter (mg CE/g MS).

2.3. DPPH assay

DPPH assay was performed by the method of Blois.[22] Briefly, 2 ml of different dilutions of the tested solution was added to 2 ml of an ethanolic solution of 1,1-diphenyl-2-picrylhydrazyl (DPPH). The contents were mixed vigorously and let stand for 30 min at room temperature in the dark. The antiradical activity was measured spectrophotometrically at 515 nm according to the following equation: % scavenged [Free radicals] = [(***A*** blank − ***A*** sample)/***A*** blank] × 100.

Where ***A*** blank is the absorbance of the control DPPH, and ***A*** sample is an absorbance in the presence of the sample extract and DPPH. The antioxidant activities were expressed as IC_{50}, defined as the concentration of the test substance necessary to cause a 50% decrease in the initial concentration of DPPH.

2.4. Chemicals

Aluminum trichloride ($AlCl_3$), gallic acid, Quercetin, and Catechin were obtained from Biochem, Chemopharma (Cosne Sur Loire, France). Alloxan, 5,5-dithiobis (2-nitrobenzoic acid) [DTNB], Nitroblue tetrazolium chloride (NBT), reduced glutathione (GSH), trichloroacetic acid (TCA), thiobarbituric acid (TBA), and hydrogen peroxide were purchased from Sigma-Aldrich.

2.5. Experimental design

Twenty-eight male adult albinos Wistar rats (aged 9–10 weeks; weighing 180–200 g) were obtained from the Pasteur Institute (Algiers, Algeria). They were fed pellet diet purchased from the Industrial Society of rodent's diet (UAB - El K seur, Bejaia, Algeria) and tap water *ad libitum*. After one month of acclimatization in a room with controlled temperature (22 ± 3 °C) and lighting (12-h light/dark cycle), rats were randomly divided into four groups of seven rats each: Group I: Normal control (NC) received saline solution at 9‰ given by intraperitoneal way; Group II: Diabetic control (DC) received alloxan solution (150 mg/kg b.w) intraperitoneally; Group III: Normal control (NC + AHA) received saline solution at 9‰ given by intraperitoneal and treated orally (gavage) by (AHA) (400 mg/kg/b.w); Group IV: Diabetic control (DC + AHA) received alloxan solution (150 mg/kg b.w) intraperitoneally and treated by aqueous extract of AHA (400 mg/kg/b.w/day) orally after one week of alloxan administration. After 30 days, the rats were sacrificed under anesthesia by cervical decapitation. Blood was collected and centrifuged at 3000×*g* for 15 min using automatic refrigerated centrifuge (DCS-16RTV, Argentina) to obtain serum and was then divided into aliquots that were stored at −20 °C until biochemical analysis. Two kinds of bottles were used, those with anticoagulant were dedicated to hematological parameters. The kidney was immediately removed and divided into two parts: the first half is stored at **-20°C** in the freezer for the subsequent preparation of the homogenate which was used for determination of oxidative stress parameters. The other half is preserved in 10% buffered formalin for the histopathological analysis. The experimental procedures were carried out according to the National Institute of Health Guidelines for Animal Care and approved by the NRP/SF 08/2012 by the Ethics Committee of the University.

2.6. Induction of experimental diabetes and measurement of fasting blood glucose

Rats kept on fasting for 12 h received a single injection of freshly dissolved alloxan in 1.0 ml of sodium citrate buffer (0.1 M, pH 4.5) intraperitoneally (*i.p*), at a rate of 150 mg/kg body weight.[23] Diabetes is determined by measurement of fasting blood glucose with glucometer (Accu-Chek® Aviva) 72 h and then on seven days after Alloxan injection. Rats with fasting glucose levels greater than 200 mg/dl are selected for our study. The blood was withdrawn from the tail vein of rats, then the measurement of fasting blood glucose concentration was determined using a glucometer (Accu-Chek® Aviva) every seven days along the experimental protocol.

2.7. Bodyweight, urine volume and protein levels determination

The animals are weighed using a precision scale daily before the induction of diabetes (beginning of the manipulation) and after the induction of diabetes, until the day of dissection of the rats. After isolating the control rats from those with diabetes in metabolic cages (Tecniplast®, LP 2100R**),** The measurement of 24 h urine test is performed. Urines are collected in a clean recipient, then urine volume is determined. The urines were immediately stored in the freezer at 4 °C for the subsequent measurement of the urinary urea and urinary creatinine concentration. The protein concentration is determined according to the method of Bradford[24] which uses Coomassie blue (G250) as a reagent. Briefly, 5 mL of Coomassie blue is added to 0.1 ml of the previously prepared kidney homogenate. After stirring and a rest period of 5 min, the reading of the optical densities is carried out at 595 nm against blank. The concentration of the proteins is determined by comparison with a standard range of bovine serum albumin (1 mg/ml) previously carried out under the same conditions.

2.8. Hematological parameters and kidney function markers determinations

Measurement of hematological compounds was assessed by an auto hematological counter (Full Automatic, Blood Cell Counter, PCE-210 N®, TOKYO). Blood urea, urinary urea concentration, blood creatinine and urinary creatinine concentration were measured using a commercial kit (Biomaghreb®, Tunis), according to the manufacturer's instructions by an auto-analyzer (Micro Lab® 200). The values of creatinine clearance were measured by the following equation: Creatinine clearance (ml/min) = [urine creatinine (mg/dL)/serum creatinine (mg/dL)] × Urinary flow.

2.9. Kidney homogenate preparation

Kidney homogenate was obtained according to Adeyemi et al.[25] Briefly, 2 g of Kidney of the groups under study were crushed. After tissue homogenization in TBS (50 mM Tris, 150 mM NaCl, pH 7.4), the cell suspension was centrifuged using an automatic refrigerated centrifuge (DCS®-16RTV, Argentina) (9000 rpm, 4 °C, 15 min). The obtained supernatant was aliquoted and then stored at −20 °C.

