# Protective Effect of Royal Jelly against Renal Damage in Streptozotocin Induced Diabetic Rats

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## ABSTRACT

**Background**: Royal jelly has been shown to have antioxidant and antidiabetic effects. The objective of this study was to evaluate the protective effect of RJ against kidney damage in streptozotocin induced diabetic rats.

**Methods:** Thirty two male Wistar rats were divided randomly into four groups (n=8 per group). Normal control and diabetic control groups received 1cc/day distilled water, normal RJ-treated and diabetic RJ-treated groups received 100mg RJ/kg body weight daily. Diabetes was induced by intraperitoneal injection of streptozotocin. At the end of the experiment, urine and kidney samples were collected for biochemical and histopathological analysis.

**Results:** The results showed that diabetes could increase levels of urine urea, total protein and albumin significantly, and could decrease the levels of creatinine and uric acid in urine. In the kidney tissue homogenates, catalase activity and antioxidant power were significantly lower, whereas malondialdehyde levels were significantly higher in diabetic group when compared with control group. Diabetic rats showed severe histological changes in kidney tissues. Treatment of diabetic rats with RJ improved significantly all of these parameters.

**Conclusion:** The present study revealed that treatment with RJ resulted in significant improvement in histopathological alterations in kidney tissue and urine parameters of diabetic rats. This could be due to its antioxidant activity and the ability of RJ for scavenging the free radicals released in diabetes. These findings suggest that RJ has protective effects on kidneys affected by diabetes mellitus.

**Keyword:** Diabetes Mellitus, Oxidative Stress, Rats, Renal, Royal Jelly, Streptozocin.

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## INTRODUCTION

Diabetic nephropathy is one of the major late complications of diabetes mellitus [1]. The first signs of diabetic nephropathy are characterized by increase in renal size, glomerular volume, and renal load followed by accumulation of glomerular extracellular matrix and an abnormal increase in the urinary albumin excretion. End-stage overt diabetic nephropathy is characterized by hypertension, proteinuria, and progressive renal insufficiency [2]. Impairment of renal function is one of the most important features of diabetes. Decreased urinary concentrations of creatinine and uric acid and increased levels of urea have been shown in diabetes [3]. Chronic hyperglycemia results in elevated production of free radicals, especially reactive oxygen species (ROS) and protein glycosylation and glucose autoxidation in all tissues [4]. Damage of enzymes and cellular organelles and increase in malondialdehyde levels can be caused by increased levels of free radicals and simultaneous decline of antioxidant defense mechanisms [5-7]. In kidneys of diabetic rats, lower activity of key antioxidant enzymes, such as catalase (CAT), superoxide dismutase (SOD) and glutathione-s-transferase, was observed [8]. Short-term effects of diabetes on kidney histology include cortical hypertrophy accompanied by glomerular mesangial hypertrophy. Increased glomerular basement membrane thickness is an indicator of long-term effects of diabetes [9].

Royal jelly (RJ) is a food item secreted from the hypopharyngeal and mandibular glands of the worker honeybees [10]. On the other hand, RJ has antioxidant activity that can

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counteract lipid peroxidation caused by free radicals under different stress conditions [11], hypoglycemia, wound healing process [12], and has been used to thwart the toxic effects of some chemical agents [13]. Abd El-Monem reported that RJ could ameliorate antioxidant status of reduced glutathione (GSH) and inhibition of MDA production induced by Malathion in rat cells [14]. Hence, in the present study the effect of RJ was investigated on renal damage and oxidative stress in streptozotocin-induced diabetic rats.

## MATERIALS AND METHODS

### *Animals*

Thirty two healthy male Wistar rats (weight, 200 ± 10 gr) obtained from Urmia University's Animal Lab were used for the study. They were housed under controlled conditions with a 12-h light/dark cycle at 21 ± 2 ˚C. They had free access to standard pellet diet and water ad libitum. This study is in accordance with the Guidance of Ethical Committee for Research on Laboratory Animals of Urmia University.

### *Induction of Experimental Diabetes*

All rats were randomly divided into four groups (n=8/group): normal control and diabetic control groups received 1cc of distilled water by gavage. Normal RJ-treated and diabetic RJ-treated groups received 100 mg RJ/kg BW. dissolved in 1cc distilled water orally. Diabetes was induced by a single intraperitoneal injection of STZ (Sigma, St. Louis, MO, USA) at 60 mg/kg BW freshly dissolved in 0.1 M citrate buffer (pH 4.6) [15, 16]. After 72 h from the administration of STZ, the tail vein blood glucose level was measured with a glucometer (Roche, Switzerland), and the rats with blood glucose levels of ≥ 300 mg/dL were considered diabetic.

