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ORIGINAL ARTICLE

# Antioxidant Effect of Royal Jelly on Immune Status of Hyperglycemic Rats

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## ABSTRACT

**Context:** Diabetes mellitus and its increasing effect on endocrine functionality have been widely observed. **Aims:** The present study aims at investigating the histopathological changes of splenic cells in diabetic rats. **Subjects and Methods:** The experiment was conducted on adult male albino rats. They were categorized into four groups: Group I (negative control; fed with standard basal diet [SBD]), Group II (positive control; fed with SBD), Group III (fed on SBD + dose of 50 mg/kg of body weight royal jelly [RJ]) and Group IV were given orally RJ 100 mg/kg of b.wt. The food intake ratio and body weight gain were monitored on a weekly basis. The blood sample and organ tissue samples were collected at the end of feeding and biochemical and immunological analysis was performed for determining the blood glucose level and immune status of the investigated rats. Superoxide dismutase activity was also determined. **Results:** The results showed increased antioxidant enzymes and lowered glucose levels in diabetic rats fed with RJ. **Conclusion:** Histopathology tissues indicated moderate to extreme cellular transformations upon treated rats. The histopathological variations in treated rat cells were found to be evident. For this reason, there is likelihood that RJ may have an effect on splenic tissue repair in diabetic rats.

**Key words:** Antioxidant enzymes, insulin resistance, royal jelly, superoxide dismutase

## SUMMARY

- Diabetes mellitus and its increasing effect on endocrine functionality have been widely observed. Recent studies indicate that developing nations in Asia and Middle East will experience a surge in the incidences of diabetes by 2030. The present study aims at investigating the functions of royal jelly (RJ) in overcoming insulin resistance through enzyme function modification leading to prevention of lipid aggregation. The results showed enhanced immunity indices, antioxidant enzymes, and lowered glucose levels in diabetic rats fed with RJ.

**Abbreviations used:** RJ: Royal jelly; SBD: Standard basal diet; SPSS: Statistical package for the social science; STZ: Streptozotocin; BWG: Body weight gain; SD: Standard deviation.

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## INTRODUCTION

In Tehran, Iran diabetes type II is prevalent with estimated occurrence rate of roughly 1% of the population annually. While there is a strong inclination, the danger is significantly increased when accompanied with lifestyle characteristics including high blood pressure, absence of physical exercise, overweight, and poor nutritional diet. There are some risk factors such as hyperglycemia, resistance to insulin, inflammation, and oxidative stress associated with diabetes mellitus. Of these factors, hyperglycemia may contribute to oxidative stress that can, in turn, lead to insulin resistance, cell dysfunction of pancreatic beta, and cellular damage. The oxidative role takes a center stage when it comes to the development as well as the progression of diabetes and other disorders including renal illnesses and atherosclerosis.[1] Vitamin E and C supplements can increase not only oxidative stress but also control glycemia in patients with diabetes Type II. Royal jelly (RJ) is a product secreted by the hypopharyngeal and mandibular glands of worker bees, which contains three main nutrients such as minerals, vitamins, and amino acids. Moreover, RJ has different biological actions including hypotensive effect, insulin action, and antitumor.[2] In addition to biological action, RJ has flavonoids that prevent lipid platelet aggregation, preoxidation, capillary permeability and fragility, and enzymes activities such as lipoxygenase and oxygenase. For that reason, there is a high likelihood that RJ may influence insulin resistance that is regarded to be the main cause of diabetes mellitus.



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## SUBJECTS AND METHODS

### Royal jelly sample

Samples of RJ used in the present study was originated from the beehive of Al-Taif region, Makkah province, KSA. These samples were harvested in October 2013 as yellow granules and dried for use as food additive. Chemical analysis of RJ including moisture, crude protein, crude fat, and ash was determined on dry weight basis.[3] Total carbohydrates were determined by difference as 100 (protein + total fat + ash).

### Experimental animals

Adult male albino rats weighing (180 ± 9 gm) were used in the present study. Animals were obtained from Laboratory Animal Centre, Department of Biochemistry, Faculty of Medicine, Umm Al-Qura University, Makkah, KSA. Biological investigation has been carried out in the Animal House Facility of the Faculty of Applied Medical Sciences, Umm Al-Qura University, where animals were housed in a clean polypropylene cage with not more than four animals per cage and maintained under standard laboratory conditions (temperature 25 ± 2°C with 12/12 h dark/light cycle). They were fed standard basal diet (SBD) (12% casein, 10% corn oil, 0.2% choline chloride, 1% vitamin mixture**,** 5% cellulose, 4% salt mixture, and up to 100% corn starch) according to NRC (1995)[4] and water *ad libitum*. The animals were acclimatized to laboratory conditions for 1 week before the experiment. All procedures described were reviewed and approved by the Animal Care and Use Bioethical Committee of Medical Sciences, Umm Al-Qura University, KSA.