2.10. Lipid peroxidation (LPO) level and reduced glutathione (GSH) assay

The lipid peroxidation (LPO) level in the kidney homogenate was measured as malondialdehyde (MDA), which is the final product of lipid peroxidation. Malondialdehyde reacts with thiobarbituric acid (TBA) as a TBA reactive substance (TBARS) to produce a red colored complex which has peak absorbance at 532 nm according to Buege and Aust.[26] A quantity of 375 µl of the TCA-BHT solution (TCA 20% and BHT 1%) are added to 375 µl of the homogenate and 150 µl of TBS (Tris 50 mM, 15 Mm NaCl, pH = 7.4). The mixture is well vortexed and then centrifuged at 1000 rpm for 10 min. Then, 400 µl of the supernatant is taken and added to 80 µl of HCl (0.6 M) and 320 µl of the Tris-TBA buffer (26 mM and 120 mM, respectively). This last mixture is incubated at 80 °C for 10 min.

Reduced glutathione (GSH) contents in kidney homogenate were estimated using a colorimetric technique, as mentioned by Ellman[27] and modified by Jollow et al.[28] In brief, 0.8 ml of kidney supernatant was added to 0.2 ml of 0.25% sulphosalicylic acid then tubes were centrifuged at 2500×*g* for 15min using automatic refrigerated centrifuge (DCS-16RTV, Argentina). The resulting supernatant (0.5 ml) was mixed with 0.025 ml of (0.01 M) DTNB and 1 ml phosphate buffer (0.1 M, pH 7.4). In the end, absorbance at 412 nm was measured and recorded. The amount of GSH was expressed as nmoles of GSH/mg protein.

2.11. Glutathione peroxidase (GPx), superoxide dismutase (SOD), catalase and glutathione-S-transferase (GST) assay

Glutathione peroxidase (GPx; E.C 1.11.1.9) activity was measured according to the procedure of Flohe and Gunzler.[29] This method is based on the reduction of hydrogen peroxide (H_2O_2) in the presence of reduced glutathione (GSH). The latter is transformed into (GSSG) under the influence of GPx. The superoxide dismutase (SOD; E.C 1.15.1.1) activity was assayed by the method of Fridovich.[30] SOD activity was evaluated by measuring its ability to inhibit the photoreduction of nitro-blue tetrazolium (NBT). Catalase activity (CAT; E.C.1.11.1.6) in kidney homogenate was measured according to the method described by Aebi,[31] using hydrogen peroxide (H_2O_2). The enzyme activity was calculated using an

extinction coefficient of 0.043mM^{-1}cm^{-1}. Glutathione-S-transferase (GST; E.C 2.5.1.18) activity was calculated by Habig et al.[32] using 1-chloro-2,4-dinitrobenzene (CDNB).

2.12. Histopathological examination

Histopathological examination was carried out according to the technique described by Drury and Wallington.[33] Histothological examination was performed at Ibn-Rochd Annaba Hospital, Department of Pathological Anatomy. The cuts (5 μm on average) were made using a microtome (Leica® RM2235). The obtained sections were stained by the haematoxylin-Eosin (H&E) technique. The preparations were then observed under an optical microscope (Leica® DM1000) and photographed. A renal scoring analysis of tubular and glomerular lesion is providing to improve pathological data.

2.13. Statistical analysis

Data were expressed as Mean ± standard error (SE). The difference between each peers group was calculated using "student-test" (Microsoft® Office Excel® 2016), and the difference between all studied groups was assessed by One-Way ANOVA using (Origin® Pro15). *P* values < 0.05 are considered statistically significant and those of *p* values < 0.01 are considered highly significant.

3. Results

3.1. Phenolic contents and antioxidant activity of the AHA extract

The quantitative assessment of chemical components of AHA aqueous extract is summed-up in (Supplementary Table 1). phytochemical analysis of AHA leaves aqueous extract showed various metabolites. Corresponding to the obtained results of the phytochemical profile, the AHA aqueous extract presented a total content of phenolic compounds of 83.59 ± 0.96 mg gallic acid/g extract. Flavonoid composition of the extract expressed as mg of quercetin equivalents per gram of the dry plant was 25.7 ± 0.95. On the other hand, our results demonstrate that the AHA aqueous extract was rich in condensed tannins (8.75 ± 0.5 mg EC/g MS). The antioxidant activity assessed by the DPPH method gave an interesting result, 71.15%; amounting to 50% of the reduction with an IC_{50} value of 30.46 ± 0.45 mg/ml.

3.2. Effect of treatments on fasting blood glucose

The blood glucose levels in normal control rats processed and not processed with AHA, as well as diabetic control rats were recapitulated in Table 1. It was observed that blood glucose levels were significantly higher in diabetic control rats (about four-fold) than in normal control rats. The anti-hyperglycemic effect of AHA induces significant decrease (approximately 50%) in blood glucose levels in treated diabetic rats compared to untreated diabetic rats [178.6 ± 19.51 and 389.6 ± 28.59 mg/dL, respectively, $p < 0.001$]. As shown in the following table, the hypoglycemic effect of AHA was significantly obvious 30 days after alloxan exposure.

3.3. Effect of treatments on urinary volume, food intake, and water consumption

Table 2 recapitulates parameters of food intake (g), water consumption (ml) and urine volume (ml) assessed in controls and diabetic groups. We observed a significant increase in urinary volume (32.8 ml ± 1.92) in diabetic rats compared to control rats (21.60 ml ± 1.09). With respect to water and food intake, there was a significant increase in water consumption (37.8 ml ± 3.76) and dietary intake (29.35 g ± 1.02) in diabetic rats compared with controls. Conversely, food intake (g), water consumption (ml) and urine volume (ml), of the treated-diabetic group with the AHA aqueous extract, were significantly normalized (22.88 ± 1.79 g, 29.4 ± 2.31 ml and 19.6 ± 2.38 ml respectively) in comparison with the untreated rats. Therefore, no difference was observed between the treated diabetic group and the normal control group.