### *Sample Collection and Biochemical Assays*

After 42 days of RJ treatment, the volume of collected urine was used to analyze creatinine, albumin, urea, total protein and uric acid levels. The rats were then sacrificed and kidneys were quickly excised off. One portion of the kidney was stored in 10 % buffered formalin solution for histopathological evaluation. The rest of the kidney sample was washed with saline and kidney homogenate was prepared in 0.5M phosphate buffer (pH 7.4). The homogenates were centrifuged and the supernatant was used for determination of MDA, ferric reducing antioxidant power (FRAP) levels and catalase activity.

### *Biochemical Analysis*

MDA levels in the kidney tissue homogenates were determined spectrophotometrically using the method described by El-Beshbishy *et al*. [17]. Briefly, 150 microliters of homogenate was added to 300 microliters of 10 % trichloroacetic (TCA) acid and was centrifuged at 1000 rpm for 10 min at 4 ˚ C. Afterwards, 300 microliters of supernatant was added to 300 microliters of 0.6 % solution of thiobarbituric acid (TBA) in a centrifuge tube and the mixture was placed in boiling water bath for 15 min. After cooling, a pink color appeared due to of MDA - TBA reaction and its absorbance was measured at a wavelength of 532 nm. The MDA values were expressed as nanomoles per milligrams of tissue. The activity of catalase enzyme in kidney tissue homogenate was measured using the method described by Pandir *et al*. [18]. The reaction mixture (1.5 mL, vol) containing 1.0 mL of phosphate buffer (0.01M; pH 7.0) and 0.1 mL of tissue homogenate was added to 0.4 mL of 2 ml $H_2O_2$. The change in absorbance was read at 240 nm in a spectrophotometer (Pharmacia, Novaspec II, and Biochrom, England). The catalase activity was expressed as units / mg of tissue protein.

Total antioxidant capacity in the kidney homogenate was evaluated using ferric reducing antioxidant power (FRAP) assay. The FRAP assay was determined as described by Benzie IFF and Strain J. method [19]. To prepare the FRAP reagent, a mixture of 25 ml acetate buffer (pH 3.6), 2.5 ml TPTZ (2, 4, 6-tripyridyl-s-triazine) in 40 mM hydrochloride acid (HCl) and 2.5 ml FeCl3.6H2O (10:1:1, v/v/v) was prepared. Briefly, 50 µl of renal homogenate was added to 1.5 ml fresh FRAP reagent in a centrifuge tube and was incubated at 37 ºC for 10 min. The absorbance of the blue colored complex was determined by spectrophotometer (Pharmacia, Novaspec II, and Biochrom, England) at 593 nm. FRAP value in tissue was expressed as µmoles/milligram of wet tissue.

Urine samples were centrifuged and the supernatant was separated to use for

determination of urea, uric acid, creatinine, total protein, and albumin. The levels of creatinine, uric acid, urea, albumin and total protein in the urine were determined spectophotometrically according to standard procedures using a commercially available diagnostic kit (Pars Azmoon, Tehran, Iran) [20].

### *Histopathological Analysis*

The tissue was fixed for 48 hour in 10 % formalin solution and was embedded in paraffin. Microtome sections (5 µm thick) were prepared from kidney samples and stained with periodic acid shiff (PAS) [21] and observed under a light microscope to evaluate the details of renal architecture in each group microscopically.

### *Statistical Analysis*

Results were expressed as means ± SEM. The differences between groups were evaluated with One-Way ANOVA and Tukey test using SPSS package (version 18). Differences were considered statistically significant at $P < 0.05$.

## RESULT

Table 1 shows the levels of total protein, albumin, urea, uric acid, creatinine in the urine of different experimental groups.

The diabetic group showed a significant elevation in urine total protein and albumin when compared to normal control and RJ groups. Oral administration of RJ to diabetic rats significantly reversed the above biochemical changes ($P < 0.05$; Table 1).

In our study, the levels of uric acid, urea and creatinine significant decreased in the urine of rats in diabetic group as compared to control and RJ groups. Diabetic rats treated with RJ showed the reversal of these parameters towards normal. On the other hand, there was no significant difference between RJ-treated diabetic, control and RJ groups ($P < 0.05$; Table 1). MDA levels in kidneys were increased in untreated diabetic rats when compared to those of the control and RJ groups ($P < 0.05$). In the RJ-treated diabetic group, mean MDA level was significantly lower when compared to untreated diabetic rats but was not significantly different from controls (Table 2; $P < 0.05$).

To understand the effect of RJ on antioxidants, we measured the activity of CAT which was significantly lower in the kidney homogenates of untreated diabetic rats as compared to the controls. The CAT activity in the kidneys was increased in the RJ-treated diabetic group compared to the diabetic group.