### Induction of diabetic rate and experimental design

Diabetes mellitus was induced in overnight fasted rats by a single intraperitoneal streptozotocin injection (65 mg/kg b.wt).[1] After 3 days, fasting blood glucose levels were measured and the animals showing blood glucose level ≥225 mg/dL were used for the study. Proved hyperglycemic rats were divided into three groups (8 rats each). In addition, a group of 8 normal rats without diabetes were included as a negative control. Investigated animal groups were fed on various diets for 45 days. The first group included normal rats fed only on SBD and served as negative control group (Group I). The second group was diabetic rats fed only on SBD and served as positive control group (Group II). The third group included diabetic rats fed on SBD plus dose of RJ 50 mg/kg of body weight (b.wt) were given orally and assigned as Group III. The last group included diabetic rats fed on SBD plus dose of RJ 100 mg/kg of b.wt were given orally and assigned as Group IV. All rats were weighed once weekly and food intake was estimated for each group.

### Food efficiency parameters

At the end of the experiment, biological evaluation of the different diets was carried out by determination of body weight gain percent (BWG%) and food efficiency ratio (FER).[5]

### Blood and organs sampling

At 24 h of the last feeding, blood samples were collected. The animals were previously anesthetized in a chamber containing diethyl ether. Two blood samples were collected from each animal into heparin-containing tube and plane tube, respectively. For serum production, collected blood samples in plane tubes were centrifuged at 3000 rpm for 10 min. The produced serum was collected and stored at −20°C until further analysis while packed RBC was used for the estimation of antioxidant enzymes.

Neutrophils were isolated from the freshly collected whole blood samples of control and diabetic rats by the standard procedure of dextran (Sigma, St. Louis, USA) sedimentation followed by centrifugation on Ficoll-Hypaue (Pharmacia, Uppsala, Sweden) as described elsewhere.[6]

### Biochemical and immunological analysis

Enzymatic colorimetric method was used to determine blood glucose according to glucose test kit (Cayman Chemical Company, Ann Arbor, Michigan, USA). Levels of immunoglobulins, CIC, Interferon-gamma (IFN-gamma), and neutrophils phagocytic activity were measured in the current study to evaluate immune status of investigated rats. Immunoglobulins quantification was performed by conventional radial immunodiffusion according to a method of Mancini using commercial kits for rat sera (ICNBiomedicals, Aurora, OH). Measurements were based on an 18-h timed diffusion for IgG and end-point determinations for other Igs.[7] IFN-gamma was measured in serum using Rat-IFN-gamma Sandwich ELISA kit (Thermo Fisher Scientific Inc., Rockford, IL, USA). The phagocytic activity of collected neutrophils from all investigated animals was evaluated by Zymosan colorimetric format of CytoSelect™ 96-Well Phagocytosis Assay. The assay uses prelabeled Zymosan particles as a phagocytosis pathogen. The assay was performed as instructed by the manufacturer (Cell Biolabs, Inc., San Diego, CA, USA). CIC concentration was measured in the serum of all investigated rats by Grinevich method (CIC-enriched fraction, precipitated with 3.5% polyethylene glycol) as previously described.[8]

### Antioxidant enzyme assessment

Superoxide dismutase activity was determined using a xanthine oxidase system to generate superoxide radials ($O^-$) as described by Catalase (CAT) enzyme activity was expressed as U of CAT/mg protein. CAT activity was determined by the method of glutathione peroxidase assayed by the method of Qari and Al-Ghamdi.[9]

### Statistical analysis

The results were expressed as mean ± standard deviation. Statistical analysis of the results was performed using software Statistical Package For Social Science, Version 20 (IBM Corp., Armonk, NY, USA) for Windows. Paired-sample *t*-test was used to compare the parameters between treated diabetic groups and diabetic group. $P < 0.05$ were considered statistically significant.

## RESULTS

The chemical composition of RJ used in this study is presented in Table 1. The effect of RJ on food efficiency parameters, biochemical and immunological analysis, and antioxidant enzyme assessment are presented through Tables 2-6.