3.4. Effect of treatments on body weight, relative kidney weight, and total protein content

Changes in body weight, relative kidney weight and total protein content of all groups under study are shown in Table 3. It has been observed that body mass gradually increased in controls groups, however, bodyweight rise was very low in diabetic rats than in control groups. This reduction in body weight of diabetic rats was significantly restored by AHA treatment. No significant difference was recorded comparing the control group with the diabetic treated group. Alloxan administration caused a significant increase in relative kidneys weight of the diabetic group (39%) compared with the control. However, treatment with AHA prevented this modification. Similarly, the results obtained in our study revealed a significant difference in total protein between the diabetic group and the control group. Thus, a significant decrease in total protein was noted in the diabetic group compared with those of control ($p = 0.008924$). Conversely, total protein of the treated-diabetic group with AHA aqueous extract was raised to near-normal values. Therefore, no difference was observed between the treated diabetic group and the normal control group in total protein content ($p = 0.33712$).

3.5. Effect of treatments on hematological parameters

As shown in Table 4, diabetic rats displayed a decrease in the number of red blood cells (RBC), hemoglobin content (Hb), hematocrit (Ht) and an increase in platelets (Plt) content appearing to

Table 1
Effects of treatment with aqueous extract of *Artemisia herba alba* on blood glucose level (mg/dL).

Blood glucose level (mg/dL)					
	Treatment				
	Day 0	7th day	14th day	21st day	30th day
Normal control	102 ± 6.12	96 ± 5.36	108 ± 3.71	95 ± 2.95	106 ± 3.33
Diabetic control	224 ± 25.65**	434 ± 25.18***	417 ± 22.10 ***	403.8 ± 19.08 ***	389.6 ± 28.59 ***
Normal control + AHA	105 ± 10.08$^{\#\#}$	96 ± 17.65$^{\#\#\#}$	105 ± 4.35$^{\#\#\#}$	98 ± 8.25$^{\#\#\#}$	109 ± 4.03$^{\#\#\#}$
Diabetic control + AHA	212 ± 23.38**	397.4 ± 42.04***	248.4 ± 53.04$^{***\#\#\#}$	201.4 ± 80.30 $^{***\#\#\#}$	178.6 ± 19.51$^{**\#\#\#}$

Values are given as average ± SE of seven (n = 7) animals in each group.
$^{**}p < 0.01$ versus normal control, $^{***}p < 0.001$ versus normal control, $^{\#\#}p < 0.01$ versus diabetic control, $^{\#\#\#}p < 0.001$ versus diabetic control.

Table 2
Effects of treatment with aqueous extract of *Artemisia herba alba* on urinary volume, food intake, and water consumption.

Parameter	Treatment			
	Normal control	Diabetic control	Normal control + AHA	Diabetic control + AHA
Water consumption (mL)	21 ± 3.31	37.8 ± 3.76*	21.40 ± 3.21$^{\#\#}$	29.4 ± 2.31$^{\#}$
Food intake (g)	19.56 ± 0.45	29.35 ± 1.02 ***	20.86 ± 0.82$^{\#\#\#}$	22.88 ± 1.79$^{\#\#\#}$
Urine volume (mL)	21.60 ± 1.09	32.8 ± 1.92**	21.40 ± 2.21$^{\#\#}$	19.6 ± 2.38$^{\#\#}$

Values are given as average ± SE of seven (n = 7) animals in each group. Each intake value is corresponding to the daily intake for seven rats expressed by the mean value of 30 experimental days.
$^{*}p < 0.05$versus normal control, $^{**}p < 0.01$ versus normal control, $^{***}p < 0.001$ versus normal control, $^{\#}p < 0.05$ versus diabetic control, $^{\#\#}p < 0.01$ versus diabetic control, $^{\#\#\#}p < 0.001$ versus diabetic control.

the control, as regards the profile of the white blood cells (WBC). The results showed that they were significantly increased compared to control (p = 0.03126). A reduction in the hemoglobin content created anemia in diabetic animals [(15.29 ± 0.702) versus (1.87 ± 0.144 g/dL) in control rats (p = 0.00014)]. Previous hematological alterations were significantly attenuated by AHA aqueous extract supplementation. Little changes were observed when the hematological profile of the treated diabetic group was compared to those of the controls.

3.6. Effect of treatments on kidney function markers

As shown in Table 5, experimental diabetes induced a significant increase (p = 0.000228) in serum creatinine and blood urea (p = 0.001882) in comparison to those of the control. However, the renal excretion of urea and creatinine were significantly decreased in diabetic rats (p = 0,00273 and p = 0,0000056, respectively) as compared to those of the normal rats, while the values of creatinine clearance were strongly lower in diabetic animals group compared to the values assessed in the normal control group (p = 0,00072). A significant improvement of those parameters was recorded with the AHA aqueous extract group.

3.7. Effect of treatments on lipid peroxidation (LPO) and reduced glutathione (GSH) levels

The lipid peroxidation level (LPO) of all groups studied was presented on Fig. 1. From the obtained results, we recorded a significant increase in the MDA level in renal homogenate of diabetic control rats compared to the control group (P ≤ 0.001). On the contrary, as regards to the results obtained after rats processing with AHA aqueous extract, it's noted that lipid peroxidation level measured as malondialdehyde (MDA) was significantly decreased in the renal homogenate of treated diabetic rats compared to those of untreated rats (p = 0,00768). Similarly, there was no significant difference when compared the treated diabetic rats with normal control rats. Additionally, Fig. 1 equally, showed that GSH levels were significantly decreased (p < 0.001) in kidney homogenate of diabetic rats compared to the normal ones. Inversely, AHA supplementation on alloxan-exposed rats exhibited a significant increase in GSH content when compared with untreated groups (p = 0,01751). Moreover, no significant changes were seen in GSH levels of treated diabetic rats with AHA aqueous extract compared to the normal control rats.

Table 3
Effects of treatment with aqueous extract of *Artemisia herba alba* on the body, total protein, absolute and relative kidney weights.

