ISSN : 0974 - 7532 Volume 7 Issue 2

Trade Science Inc.

Research & Reviews in

BioSciences

Regular Paper

RRBS, 7(2), 2013 [58-65]

# *Centaurium umbellatum* attenuates nephrotoxicity induced by streptozotocin in adult rats

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## ABSTRACT

Hyperglycemia-mediated oxidative stress plays a crucial role in the progression of diabetic nephropathy. Hence, the objective of the present study was to explore the renoprotective effect of *Centaurium umbellatum* (CU) by assessing renal functions, markers of oxidative stress and antioxidant status in streptozotocin (STZ)-induced diabetic rats. Administration of CU to diabetic rats showed a significant normalization of urea, uric acid, creatinine, blood urea nitrogen (BUN) levels and creatinine/BUN ratio in comparison with diabetic rats. The elevated levels of renal lipid peroxidation (MDA) and protein carbonyls (PCO) in diabetic rats were also reversed without reaching normal values. Furthermore, CU treatment revealed a significant improvement in catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx) with a significant decline in glutathione (GSH) and ascorbic acid (Vit C) levels in kidneys of diabetic rats. Histological studies confirmed that CU effectively protected the kidneys against hyperglycemia-mediated oxidative damage. These findings demonstrated the renoprotective effects of CU by attenuating markers of oxidative stress in renal tissues of diabetic rats. 

## KEYWORDS

*Centaurium umbellatum*;
Streptozotocin induced diabetes;
Renal damage;
Antioxidant;
Status.

## INTRODUCTION

Vascular complications of *diabetes mellitus* (nephropathy, retinopathy, and neuropathy) are likely to be mediated by the associated hyperglycemia-induced intense oxidative stress, leading to increased protein glycation and subsequent production of free radicals and reactive oxygen species[1]. Renal impairment is commonly associated with diabetes. The most common long-term complication in these patients is diabetic nephropathy (DN) accounting for nearly 44% of new cases at the end stage of renal disease[2]. However, in the early course of diabetes, the presence of hyperglycemia, normal urinary protein excretion, renal hypertrophy and glomerular lesions often indicates the beginning of occurring DN. In fact, according to Mauer *et al.*[3], these changes are characteristics of both stages one and two of DN progression. Although these disturbances might be self limiting and tend to improve in response to glycemic control with oral hypoglycemic agents (OHA), a complete normalization does not usually occur. In addition, OHA are associated with seri-

ous side effects like hypoglycemic coma, hematological and hepato-renal perturbations[4]. Compared to modern allopathic medicines, herbal preparations are considered to be more cost effective and safe, with no untoward side effects[5]. This unique characteristic contributed to the world wide interest in herbal medicines as an alternative therapy to commercially available synthetic drugs. Hence, the continuous research on herbal formulation with strong antidiabetic and antioxidative properties is in progress to curtail these early changes, and possibly prevents renal failure.

*Centaurii herba* (*Centaurium umbellatum*), belonging in Gentianaceae family, is a medicinal plant used in numerous countries combined with other plants[6]. Studies in experimental animals have shown that plant mixture extract containing *Centaurium umbellatum* and other plants like *Vaccinium myrtillus* L. and *Taraxacum officinale* Web. have a variety of pharmacological functions such as: antihyperglycemic, free radical scavenging and antioxidant activities[7-9]. Phytochemical studies on this plant revealed the presence of phenolic acids[6].

To our knowledge, no studies were carried out on protective effects of *Centaurium umbellatum* (CU) in early diabetic nephropathy. Therefore, in the current study, attempts have been made to rationalize the scientific validity for the effectiveness of CU extract in the management of renal abnormalities using streptozotocin (STZ)-induced diabetic model in rats. Our results could serve as a step towards the development of an effective herbal therapy for the management of diabetes-induced renal impairments.

## MATERIALS AND METHODS

### Chemicals

STZ was purchased from Sigma Chemicals Co. (St. Louis, MO, USA); all other chemicals of analytical grade were purchased from standard commercial suppliers.

### Plant material

*Centaurium umbellatum* (CU) plants (family: *Gentianaceae*) were collected from North Tunisia during May and June (2009). Botanical identification was carried out by Emeritus Professor Abdelhamid Nabli, botanist at the Faculty of Science, University of Tunis El Manar, according to the flora of Tunisia. Voucher specimen of *C. umbellatum* was deposited at the Faculty of Pharmacy (Monastir, Tunisia).