### Histopathological results

#### *Spleen*

The spleen of control negative group which has normal size and structure as shown in Figure 1. The spleen of diabetic rats was bigger than the normal size as shown in Table 3, with massive extra vacation and congestion that demonstrated impairment in lymphatic structure of

**Table 1:** Chemical composition of royal jelly (g/100 g w/w)

| RJ composition | Protein (%) | Fat (%) | Ashes (%) | Moisture (%) | CHO (%) | Energy (%) |
|---|---|---|---|---|---|---|
| Composition/gram | 12.5 | 5 | 1.0 | 67 | 14.5 | 153 |

RJ: Royal jelly; CHO: Carbohydrates

exposed rats as shown in Figures 2-4. As shown in Figures 5 and 6, the impairment was attenuated in treated group.

### *Liver*

Figures 7-12 demonstrate the standard histological structure of liver cells formed in the form of liver cords, blistering from the core veins. However, the histological examination of groups 8, 9, 10, and 11 demonstrate a disoriented lobular pattern, piecemeal necrosis, and interface inflammation.

**Table 2:** Effect of royal jelly on body weight gain percentage, food intake/g, and food efficiency ratio of hyperglycemic rats

| Groups | BWG % | FI | FER |
|---|---|---|---|
| Nondiabetic rats (Group I) | 13.6±1.98** | 16.3±2.3* | 0.045±0.01** |
| Diabetic rats | | | |
| No RJ (Group II) | 3.3±0.77 | 11.22±2.11 | 0.01±0.005 |
| RJ (50 mg/kg b.wt) (Group III) | 4.86±1.22 | 14.33±0.74 | 0.022±0.004 |
| RJ (100 mg/kg b.wt) (Group IV) | 7.42±1.53* | 16.17±0.38* | 0.031±0.003 |

Data are expressed as mean±SD of eight experiments. $P<0.05$ was considered statistically significant. Parameters of Group II were compared to that of Group I and parameters of Group III and Group IV were compared to that of Group II. *$P\leq0.05$ significant change; **$P\leq0.01$ significant change; ***$P\leq0.001$ significant change. BWG: Body weight gain; FI: Food intake; FER: Food efficiency ratio; SD: Standard deviation; RJ: Royal jelly

## DISCUSSION

Shidfar *et al*. reported that RJ intake tended to reduce serum glucose and insulin levels and homeostatic model assessment-insulin resistance values compared with those by placebo intake, although differences between the two groups were not statistically significant. Moreover, in the RJ intake group, total antioxidant capacity in serum was significantly increased in male and female patients.[10] RJ has potent ability to improve hyperinsulinemia and insulin resistance in fructose-drinking rats.[11] Recent studies indicate that developing nations in Asia and Middle East will experience a surge in the incidences of diabetes by 2030. These reports suggest that RJ supplementation ameliorates hyperglycemia and insulin resistance associated with type 2 diabetes; however, the molecular mechanisms involved are unclear. Since direct investigation of molecular mechanisms for improving Type 2 diabetes associated with RJ supplementation in humans is difficult, using inbred animal models is essential for such investigations.[12]

**Table 3:** Biological effect of royal jelly on spleen and liver weight to body weight ratio for hyperglycemic rats

| Groups | Nondiabetic rats (Group I) | Diabetic rats | | |
|---|---|---|---|---|
| | | Diabetic rats | RJ (50 mg/kg b.wt) (Group III) | RJ (100 mg/kg b.wt) (Group IV) |
| S/b.wt | 0.39±0.04** | 0.51±0.03 | 0.44±0.02 | 0.41±0.01* |
| L/b.wt | 2.76±0.03* | 3.14±0.08 | 2.85±0.14 | 2.05±0.05* |

Data are expressed as mean±SD of eight experiments. $P<0.05$ was considered statistically significant. Parameters of Group II were compared to that of Group I and parameters of Group III and Group IV were compared to that of Group II. *$P\leq0.05$ significant change; **$P\leq0.01$ significant change; S/b.wt: Spleen weight/body weight ratio; L/b.wt: Liver weight/body weight ratio; RJ: Royal jelly

**Table 4:** Biological effect of royal jelly on glucose level for hyperglycemic rats

| Groups | Nondiabetic rats (Group I) | Diabetic rats | | |
|---|---|---|---|---|
| | | Diabetic rats no RJ (Group II) | RJ (50 mg/kg b.wt) (Group III) | RJ (100 mg/kg b.wt) (Group IV) |
| Glucose (mg/dl) | 101.9±8.1** | 237.6±8.02 | 222.43±12.14* | 181.35±7.46** |

Data are expressed as mean±SD of eight experiments. $P<0.05$ was considered statistically significant. Parameters of Group II were compared to that of Group I and parameters of Group III and Group IV were compared to that of Group II. *$P\leq0.05$ significant change; **$P\leq0.01$ significant change; RJ: Royal jelly; SD: Standard deviation