Parameter	Treatment			
	Normal control	Diabetic control	Normal control + AHA	Diabetic control + AHA
Body weight (g)	289.6 ± 13.36	210.4 ± 08.69***	294,4 ± 15.75$^{\#\#\#}$	248.6 ± 12.43$^{\#\#}$
Absolute kidney weight (g)	1.87 ± 0.30	2.17 ± 0.34***	1.86 ± 0.21$^{\#\#\#}$	1.92 ± 0.43$^{\#\#}$
Relative kidney weight (%)	0.64 ± 0.022	1.03 ± 0.039***	0.63 ± 0.013$^{\#\#\#}$	0.77 ± 0.034$^{**\#\#\#}$
Total protein (g/L)	73.2 ± 4.77	62.8 ± 5.66**	77.72 ± 1.64$^{\#\#}$	73.75 ± 3.09$^{\#\#}$

Values are given as average ± SE of seven (n = 7) animals in each group. Body weight value is corresponding to the daily weighting for seven rats expressed by the mean value of 30 experimental days. Absolute kidney weight, Relative kidney weight and Total protein are measured only after sacrifice at the end of the 30 experimental days.
$^{**}p < 0.01$ versus normal control, $^{***}p < 0.001$ versus normal control, $^{\#\#}p < 0.01$ versus diabetic control, $^{\#\#\#}p < 0.001$ versus diabetic control.

Table 4
Effects of treatment with aqueous extract of *Artemisia herba alba* on hematological parameters.

Parameter	Treatment			
	Normal control	Diabetic control	Normal control + AHA	Diabetic control + AHA
WBC(10^3/μL)	8.67 ± 2.512	12.35 ± 1.804 *	7.554 ± 2.941$^{\#\#}$	5.546 ± 1.046$^{**\#}$
RBC(10^6/μL)	9.576 ± 0.209	1.3075 ± 0.470***	9.264 ± 0.242$^{\#\#\#}$	8.37 ± 0.604$^{***\#\#\#}$
Hb (g/dL)	15.29 ± 0.702	1.87 ± 0.144***	14.95 ± 0,680$^{\#\#\#}$	14.22 ± 0.825$^{*\#\#\#}$
Ht (%)	46.74 ± 1.429	24.68 ± 3.131***	44.84 ± 1.660$^{\#\#}$	42.68 ± 2.107$^{**\#\#\#}$
MCHT (pg)	16.02 ± 0.630	19.42 ± 3.169*	16.14 ± 0.694$^{\#}$	17 ± 0.860$^{*\#\#\#}$
MCHC (g/dL)	32.86 ± 0.730	28.4025 ± 2.669**	33.52 ± 0.855$^{\#\#}$	33.32 ± 1.112$^{\#\#}$
Plt (10^3/μL)	946.8 ± 40.782	1008.25 ± 11.898**	810.6 ± 127.723$^{\#}$	740.6 ± 67.013$^{***\#\#\#}$
LT (10^3/μL)	4.87 ± 1.240	1.58 ± 0.358***	4.186 ± 1.148$^{\#\#}$	3.186 ± 0.763$^{**\#\#}$

Values are given as average ± SE of seven (n = 7) animals in each group. Measured hematological parameters are done after sacrifice at the end of the 30 experimental days. WBS, white blood cell; RBC, red blood cell; Hb, hemoglobin content; Ht, hematocrit; MCHT, mean corpuscle hemoglobin content; MCHC, mean corpuscular hemoglobin concentration; Plt, platelet; LT, lymphocytes. $^{*}p < 0.05$versus normal control, $^{**}p < 0.01$ versus normal control, $^{***}p < 0.001$ versus normal control, $^{\#}p < 0.05$ versus diabetic control, $^{\#\#}p < 0.01$ versus diabetic control, $^{\#\#\#}p < 0.001$ versus diabetic control.

Table 5
Effects of treatment with aqueous extract of *Artemisia herba alba* on the kidney markers parameters.

Parameter	Treatment			
	Normal control	Diabetic control	Normal control + AHA	Diabetic control + AHA
Serum creatinine (mg/dL)	0.48 ± 0.05	0.862 ± 0.07***	0.475 ± 0.02$^{\#\#\#}$	0.65 ± 0.05$^{***\#\#\#}$
Urine creatinine (mg/dL)	235.5 ± 38.12	12.75 ± 2.5***	215 ± 28.15$^{\#\#\#}$	83.75 ± 5.73$^{***\#\#\#}$
Serum urea (mg/dL)	4.225 ± 0.64	13.45 ± 3.10***	4.225 ± 0.22$^{\#\#\#}$	6.525 ± 1.21$^{**\#\#\#}$
Urine urea (mg/dL)	43.328 ± 3.49	19.292 ± 5.14***	40.006 ± 0.59$^{\#\#\#}$	28.358 ± 1.55$^{***\#\#\#}$
Urine volume (ml)	21.6 ± 1,09	32.8 ± 1.92**	21.4 ± 2.21$^{\#\#}$	19.6 ± 2.38$^{\#\#}$
Urinary flow (ml/min)	0.015 ± 0.0002	0.022 ± 0.0001***	0.014 ± 0.0001$^{\#\#\#}$	0.013 ± 0.0009$^{\#\#\#}$
Creatinine clearance (ml/min)	7.359 ± 0.021	0,336 ± 0.001***	6.726 ± 0.063$^{\#\#\#}$	1.753 ± 0.012$^{***\#\#\#}$

Values are given as average ± SE of seven (n = 7) animals in each group. Measured parameters were done as following: Serum creatinine; Urine creatinine; Serum urea; Urine urea; Urine volume: only one-time measurement conducted at the **sacrifice day-1.**
$^{**}p < 0.01$ versus normal control, $^{***}p < 0.001$ versus normal control, $^{\#\#}p < 0.01$ versus diabetic control, $^{\#\#\#}p < 0.001$ versus diabetic control.

3.8. Effect of treatments on antioxidants enzymes activities

In the present study, significant reductions were observed on antioxidant enzymes activities; Superoxide Dismutase SOD (45%), Catalase CAT (47.5%), GST (19%) and Glutathione Peroxidase GPx (90.82%) in the kidney of alloxan-exposed rats compared with control groups Fig. 2. Conversely, the activities of those antioxidant enzymes were significantly normalized in diabetic rats supplemented with AHA aqueous extract compared to the untreated diabetic rats, which obviously reflected the antioxidant properties of the AHA aqueous extract.