### Preparation of Centaurium leaf extracts

Leaves of *Centaurium umbellatum* were washed quickly in running water, dried in an oven at 40°C and then finely powdered in a Willey mill. The powder was extracted with distilled water (50 g powder/500 ml water) by boiling under reflux for 20 min. The decoction obtained was centrifuged, filtered, frozen at -20°C and then lyophilised (Free Zone® Dry 4.5, USA) to yield approximately 10% (w/w) of the tansy extract, and was stored at -20 °C until used.

### Animals

In the present study adult male rats of Wistar strain weighing 230–250 g, purchased from the Central Pharmacy (SIPHAT, Tunis, Tunisia), were used. They were housed in an air-conditioned room at 22 ± 3°C with a lighting schedule of 12 h light and 12 h dark. A standard pellet diet (SICO, Sfax. Tunisia) and tap water were supplied *ad libitum*. The animals were maintained in accordance with the international guidelines for Care and use of laboratory animals[11] and approved by the Local Animal Care Committee at Sfax University.

### Experimental design

Freshly prepared STZ (dissolved in citrate buffer of pH 4.5) at a dose of 65 mg/kg was administered intraperitoneally to adult rats. Three days after STZ administration, the fasting blood glucose level was measured and rats with persistent hyperglycemia over 250 mg/dl were considered as diabetics and selected for experimentation. The experimental animals were divided into four groups of seven rats each and detailed as follows: Group 1 (C) served as control rats; group 2 (C + CU) served as control rats daily administered with CU extract (200 mg/kg bw) intraperitoneally for 30 days; group 3 (STZ) served as streptozotocin–induced diabetic rats; group 4 (STZ + CU) served as diabetic rats administered daily with CU extract (200 mg/kg bw) by intraperitoneal way for 30 days.

24 hours before sacrifice, each rat was placed in metabolic cage to collect 24-h urine volume. After measuring urine volumes, samples were centrifuged at 5000×g for 5 min in order to eliminate waste droppings. The supernatants were collected and stored at

Regular Paper

-20°C until their use. Urinary proteins were assessed by Labstix Reagent Strips (ref. Bayer 2810A). This test was based on the color change of the indicator tetrabromophenol blue. A positive reaction was indicated by a color change from yellow through green and then to greenish-blue. Proteinuria was analysed qualitatively. Its amount was estimated as follow: negative, trace, 30, 100, 300 and 2000 mg/dl.

At the end of the treatment period, the rats were fasted overnight and killed by cervical decapitation to avoid animal stress. The blood was collected without anticoagulant for serum separation. Kidneys from control and experimental groups of rats were excised and cleaned. Some kidneys were rinsed with ice-cold saline, homogenized in 10% (w/v) of phosphate buffer (0.1 M, pH 7.4) and centrifuged at 10.000 × g for 10 min at 4°C. The supernatants were collected and stored at - 80°C until analysis. Other samples were immediately fixed in 10% formalin solution for histological studies.

**Biochemical analysis**

**(a) Estimation of renal biomarkers**

Serum urea, uric acid and creatinine levels were estimated using commercially available diagnostic kits (Biomagreb, Tunisia, ref: 20143, 20091 and 20151, respectively). Blood Urea Nitrogen (BUN) was calculated using the following equation: [urea (mg/dl)] / 2.14.

**(b) Estimation of renal oxidative stress and antioxidant parameters**

The clear supernatant of renal tissue was used for assays of lipid peroxidation (MDA content), protein carbonyls (PCO) and endogenous antioxidant enzymes like reduced glutathione (GSH), superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx).

**(A) Lipid peroxidation (MDA) level**

Lipid peroxidation was estimated colorimetrically by measuring thiobarbituric acid reactive substances (TBARS) in kidney tissues by the method of Draper and Hadley[12]. 0.5 ml of tissue homogenate was treated with 1 ml of TCA reagent (30%) and centrifuged at 4000 × *g* for 10 min. 1 ml of 0.67% thiobarbituric acid (TBA) and 0.5 ml of clear supernatant were placed in water bath at 90°C for 15 min and cooled. The absorbance of samples was measured against blank at 532 nm. Values were expressed as nmol/g of tissue.