Total antioxidant capacity in kidney tissue samples were analyzed using FRAP assay. Kidneys of diabetic rats showed significant decrease in antioxidant capacity in comparison with control rats. The antioxidant capacity in the kidneys of treated diabetic rats was higher than those of the diabetic group (Table 2; $P < 0.05$).

**Table 1.** Effect of RJ on levels of urea, uric acid, creatinine and albumin in the urine of control and diabetic rats, n=8/group.

| Groups | Urea (mg/dl) | Ureic acid (mg/dl) | Cr (mg/dl) | ALB (mg/dl) | TP (mg/l) |
|---|---|---|---|---|---|
| **Control** | 136.07±3.93 a | 7.86±0.45 a | 8.99±0.37 a | 6.65±0.46 a | 71±9.5 a |
| **RJ** | 137.89±2.86 a | 8.01±0.41a | 8.83±0.24 a | 6.91±0.18 a | 61±3.7 a |
| **Diabetic** | 106.79±3.12 b | 4.75±0.32 b | 6.33±0.12 b | 17.76±2.30 b | 176±3.7 b |
| **Diabetic/RJ** | 120.02±3.52 c | 7.76±0.57 a | 8.94±0.97 a | 6.71±1.16 a | 66±6.9 a |

Data are presented as mean ± SE for 8 rats in each group. Values with different letters indicate significant differences among groups at $P < 0.05$.

**Table 2.** Effect of royal jelly administration on the malondialdehyde (MDA), ferric reducing antioxidant power (FRAP) and catalase (CAT) in kidney tissue samples, n=8/group.

| Groups | **MDA** (µmol/gr protein) | **CAT** (units/mg tissue) | **FRAP** (µm/mg tissue) |
|---|---|---|---|
| **Control** | 13.3 ± 0.54 a | 2.1 ± 0.15 a | 6.71 ± 0.51 a |
| **RJ** | 13.1±1.11 a | 2.2 ± 0.09 a | 6.98 ± 0.39 a |
| **Diabetic** | 31.5±1.50 b | 1.1 ± 0.12 b | 2.83 ± 0.14 b |
| **Diabetic/RJ** | 17.6±1.23 a | 1.8 ± 0.15 a | 7.49 ± 0.80 c |

Data are presented as mean ± SE for 8 rats in each group. Values with different letters indicate significant differences among groups at $P < 0.05$.

### *Histopathology Findings*

Histological study of the normal kidneys of the control rats revealed normal glomeruli surrounded by the Bowman's capsule, distal and proximal convoluted tubules without any changes (Fig. 1). The diabetic group demonstrated atrophy of glomerular capillaries (2a), dilation of Bowman's space (urinary space) (2b), and acute tubular necrosis (2c) (Fig. 2). Fig. 3 shows the renal tissues of diabetic rats exhibiting severe tubular epithelial necrosis (3a), glomerular focal necrosis (3b), and massive necrosis in the proximal convoluted tubules (3c). The diabetic group that were treated with RJ showed features of healing similar to normal glomerulus and the absence of necrotic cells in the proximal convoluted tubule could be easily appreciated (Fig. 4).

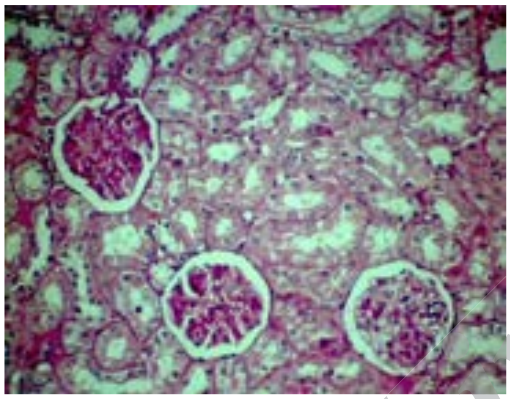

**Figure 1.** Photomicrograph of control rat kidney section showing normal renal histoarchitecture with well developed distal and proximal convoluted tubules and normal glomerulus and Bowman's capsule (PAS, ×100).

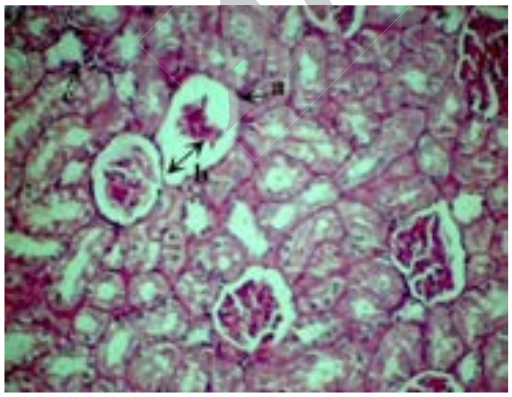

**Figure 2.** Photomicrograph of kidney in a untreated diabetic rat showing atrophy of glomerular capillaries (a), glomerular focal necrosis (b) and necrosis in the proximal convoluted tubule (c) (PAS, × 100).