3.9. Histopathological results

Microscopic observation of Haematoxylin and Eosin (H&E) stained kidney samples of the control group shows a normal architecture of renal tubules and a normal Bowman's capsules (Fig. 3-A). In this control group, no morphological changes were observed in the glomerular size and in basement membrane and no renal lesions were recorded. In the same, control group treated with the AHA aqueous extract showed no morphological changes in the kidney, and no toxic effect of our aqueous extract on the kidneys was recorded (Fig. 3-B).

Kidney histo-architecture the of alloxan-treated rats showed severe degeneration of the renal tubules, increased glomerular space, a glomerular hypertrophy with a transparent fluid (Fig. 3-C) and thickening of the basement membrane. Conversely, the kidney histo-architecture of diabetic rats supplemented with AHA aqueous extract (400 mg/kg) showed almost normal morphology. Thus, the AHA therapy significantly reduced renal tubule degeneration and glomeruli swelling (Fig. 3-D).

4. Discussion

From obtained results, lipid peroxidation level (LPO) has been significantly increased in the renal homogenate of diabetic rats which go beyond previous reports, showing that MDA levels are up-generated over the baseline values in alloxan-induced diabetic rats. This is consistent with what has been found by Joydeep and Parames[34] wherein they have demonstrated that MDA levels increased to 9.83 nmol/mg protein three weeks following alloxan exposure. This increase in lipid peroxidation level may play a critical role in the development of diabetic nephropathy. From the obtained results, we recorded a significant decrease in activity of all enzymes, involved in the defense system, in the diabetic group compared to the controls ones. Mentioned activities alterations of those antioxidant enzymes increased susceptibility to nephropathy and reflected the contribution of oxidative stress on renal functions losses spotted in this study during diabetes. Mohanasundaram et al.[13] also noted a lessening activity of antioxidant enzymes in streptozotocin-induced diabetic rats. Enzymatic activity dysfunction may be due to the reactive oxygen species that cause proteins oxidation when they highly generated in oxidative stress situation.[35,36] Enzymatic activity enhancement may be due to the antioxidant properties of AHA leaves aqueous extract. The natural antioxidant molecules scavenge free radicals which allowing the maintenance of antioxidants/free radicals ratio in a near-normal manner.[37] From these results, it is clear that the choice of AHA

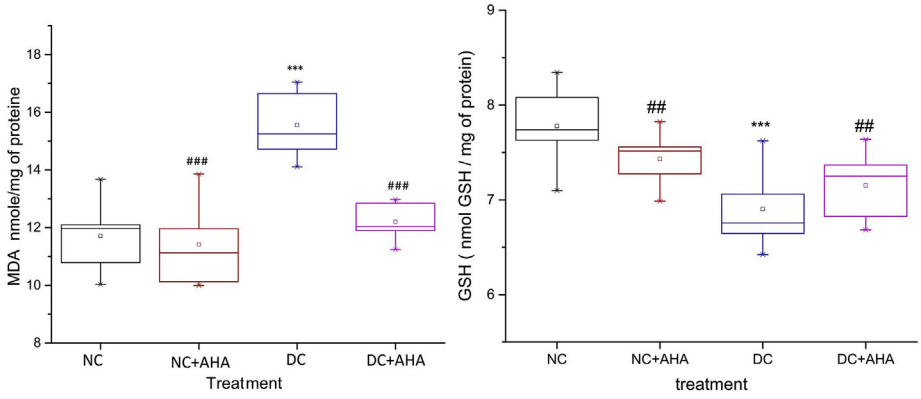

Fig. 1. Effects of treatment with aqueous extract of *Artemisia herba alba* on Malondialdehyde (MDA) and Reduced Glutathione (GSH) levels.
Values are given as meant ±SD of seven animals in each group
$^{***}p <$
0.001 versus normal control, $^{\#\#}p < 0.01$ versus diabetic control, $^{\#\#\#}p < 0.001$ versus diabetic control.

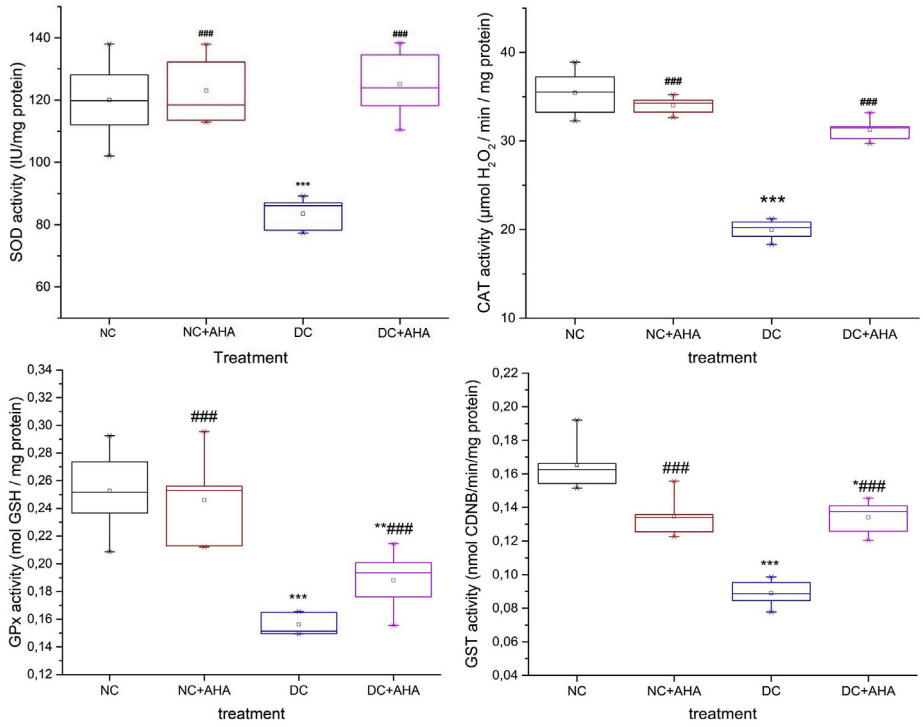

Fig. 2. Effects of treatment with aqueous extract of *Artemisia herba alba* on Superoxide dismutase (SOD), Catalase (CAT), Glutathione peroxidase (GPx), Glutathione-S-transferase (GST).
Values are given as meant ±SD of seven animals in each group.
$^{*}p < 0.05$versus normal control, $^{**}p < 0.01$ versus normal control, $^{***}p < 0.001$ versus normal control, $^{\#\#\#}p < 0.001$ versus diabetic control.