**(B) Protein carbonyl (PCO) level**

This method depends on the formation of a Schiff base from the reaction of dinitrophenylhydrazine with protein carbonyls to form protein hydrazones which was measured spectrophotometrically, as described by Reznick and Packer[13]. Briefly, after precipitation of protein with 0.5 ml of 20% trichloroacetic acid, the pellet was resuspended in 10 mM DNPH in 2 N HCl. Next, after the washing procedure with 1:1 ethanol-ethylacetate, the final palette was dissolved in 6 M guanidine. The carbonyl group was determined from the absorbance at 370 nm. The carbonyl content was calculated in terms of nmol/mg of protein.

**(C) Reduced glutathione (GSH) level**

The GSH level in the kidney was estimated using the method described by Ellman[14] modified by Jollow *et al.*[15]. Briefly, the renal homogenate was mixed with 4% w/v sulfosalicylic acid in ratio of 1:1 and centrifuged at 4 °C for 10 min at 3500 × *g*. 0.2 ml of the obtained supernatant was mixed with 1 ml of 0.1 M potassium phosphate buffer (pH 7.4) and 0.4 ml of 10 mM freshly prepared DTNB [5, 52 -dithiobis (2-nitrobenzoic acid)]. The reaction mixture was incubated for 10 min and absorbance of yellow colored complex was noted spectrophotometrically at 412 nm. The results were expressed as µg/g of tissue.

**(D) Ascorbic acid (Vit C) level**

Ascorbic acid (Vit C) determination was performed as described by Jacques-Silva *et al.*[16]. Protein in kidney homogenate extract was precipitated in 10 volumes of a cold 4% trichloroacetic acid solution. An aliquot of supernatant (300 µl) was adjusted with $H_2O$ to a final volume of 1 ml and incubated at 38 °C for 3 h, then 1 ml of $H_2SO_4$ 65% (v/v) was added to the medium. The reaction product was determined using color reagent containing 4.5 mg/ml dinitrophenyl hydrazine and $CuSO_4$ (0.075 mg/ml). The data were expressed as µmol ascorbic acid/g of tissue.

**(E) Catalase (CAT) activity**

The activity of CAT was determined by the method of Aebi[17]. The reaction mixture contained 20 µl of kidney homogenate, 1.880 ml of phosphate buffer (0.1 M, pH 7.5) and 0.1 ml of 0.5 M hydrogen peroxide ($H_2O_2$). Changes in absorbance were recorded at

240 nm. CAT activity was expressed as μM of $H_2O_2$ consumed/min/mg of protein.

**(F) Superoxide dismutase (SOD) activity**

SOD was assayed by the method of Beauchamp and Fridovich[18]. Briefly 0.05 ml of kidney homogenate was diluted with 1.8422 ml of potassium phosphate buffer (pH 7.4). The activity of the enzyme in the supernatant was determined by adding 1 ml of $Na_2$EDTA-Methionine, 85.2 μl of NBT (2.64 mM) and 22.6 μl of Riboflavine (0.26 mM) to 50 μl of supernatant in a total volume of 3 ml. After incubation at ambient temperature for 20 min under light, the intensity of the colour was measured at 580 nm against blank. One unit of enzyme activity is defined as the enzyme quantity which gives 50% inhibition of NBT reduction under the assay conditions and expressed as specific activity in units/mg of protein.

**(G) Glutathione peroxidase (GPx) activity**

The activity of GPx was measured by the method described by Flohe and Gunzler[19]. Briefly, the reaction mixture contained 0.1 ml phosphate buffer (0.1 M, pH 7.4), 0.2 ml of tissue homogenized in phosphate buffer (0.1 M, pH 7.4), 0.2 ml glutathione (4 mM) and 0.5 ml $H_2O_2$ (5 mM). The contents were incubated for 10 min at 37 °C, 1 ml 5% TCA was then added to precipitate proteins and centrifuged at 3200 × *g* for 20 min. The supernatant was assayed for glutathione content using DTNB (5,52 -dithiobisnitrobenzoic acid) reagent (10 mM). The activity was expressed as μmol of GSH consumed/min/mg of protein.

**(H) Total protein level**

The renal total protein content was estimated by Lowry's method[20] using bovine serum albumin (BSA) as a standard. Briefly, 0.2 ml of diluted homogenate was added to a same volume of Lowry's reagent. The mixture was treated with 0.2 ml of Folin–Ciocalteu reagent diluted to ½ and then incubated in the dark for 30 min. The standard curve was plotted using BSA. The protein content was determined spectrophotometrically at 500 nm.