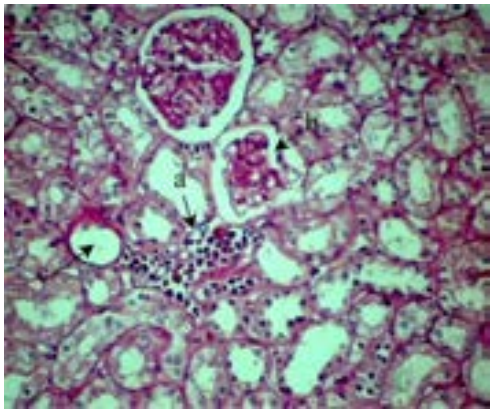

**Figure 3.** Histopathological image of kidney from diabetic rats. Figure showing tubular epithelial necrosis (a), dilation of Bowman capsular space (b), and massive necrosis in the proximal convoluted tubules (c) (PAS, ×100).

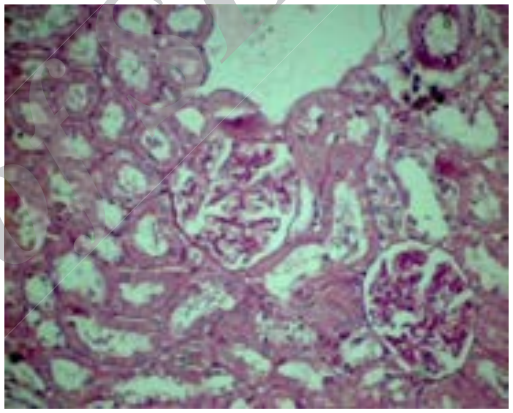

**Figure 4.** Photomicrographs of diabetic treated with RJ rat shows normal morphological architecture and significant degenerative cells reduction (PAS, ×100).

## DISCUSSION

In diabetic rats we observed decreased levels of creatinine, uric acid, urea and increased levels of albumin and total protein but treatment with RJ reversed these parameters to near normal levels.

Urinary albumin rose in diabetic rats owing to protein leak from the kidney as a consequence of metabolism and cell turnover. The release of these proteins was increased due to renal function impairment as is usually seen in chronic diabetes [22]. Gupta R. *et al.* observed an increase in urinary protein level in diabetic rats [23]. Murugan P. and Pari L. reported significant increase in concentration of urinary urea and significant decreases in urinary uric acid and createnine levels in untreated diabetic

rats [20]. Our findings are in agreement with the results of the mentioned studies.

El-Nekeety AA. *et al.* stated that rats treated with fumonisin showed significant increase in serum createnine and uric acid levels while the addition of RJ to fumonisin resulted in significant improvement in all tested parameters towards normal values [13]. In the current study, treatment with RJ reversed urine createnine and uric acid to near normal which could be due to decreased metabolic disturbances of other pathway such as nucleic acid metabolism and protein as evidenced by improved glycemic control.

In a study by Gomathi D. *et al.*, the CAT activity was significantly decreased in the kidney tissues of the diabetic group as compared to the controls, but lipid peroxidation (MDA) levels were significantly increased in the kidney tissue of the diabetic group compared to controls [24]. Our results on MDA levels and CAT activity are in agreement with this report.

A previous study revealed that RJ caused significant recovery in antioxidant status of glutathione (GSH), superoxide dismutase (SOD) and CAT and a significant inhibition of malondialdehyde (MDA) production in renal tissue of rats treated with cisplatin [25]. Guo *et al.* stated that RJ peptides have strong hydroxyl radical scavenging quality. Accordingly, Guo H *et al.* reported that RJ peptides content show antioxidant activity, preventing MDA production [26]. Our present study demonstrated significant decrease in MDA levels in kidney tissues and significant increase in the activity of kidney tissue CAT in RJ-treated diabetic group.

Cakatay U. and Kayali R. showed that total antioxidant capacity in the plasma of chronic diabetic rats was decreased significantly [27]. The present work indicated the same results and showed significant reduction in kidney homogenate's FRAP in untreated diabetic rats. Treatment with RJ improved significantly antioxidant power of kidney homogenate samples in RJ-treated diabetic rats when compared with the untreated diabetic one.

Our results are consistent with the results of Zafar *et al.* who reported that diabetes caused severe histological changes in renal tissues of rats [28].

## CONCLUSION

The present study revealed that treatment with RJ resulted in significant improvement in histopathological alterations in kidney tissue and urine parameters of diabetic rats (urea, uric acid, createnine, total protein and albumin). This could be due to its antioxidant activity and the ability of RJ for scavenging the free radicals released in diabetes.

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