leaves aqueous extract against alloxan-induced oxidative stress is well justified. Nitin et al.[38] demonstrated that GSH levels were decreased to approximate 10 µg/100 g protein in STZ-induced-diabetic rats, which is in concordance with our results. GSH decrease might be attributed to increased use of GSH by GPx in detoxification of H_2O_2 up-generated by alloxan, and also due to the hyperglycemia itself. In that case, the excess cellular glucose is reduced to sorbitol by aldose reductase, which decreases the level of intracellular NADPH required for regenerate GSH from oxidized GSH.[39] Our results demonstrated that the supplementation of *Artemisia herba-alba* aqueous extract improves the GSH levels in alloxan-exposed rats to near-normal values. The important role of AHA aqueous extract against GSH depletion may attribute to its hypoglycemic properties led to significant decreases of excess cellular glucose which minimizes the sorbitol pathway, this may prevent the overuse of intracellular NADPH needed to regenerate GSH. Our results demonstrated that alloxan-exposed rats exhibit a decreased level of RBC, Hb, Ht, and an increased Plt levels creating anemia in rats. These results are in agreement with Tijani et al.[40] who reported that Diabetes Meletus (DM) is associated with increased risk of anemia in rats. Anemia observed in alloxan-exposed rats could be attributed to the increased elimination of erythrocytes. Cells membranes deterioration due to the alloxan-exposure could cause RBC hemolysis. Treatment by AHA refine the reduction of hematological parameters and could have an anti-inflammatory effect. These effects can be attributed to flavonoid present in the AHA aqueous extract. Our results put in case a significant weight loss on alloxan-exposed rats as compared to normal rats, which ties well with previous studies wherein Farzad et al.[41] demonstrated that STZ-induced-diabetic rats are characterized by a significant loss of body weight. The decrease in body weight in diabetes mellitus is usually attributed to the stimulation of gluconeogenesis. Indeed, abnormal carbohydrate metabolism results in catabolism acceleration of proteins and fats which leads to an increase in muscle atrophy and loss of tissue protein42. In this study, the significant increase in body mass recorded after administration of AHA extract in diabetic rats is probably due to the regulation of glucose homeostasis by the plant extract. In our study, the renal damage markers such as urea and serum creatinine levels have been increased significantly in diabetic rats as compared to normal ones. Our results are in agreement with those reported by Hossein et al.,[43] who found that these markers are significantly elevated in STZ-induced diabetic rats. The significant increase of those renal injury markers in diabetic rats group may due to the intense degradation of proteins. Conversely, we have noted a significant reduction in urinary volume, serum creatinine, and urea in diabetic rats after AHA administration, which obviously shows that protein degradation was strongly reduced. Our results tie well with previous studies of Bárbara Bruna et al.,[44] who demonstrated that creatinine clearance was strongly decreased to approximate (1.32 ml/min) after 24-h of urine collection in rats. The decrease in creatinine clearance noted in our study maybe do to the alloxan accumulation in tubular cells expressed the Glucose Transporter 2 (GLUT2), which results in glomerular injury and nephrotoxicity situation led in a low creatinine clearance activity. At the opposite, AHA treated-rats showed an important increase in creatinine clearance as compared to untreated-ones. This is probably due to the improvement of glomerular clearance activity by the abolish of the cells injury caused by the alloxan injection. In order to comfort our previous results, a renal-histopathological examination of all groups under study was carried out. kidney-histopathological examination revealed that alloxan-exposed rats have several changes in renal architecture, such as glomerular hypertrophy, increased glomerular space and tubular damage. Our results were broadly in line with Pourghasem et al.[45] who suggested that alloxan-induced diabetic rats exhibit an important change in kidney