**Assays for renal histology**

Some kidneys, collected from control and tested rats, were fixed in 10% formalin solution for 48 h. They were subjected to standard routine tissue processing technique and embedded in paraffin. Sections of 5 μm thickness were cut from each block and stained with hematoxylin–eosin (H&E) for histological examination. Six slides from each kidney were studied by light microscopy. They were examined and scored for severity of histological changes in kidney sections using scores on a scale absent (-), weak (+), moderate (++) and intense (+++).

**Analysis of data**

ANOVA, followed by a post hoc Fisher's protected least significant difference (PLSD) test, were used to test significance of differences between groups [(STZ, STZ + CU, C + CU) *vs.* (C)] and [STZ + CU] vs. [STZ]. A *P* value of ≤ 0.05 was considered significant. The procedures were carried out using the statistical package program Stat view 5 Software for Windows (SAS Institute, Berkley, CA). Values are displayed as means ± SD.

## RESULTS

**Changes in renal function related parameters**

**(a) Urea, uric acid, creatinine and BUN levels**

Renal function was evaluated by measuring the serum levels of urea, uric acid, creatinine, BUN, and BUN/creatinine. As shown in TABLE 1, streptozotocin treatment produced a significant ($p<0.001$) rise in serum levels of urea, uric acid, creatinine and BUN in comparison with control group. Interestingly, thirty days treatment with CU extract reduced significantly ($p<0.001$) the elevated serum parameters in diabetic rats and reached control values for some ones (uric acid and creatinine). No statistical differences were shown between CU treated control rats and control rats.

**(b) 24 hours urine volume and urinary protein levels**

We found that 24 hours urine volume in diabetic and CU treated diabetic rats was 4 fold higher than that of controls. Protein appeared in trace (<30 mg/dl) both in diabetic and CU treated diabetic groups. In control group treated with CU, these parameters were not significantly changed, when compared to controls (TABLE 1).

**Renal lipid peroxidation and protein carbonyls**

TABLE 2 showed renal MDA and PCO levels in diabetic rats which were respectively 3 fold and 6 fold

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higher than those of controls. Treatment of diabetic rats with CU extract for 30 days resulted in a marked decrease ($p < 0.001$) in kidney MDA and PCO levels compared to those of diabetic group. No significant changes were observed in control group treated with CU compared to control group.

**TABLE 1 : Serum levels of urea, uric acid, creatinine, BUN, urinary protein, 24 hours urine volume and BUN/creatinine ratio in adult rats (control and experimental groups).**

| Groups | Control (C) | Diabetes (STZ) | Diabetes + CU (STZ + CU) | Control + CU (C + CU) |
|---|---|---|---|---|
| Urea [a] | 29.50 ± 4.50 | 160.76 ± 9.16$^{***}$ | 40.83 ± 6.69$^{*+++}$ | 25.23 ± 3.64 |
| Uric acid [b] | 4.07 ± 0.16 | 12.21 ± 0.15$^{***}$ | 4.10 ± 0.06 $^{+++}$ | 4.20 ± 0.11 |
| Creatinine [c] | 7.07 ± 0.82 | 13.57 ± 1.18$^{***}$ | 7.57 ± 1.04$^{+++}$ | 7.11 ± 0.62 |
| BUN [d] | 13.78 ± 2.10 | 75.12 ± 4.28$^{***}$ | 19.08 ± 3.12$^{*+++}$ | 11.97 ± 1.70 |
| BUN/Creatinine | 1.98 ± 0.37 | 5.56 ± 0.48$^{***}$ | 2.59 ± 0.76$^{+++}$ | 1.67 ± 0.20 |
| 24h urine volume [e] | 7.5 ± 0.8 | 30 ± 1.2$^{***}$ | 28 ± 1.5$^{***}$ | 8.2 ± 0.6 |
| Urinary protein [f] | Negative | Negative | Trace | Trace |

**Data are expressed as mean ± S.D. (n = 7); Treated groups (STZ); (STZ + CU); (C + CU) *vs* control group (C): $^{*}p < 0.05$; $^{***}p < 0.001$; (STZ + CU) group *vs* (STZ) group: $^{+++}p < 0.001$; [a, b, c, d, f]: (mg/dl); [e]: (ml).**

