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

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**A B S T R A C T**

**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 herba-alba* aqueous extract supplementation reduces alloxan-induced free radical generation, potentiates the antioxidant defense system and alleviates renal sensitivity to oxidative stress.

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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. Chronic hyperglycemia is associated with the development of serious long-term complications include cardiovascular disease, chronic renal failure, retinal damage. Chronic renal failure represents one of the most common and critical complications that appear in diabetic patients. According to the Centres for Disease Control and Prevention (CDC), 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. 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
mitochondrial electron-transfer chain. ROS overproduced causing lipid peroxidation, antioxidant dysfunction, cell membrane loss and organ damage. Hyperglycemia-induced oxidative stress is a molecular mechanism involved in the genesis of diabetic renal complications. Numerous studies such as those of Beisswenger et al. have shown that oxidative stress increases susceptibility to diabetic nephropathy. In addition, Witko-Sarsat et al. demonstrated that advanced oxidation protein products are oxidation signs in patients with renal impaired. 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. Mohan sandaram et al., reported a regularize levels of antioxidant enzymes in streptozotocin-induced diabetic treated by a hydroethanolic extract of Senna dialata. Patel et al., reported that pomegranate (Punica granatum Linn.) leaves attenuate hyperglycemia mediated hyperlipidemia and oxidative stress in streptozotocin induced diabetic rats. Nakashima et al., have shown that linagliptin reduces renal oxidative stress and albuminuria in diabetic mice induced by STZ, Marques et al. also have shown that Sitagliptin prevents renal inflammation and apoptosis induced by diabetic 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 (GLUT2) 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. The objective of this study was to analyze the role 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. 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. 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 Na2CO3 (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 Gallic acid, a standard range of this acid was achieved [(y = 0.0046x + 0.0263 (R² = 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 Gallic acid equivalent per gram of dry vegetable matter (mg EAG/g MS). The phenol content is expressed relatively to a reference standard of Gallic acid compound. A standard range of this acid was achieved [(y = 0.0046x + 0.0263 (R² = 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 Gallic 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 AlCl3. The total flavonoids were determined by the method described by Pourmorad et al. Briefly, 0.5 ml of the extract solution (1 mg/ml) is added to 1.5 ml of methanol, 0.1 ml of 10% AlCl3, 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 (R² = 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 Jukkunen-titto21. 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 (R² = 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. 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 sample) - (A blank)] × 100.

Where A sample 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 IC50, 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 (AlCl3), 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), tri-chloroacetic acid (TCA), thiobarbituric acid (TBA), and hydrogen peroxide were purchased from Sigma-Aldrich.
2.7. Bodyweight, urine volume and protein levels determination

Cages (Tecniplast®) isolating the control rats from those with diabetes in metabolic studies. Glucose concentration was determined using a glucometer (Accu-Chek® Aviva) every seven days along the experimental protocol. 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-16RVT, 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 an 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.

2.8. Hematological parameters and kidney function markers determinations

Measurement of hematological compounds was assessed by an auto hematocounter (Full Automatic, Blood Cell Counter, PCE-210 N6, 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. 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-16RVT, 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. 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 and modified by Jollow et al. In brief, 0.8 ml of kidney supernatant was added to 0.2 ml of 0.25% sulfosalicylic acid then tubes were centrifuged at 2500 g for 15 min using automatic refrigerated centrifuge (DCS-16RVT, 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 nmols of GSH/mg protein.

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

Glutathione peroxidase (GPx; EC 1.11.1.9) activity was measured according to the procedure of Flohe and Gunzler. This method is based on the reduction of hydrogen peroxide (H₂O₂) in the presence of reduced glutathione (GSH). The latter is transformed into (GSSG) under the influence of GPx. The superoxide dismutase (SOD; EC 1.15.1.1) activity was assayed by the method of Fridovich. SOD activity was evaluated by measuring its ability to inhibit the photo reduction of nitro-blue tetrazolium (NBT). Catalase activity (CAT; EC 1.11.1.6) in kidney homogenate was measured according to the method described by Aebi using hydrogen peroxide (H₂O₂). The enzyme activity was calculated using an
extinction coefficient of 0.043 mM$^{-1}$ cm$^{-1}$. Glutathione-S-transferase (GST; E.C 2.5.1.18) activity was calculated by Habig et al.$^{31}$ using 1-chloro-2,4-dinitrobenzene (CDNB).

12. Histopathological examination

Histopathological examination was carried out according to the technique described by Drury and Wallington.$^{33}$ Histopathological 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 stained sections were obtained 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 pair 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
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.

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 control rats (p = 0.000014). 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.

Table 3

Effects of treatment with aqueous extract of Artemisia herba alba on body weight, total protein, and absolute 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 ± 0.014****     |
| 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.

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 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*        |
| Pt (10^3/μL)               |                                | 946.8 ± 40.782 | 100825 ± 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; Pt, 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.
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 increased susceptibility to nephropathy which go beyond previous reports, showing 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 antioxidant enzymes in the diabetic group 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 capsule (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 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. 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. Enzymatic activity enhancement may be due to the antioxidant properties of AHA leaves aqueous extract.
leaves aqueous extract against alloxan-induced oxidative stress is well justified. Nitin et al. 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 H2O2 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. 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. 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. 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 protein. 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., 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., who demonstrated that creatinine clearance was strongly decreased to approximate (1.32 ml/min) after 24-h of urine collection in rats. Our results put in case a significant weight loss on alloxan-exposed rats as compared to normal rats.

![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 mean ±SD of seven animals in each group.](image-url)
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.

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Appendix A. Supplementary data

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

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