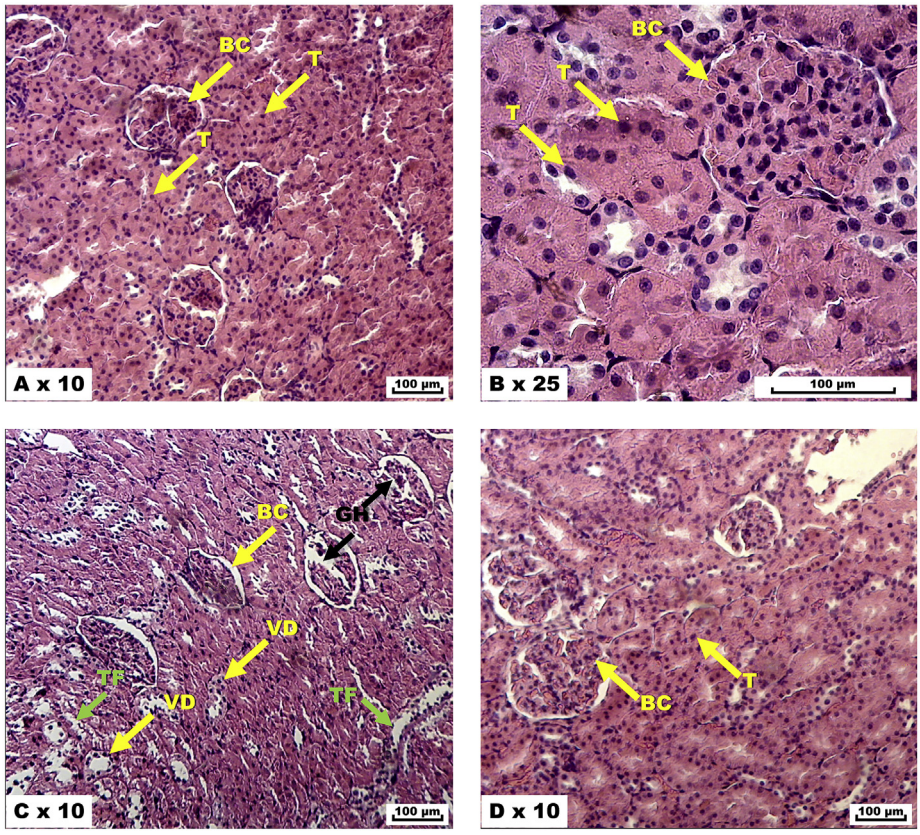

Fig. 3. Histologic sections stained with haematoxylin-Eosin (H&E) show the kidney of the experimental groups (**A-**normal control rats, **B-**normal treated rats, **C-**diabetic control rats, **D-**diabetic treated rats). The histoarchitecture of the kidney in group **A** and **B** shows a normal architecture of renal tubules **(T)** and a normal Bowman's capsules **(BC)**. Significant changes with signs of inflammation on group **C** in wish we noted an increased Bowman's space **(BC)**, an inflammation of the renal tubules with vacuole degenerative (**VD**), transparent fluid (**green arrow)** and glomerular hypertrophy (**black arrow**). While the kidney section of group **D** showed a moderate degree of glomerular hypertrophy, a near-normal architecture of renal tubules **(T)** and decreased Bowman's space.

histoarchitecture. The histological examination of AHA-treated diabetic rats showed a substantial improvement of tubular and glomerular damage to a near-normal physiological statement. This mitigating of previously alterations may be attributed to the antioxidant properties of AHA. As beforehand discussed in this study, the AHA aqueous extract-supplementation resulted in antioxidants/free radical's ratio preservation by an enhancement of antioxidant enzymes activity and free radicals scavenging, which soften those previous alterations, thus obviously attesting protective role in the renal injury during diabetes statement. The main hypothesis of this research work is obviously consistent with the basic results obtained which show that: *Artemisia herba-alba* reduced the production of free radicals induced by alloxan; increased the antioxidant defense; and mitigated the renal sensitivity to oxidative stress. In this study, the protective effects of *Artemisia herba-alba* leave aqueous extract against renal damage under diabetes condition is well justified.

Declaration of competing interest

The authors report no conflict of interest.

Acknowledgment

The present work was supported by DG-RSDT (General Directorate of Scientific Research and Technological Development-Algeria) via the Laboratory of Biochemistry and Environmental Toxicology, Faculty of Sciences, University of Badji Mokhtar-Annaba, Algeria.

We would like to thank BOUZIANE ISMAIL for proofreading the manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jtcme.2020.01.001.

References

1. Zheng XK, Li YJ, Zhang L, et al. Antihyperglycemic activity of *Selaginella tamariscina* (Beauv.) spring. *J Ethnopharmacol*. 2011;133(2):531–537.
2. Roberts AW. Cardiovascular risk and prevention of diabetes mellitus. *Clin Med*. 2010;10:495.
3. White KE, Bilous RW. Type 2 diabetic patients with nephropathy show structural-functional relationships that are similar to type 1 disease. *J Am Soc Nephrol*. 2000;11:1667–1673.
4. Centers for Disease Control and Prevention (CDC). *National Chronic Kidney Disease Fact Sheet: General Information and National Estimates on Chronic Kidney Disease in the United States*. 2014.
5. Gorriz JL, Martinez-Castelao A. Proteinuria detection and role in native renal disease progression. *Transp Rev*. 2012;26:3–13.
6. Bonnefont-Rousselot D, Beaudeux J-L, Thérond P, et al. Diabète sucré, stress oxydant et produits de glycation avancée. *Ann Pharm Fr*. 2004;62(3):147–157.
7. Haghani K, Bakhtiyari S, Doost Mohammadpour J. Alterations in plasma glucose and cardiac antioxidant enzymes activity in streptozotocin-induced diabetic rats: effects of *Trigonella foenum-graecum* extract and swimming training. *Can J Diabetes*. 2016;40(2):135–142.
8. Çakatay U. Protein oxidation parameters in type 2 diabetic patients with good and poor glycaemic control. *Diabetes Metab*. 2005;31(6):551–557.
9. Forbes JM, CoughlanMT, Cooper ME. Oxidative stress as the major culprit in kidney disease in diabetes. *Diabetes*. 2008;57:1446–1454.