**TABLE 2 : Malondialdehyde (MDA), protein carbonyl (PCO), glutathione (GSH), ascorbic acid (Vit C) levels and antioxidant enzyme activities (CAT, SOD and GPx) in kidney of adult rats (control and experimental groups).**

| Groups | Control (C) | Diabetes (STZ) | Diabetes + CU (STZ + CU) | Control + CU (C + CU) |
|---|---|---|---|---|
| MDA [a] | 65.25 ± 6.95 | 182.80 ± 21.16$^{***}$ | 82.38 ± 16.54$^{*+++}$ | 63.66 ± 7.18 |
| PCO [b] | 2.12 ± 0.18 | 13.13 ± 0.21$^{***}$ | 3.32 ± 0.07 $^{*+++}$ | 2.17 ± 0.14 |
| GSH [c] | 110.55 ± 11.92 | 75.90 ± 12.13$^{***}$ | 99.59 ± 17.01$^{+++}$ | 109.13 ± 10.52 |
| Vit C [d] | 163.47 ± 10.91 | 118.72 ± 9.11$^{***}$ | 146.11 ± 6.69$^{**+++}$ | 165.75 ± 15.47 |
| CAT [e] | 94.58 ± 11.78 | 74.31 ± 5.52$^{**}$ | 86.02 ± 6.22$^{+}$ | 91.90 ± 13.06 |
| SOD [f] | 509.72 ± 31.75 | 184.00 ± 31.22$^{***}$ | 330.85 ± 21.32$^{***+++}$ | 501.24 ± 36.82 |
| GPx [g] | 7.26 ± 0.21 | 1.49 ± 0.13$^{***}$ | 4.90 ± 0.33$^{*+++}$ | 7.55 ± 0.34 |

**Data are expressed as mean ± S.D. (n = 7); Treated groups (STZ); (STZ + CU); (C + CU) *vs* control group (C): $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$; (STZ + CU) group *vs* (STZ) group: $^{+}p < 0.05$; $^{+++}p < 0.001$; [a] : (nmol/g tissue); [b]: (nmol/mg protein); [c]: (µg/g tissue); [d]: (µmol/g tissue); [e]: (µmol $H_2O_2$/min/mg protein); [f]: (U/mg protein); [g]: (nmol GSH/min/mg protein).**

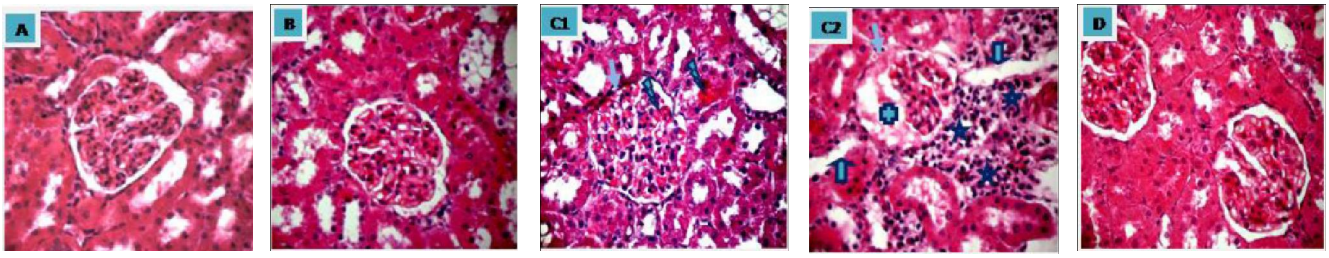


**Fig. 1**
Kidney histological sections in adult rats (control and experimental groups). Optic microscopy; hematoxylin–eosin stain; magnification A, B, C1, C2, D: (400×). (A) control group, (B) CU treated control group, (C1, C2) STZ diabetic group, (D) CU treated diabetic group.

→ : reduced glomerular space, ↘ : vascular congestion, ➡ : tubular vacuolization, ★ : infiltration of leukocytes, ✚ : glomerular lesions.

## Renal GSH and ascorbic acid (Vit C) contents

As reported in TABLE 2, the contents of GSH and Vit C in kidneys of diabetic rats were significantly ($p < 0.05$) reduced by 31% and by 27% when compared to those of controls. CU extract treatment restored partially GSH and Vit C contents in kidney of diabetic group without reaching normal values. CU extract, administered alone to rats during 30 days, did not change both GSH and Vit C kidney contents.

## Renal antioxidant enzyme activities

TABLE 2 showed the activities of enzymatic anti-

oxidants (CAT, SOD and GPx) in kidneys of different groups of rats. CAT, SOD, and GPx decreased significantly ($p < 0.01$) in diabetic rats by 21%, 64% and 79% respectively. Administration of CU extract significantly ($p < 0.05$) improved the activities of these enzymes in diabetic treated rats and did not alter enzyme activities in control group.

### Renal structural changes

Upon kidney histological examination of both control and CU treated control rats, renal tissue architecture showed normal glomeruli and tubules (Figure 1, A and B). While, kidney in diabetic rats exhibited: a reduced glomerular space, glomerular lesions, a vascular congestion, a tubular vacuolization and an infiltration of leucocytes (Figure 1, C1 and C2). The severe kidney damages, shown in diabetic rats, significantly decreased when CU was administered to diabetic rats for 30 days. So, histological pattern in the latter group became similar to that of control groups (Figure 1, D). The histopathological changes are graded and summarized in TABLE 3.

**TABLE 3 : Scores of the histological changes in the kidney sections.**

| Groups | Control (C) | Diabetes (STZ) | Diabetes +CU (STZ + CU) | Control + CU (C+ CU) |
|---|---|---|---|---|
| Reduced glomerular space | - | +++ | - | - |
| Vascular congestion | - | + | - | - |
| Leucocytes infiltration | - | +++ | - | - |
| Tubular vacuolization | - | ++ | - | - |

**Scoring was categorized as none (-), mild (+), moderate (++) and severe (+++).**

## DISCUSSION

With the increasing number of diabetic patients and limited therapeutic options, diabetic nephropathy (DN) is a long term complication of *diabetes mellitus*. The precise mechanism of DN is not yet fully understood and the effective blockage of the progression of nephropathy remains as a therapeutic challenge. From this point, our attention has been focused on *Centaurium umbellatum*, a medicinal plant known for its antihyperglycemic activity in diabetic animals. We demonstrated that CU attenuated early diabetic nephropathy syndrome in STZ induced diabetic rats characterized by normal proteinuria and renal impairment.

The first finding of interest in our study concerns the effects of CU extract on the renal biomarkers. In fact, we demonstrated that STZ-induced diabetic rats exhibited higher serum urea and uric acid levels than those of control group. A significant elevation in serum urea and uric acid levels indicated, according to Shinde and Goyal[21], an impaired renal function of diabetic animals. Thus, it would appear that CU treatment lowered the serum urea and uric acid levels by enhancing the renal function. Our results were in agreement with other previous studies using mesocarp extract of *Balanites aegyptiaca*[22].

Besides, both BUN and creatinine were accumulated in the blood when the kidney function was disturbed, and the ratio of BUN/creatinine increased also, indicating that dehydration was present. In our study, serum BUN levels in STZ induced diabetic rats increased above normal reference values. CU treated diabetic rats provoked a significant decrease in BUN, serum creatinine levels and BUN/creatinine ratio, showing the return of BUN levels to the normal reference range. These results confirmed the protective effects of CU extracts.

Furthermore, in the current study, protein appeared in trace in the urine of STZ-induced diabetic rats. The low amounts of protein in the urine, determined qualitatively, indicated the early stage of DN. As reported by Sinha *et al.*[23], mild proteinuria has been observed in experimental rats four weeks after STZ induction of diabetes. DN is characterized by an increase of urine protein excretion and renal dysfunction. Many studies have shown that the magnitude of urine protein level is associated with a graded increase in the risk of progression to end-stage renal disease and cardiovascular events[24]. Administration of CU extract in diabetic rats had no effect on urinary protein levels.

The second finding of interest in our study concerned the effects of CU extract on the oxidative stress which was a key pathogenic factor in the development of diabetic nephropathy[25]. It has been suggested by our previous study[26] that high glucose-induced tissue damage was associated with excessive production of reactive oxygen species (ROS) under hyperglycemic conditions. In this context, lipid peroxides and protein carbonyls are the secondary products of oxidative stress considered as a result of the toxic effects of ROS during diabetes. Our results showed an enhanced production of MDA and PCO in STZ induced diabetic rats. These

Regular Paper

changes were improved by *Centaurium umbellatum* extract indicating that this plant had a renal protective role against oxidative damage in early stage of diabetic nephropathy. This normalization might be accomplished by the presence of phenolic acids in CU extract, as previously reported by Hatjimanoli and Debelmas[6]. According to Soetan[27], these compounds have antioxidant activities.