10. Beisswenger PJ, Drummond KS, Nelson RG, et al. Susceptibility to diabetic nephropathy- thy is related to dicarbonyl and oxidative stress. *Diabetes*. 2005;54:3274–3281.
11. Witko-Sarsat V, Friedlander M, Capeillere-Blandin C, et al. Advanced oxidation protein products as a novel marker of oxidative stress in uremia. *Kidney Int*. 1996;49:1304–1313.
12. Coman C, Rugina OD, Socaciu C. Plants and natural compounds with antidiabetic action. *Not Bot Horti Agrobot Cluj-Napoca*. 2012;40(1):314–325.
13. Sugumar Mohanasundaram, Victor Arokia Doss D, Prasad Maddisetty PN. Hepato-renal protective effects of hydroethanolic extract of Senna alata on enzymatic and nonenzymatic antioxidant systems in streptozotocin-induced diabetic rats. *Integr Med Res*. 2016;(5):276–283.
14. Patel AN, Bandawane DD, Mhetre NK. Pomegranate (Punica granatum Linn.) leaves attenuate disturbed glucose homeostasis and hyperglycemia mediated hyperlipidemia and oxidative stress in streptozotocin induced diabetic rats. *Eur J Integr Med*. 2014;6(3):307–321.
15. Nakashima S, Matsui T, Takeuchi M, et al. Linagliptin blocks renal damage in type 1 diabetic rats by suppressing advanced glycation end products-receptor axis. *Horm Metab Res*. 2014;46:717–721.
16. Marques C, Mega C, Goncalves A, et al. Sitagliptin prevents inflammation and apoptotic cell death in the kidney of type 2 diabetic animals. *Mediat Inflamm*. 2014, 538737.
17. Lenzen S. The mechanisms of alloxan- and streptozotocin-induced diabetes. *Diabetologia*. 2008;51(2):216–226.
18. OMS OMS. *Guidelines on Good Agricultural Practices and Good Harvesting Practices*. vol. 84. for medicinal plants BPAR; 2003.
19. Donald S, Prenzler PD, Antolovich M, et al. *Phenolic Content and Antioxidant Activity of Olive Extracts*. vol. 73. 2001:73–84.
20. Pourmorad F, Hosseinimehr SJ, Shahabimajd N. *Antioxidant Activity, Phenol and Flavonoid Contents of Some Selected Iranian Medicinal Plants*. vol. 5. 2006: 1142–1145. June.
21. Levin CE, Wong GK, Julkunen-tiitto R. *Phenolic Constituents in the Leaves of Northern Willows : Methods for the Analysis of Certain Phenolics*. 1985:213–217.
22. Blois MS. Antioxidant determinations by the use of a stable free radical. *Nature*. 1958;26:1199–1200. https://doi.org/10.1038/1811199a0.
23. Šoltésová D, Herichová I. On the mechanisms of diabetogenic effects of alloxan and streptozotocin. *Diabetol Metab Endokrinol Výživa*. 2011;14(3):130–138.
24. Bradford M. *A Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein-Dye Binding*. 1976.
25. Adeyemi DO, Ukwenya VO, Obuotor EM, et al. Anti-hepatotoxic activities of *Hibiscus sabdariffa* L. in animal model of streptozotocin diabetes-induced liver damage. *BMC Complement Altern Med*. 2014;14:277.
26. Buege JA, Aust SD. Microsomal lipid peroxidation methods. *Enzymologia*. 1978;52:302–310.
27. Ellman GL. Tissue sulphydryl groups. *Arch Biochem Biophys*. 1959;82:70–77.
28. Jollow DJ, Mitchell JR, Zampaglione N, et al. Bromobenzene induced liver necrosis. Protective role of glutathione and evidence for 3, 4-bromobenzene oxide as a hepatotoxic metabolite. *Pharmacology*. 1974;1:151–169.
29. Flohe L, Gunzler WA. Assays of glutathione peroxidase. In: *Methods in Enzymology*. vol. 105. Orlando: Academic Press; 1984:114–121.
30. Fridovich I. Assaying for superoxide dismutase activity: some large consequences of minor changes in conditions. 1987;566:559–566.
31. Aebi H. Catalase in vitro. *Meth Ensymol*. 1984;105, 121e6. 45.
32. Habig WH, Pabst MJ, Jakoby WB. Glutathione-S-transferases. The first enzymatic step in mercapturic acid formation. *J Biol Chem*. 1974;249:7130–7139.
33. Drury RAB, Wallington EA. *Carleton's Histological Technique*. fifth ed. Oxford: Oxford University Press; 1980.
34. Das Joydeep, Parames C, Sil. Taurine ameliorates alloxan-induced diabetic renal injury, oxidative stress-related signaling pathways, and apoptosis in rats. *Amino Acids*. 2012;43:1509–1523.
35. Bouasla Asma, Bouasla Ihcène, Boumendjel Amel, et al. Prophylactic effects of pomegranate (*Punica granatum*) juice on sodium fluoride-induced oxidative damage in liver and erythrocytes of rats. *Can J Physiol Pharmacol*. 2016;94: 1–10.
36. Sekiou O, Boumendjel M, Taibi F, Boumendjel A, Messarah M. Mitigating effects of antioxidant properties of *Artemisia herba alba* aqueous extract on hyperlipidemia and oxidative damage in alloxan-induced diabetic rats. *Arch Physiol Biochem*. 2018:1–11.
37. Zemmouri H, Sekiou O, Ammar S, et al. *Urtica dioica* attenuates ovalbumin-induced inflammation and lipid peroxidation of lung issues in rat asthma model. *Pharm Biol*. 2017;55(1):1561–1568.
38. Mestry Snehal Nitin, Dhodi Jayesh Bachu, Balbhim Kumbhar Sangita, et al. Attenuation of diabetic nephropathy in streptozotocin-induced diabetic rats by *Punica granatum* Linn. leaves extract. *J Tradit, Complementary Med*. 2017;7: 273–280.
39. Gamage AM, Lee KO, Gan Y-H. Effect of oral N-acetyl cysteine supplementation in type 2 diabetic patients on intracellular glutathione content and innate immune responses to *Burkholderia pseudomallei*. *Microbes Infect Inst Pasteur*. 2014;16(8):661–671.
40. Shittu Shehu-Tijani Toyin, Oyeyemi Wahab A, J Lasisi Taye, et al. Aqueous leaf extract of Ocimum gratissimum improves hematological parameters in alloxan-induced diabetic rats via its antioxidant properties. *Int J Appl Basic Med Res*. 2016. https://doi.org/10.4103/2229-516X.179016.
41. Nazem Farzad, Farhangi Negin, Neshat-Gharamaleki Mehrdad. Beneficial effects of endurance exercise with *Rosmarinus officinalis* Labiatae leaves extract on blood antioxidant enzyme activities and lipid peroxidation in streptozotocin-induced diabetic rats. *Can J Diabetes*. 2015;39, 229e234 Contents.
42. Daisy P, Feril G, KANI J. Evaluation of antidiabetic activity of various extracts of *Cassia auriculata* line. Bark on streptozotocin-induced diabetic Wistar rats. *Int J Pharm Pharm Sci*. 2012;4(4):312–318, 87.
43. Ashraf Hossein, Heidari Reza, Nejati Vahid, et al. Aqueous extract of *Berberis integerrima* root improves renal dysfunction in streptozotocin-induced diabetic rats. *Avicenna J Phytomed*. 2013;3(1):82–90. Winter 2013.
44. de Castro Bárbara Bruna Abreu, Colugnati Fernando Antonio Basile, Cenedeze Marcos Antonio, et al. Standardization of renal function evaluation in Wistar rats (*Rattus norvegicus*) from the Federal University of Juiz de Fora's colony. *J Bras Nefrol*. 2014;36(2):139–149.
45. Pourghasem Mohsen, Nasiri Ebrahim, Shafi Hamid. Early renal histological changes in alloxan-induced diabetic rats. *Int J Mol Cell Med Winter*. 2014;3(1).