The inexorable generation of reactive free radicals and lipid peroxides during diabetes mediated oxidative stress could be correlated with the decline of non-enzymatic antioxidants levels like vitamin C and GSH. The latter is mainly involved in the synthesis of important macromolecules and in the protection against ROS[28]. In our study, a marked decrease in renal GSH was observed in the STZ-induced diabetic rats. GSH is required for the recycling of vitamin C and acts as a substrate for glutathione peroxidase (GPx) and glutathione-S-transferase (GST), which are involved to prevent the deleterious effects of oxygen radicals[29]. Thus, the observed decrease in GSH level in kidney of diabetic rats might be due to either its enhanced utilization in order to scavenge free radicals and to increase consumption by GPx and GST or to the increased oxidation of GSH formation. Upon CU treatment, the content of GSH was increased in diabetic rats. This might be a factor responsible for inhibition of lipid peroxidation and protein oxidation through GSH redox cycle and for a direct detoxification of ROS generated.

Another non-enzymatic antioxidant, ascorbic acid is considered as an important component of the cellular defense against oxygen toxicity and lipid peroxidation induced by free radical potential. As reported by Samuel *et al.*[30], reduced levels and altered metabolic turnover of ascorbic acid have been shown in diabetic patients. In our study, the observed decrease of ascorbic acid in the kidney of STZ-induced diabetic rats supported these findings. It has been shown that the high extracellular glucose concentration in diabetes may further impair the cellular uptake of ascorbic acid and accentuates the problems which are associated with its deficiency[30]. On the other hand, the enhanced kidney ascorbic acid levels, caused by CU extracts, brought a consolidation of kidney antioxidant status and its protection from free radical damage. This correlated with the observed reduction in the level of lipid peroxidation and protein oxidation. We suggested that the increase in ascorbic acid content of CU treated diabetic rats resulted probably from the high ascorbic acid content of CU.

Chronic hyperglycemia often associated with a notable decline in the levels of intracellular antioxidants and an elevation of the formation of pro-oxidants like ROS eventually resulted in renal dysfunction. There are numerous enzymatic and non-enzymatic sources of ROS in the diabetic kidney, such as transition metal-catalyzed Fenton reactions, polyol pathway flux, mitochondrial respiratory chain deficiencies, peroxidases and autoxidation of glucose[31]. Such glucotoxicity is possibly related to the vulnerability of kidney tissues to oxidative stress. Due to these events, an imbalance of oxidant/antioxidant defense systems results in alterations in the activity of antioxidant enzymes, such as SOD, CAT and GPx. In our study the activities of the key enzymatic antioxidants were reduced in kidney during oxidative stress in diabetic rats and improved after CU treatment. Our results were in agreement with previous study of Kuhad *et al.*[32] who reported the protective effects of sesamol, a natural compound with antioxidant activities, against oxidative stress induced in diabetic rats.

The renal tissue defensive effects of CU extract in experimental groups of rats was ascertained by histological studies. The diabetogenic potential of streptozotocin is responsible for a progressive development of the renal tissue lesions. Consequently, the major morphological abnormalities were observed in diabetic kidney tissues like: a glomerular space reduction, glomerular lesions, a vascular congestion, a tubular vacuolization and leucocytes infiltration. Such alterations were not found in kidneys of diabetic rats treated with CU, confirming its protective capacity during diabetes. There are no such studies available on the protective effects of CU extract on the structure of kidney tissue during experimental diabetes. Hence, the present investigation should be considered an innovative assessment for the protective effects of CU in kidney of streptozotocin induced diabetic rats.

*Centaurium umbellatum* a traditional herbal medicine, may be used as an adjuvant therapy in the treatment of diabetes and also may be helpful to prevent and/or delay the onset of diabetes-induced renal injury. Further studies will be required to identify the major active constituents of CU which are responsible for the beneficial renal effects observed in the present study.

In conclusion, *Centaurium umbellatum* showed an anti-diabetic effect *via* reducing hyperglycemia in order to regulate antioxidant protection system and to attenuate alterations observed in glomeruli and renal tubules of STZ diabetic rats, consequently there are less damages in renal tissue. Besides, the antioxidative properties of CU for alleviation of diabetic nephropathy could not be excluded.

## ACKNOWLEDGEMENTS

This work was supported by the DGRST grants (Appui à la Recherche Universitaire de base, ARUB UR 11/ES70), Tunisia. The authors are indebted to Miss Dalenda Kchaou for her assistance in histological techniques.

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