**Table 5:** Effect of royal jelly on some immunity indices of hyperglycemic rats

| Groups | CIC (mg/ml) | Phagocytic activity (%) | IgM (mg/ml) | IgA (mg/ml) | IgG (mg/ml) | IFN-gamma (ng/ml) |
|---|---|---|---|---|---|---|
| Nondiabetic rats (Group I) | 0.58±0.09** | 95.41±7.6** | 22.9±2.9*** | 16.9±2.1 | 69.71±6.2** | 358.9±18.9*** |
| Diabetic no RJ (Group II) | 0.92±0.08 | 50.72±5.7 | 47.91±4.8 | 19.8±2.2 | 145.8±11.9 | 660.2±25.4 |
| Diabetic RJ (50 mg/kg b.wt) (Group III) | 0.77±0.12* | 74.48±4.3* | 36.29±3.3* | 19.1±1.2 | 112.43±9.8* | 452.3±54.9** |
| Diabetic RJ (100 mg/kg b.wt) (Group IV) | 0.63±0.13** | 88.19±8.4** | 27.21±1.9** | 18.07±1.7 | 83.53±6.3** | 410.7±34.3*** |

Data are expressed as mean±SD of eight experiments. $P<0.05$ was considered statistically significant. Parameters of Group II were compared to that of Group I and parameters of Group III and Group IV were compared to that of Group II. *$P\leq0.05$ significant change; **$P\leq0.01$ significant change; ***$P\leq0.001$ significant change. RJ: Royal jelly; SD: Standard deviation; CIC: Circulating immune complexes; IFN: Interferon

**Table 6:** Effect of royal jelly on the activity of antioxidant enzymes of hyperglycemic rats

| Groups | SOD (U/mg) protein | CAT (nmol/min/mg) protein | GSH-Px (nmol/min/mg) protein |
|---|---|---|---|
| Nondiabetic rats (Group I) | 66.18±3.49** | 0.198±0.04** | 0.75±0.02** |
| Diabetic rats | | | |
| No RJ (Group II) | 49.95±3.13 | 0.136±0.02 | 0.36±0.08 |
| RJ (50 mg/kg b.wt) (Group III) | 57.16±4.41** | 0.164±0.05** | 0.64±0.02** |
| RJ (100 mg/kg b.wt) (Group IV) | 63.57±5.28** | 0.179±0.02** | 0.72±0.05** |

Data are expressed as mean±SD of eight experiments. $P<0.05$ was considered statistically significant. Parameters of Group II were compared to that of Group I and parameters of Group III and Group IV were compared to that of Group II. *$P\leq0.05$ significant change; **$P\leq0.01$ significant change; RJ: Royal jelly; SD: Standard deviation; SOD: Superoxide dismutase; CAT: Catalase; GSH-Px: Glutathione peroxidase

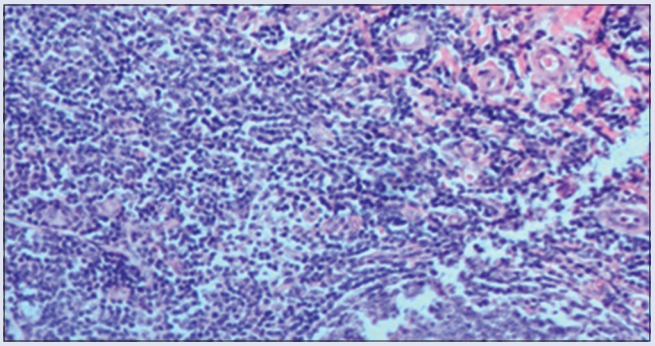

**Figure 1:** A photomicrograph of a section of the spleen of a rat from control –ve group showing the area of red pulp with normal congestion (upper right side) (H and E, ×100)

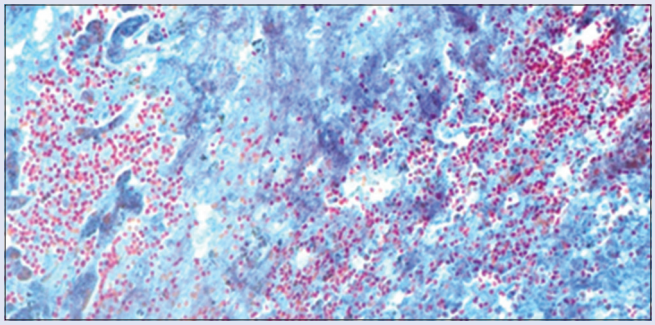

**Figure 2:** A photomicrograph of a section of the spleen of a rat from control +ve group showing focal area of necrosis surrounded by extravasated red blood cells (H and E, ×100)

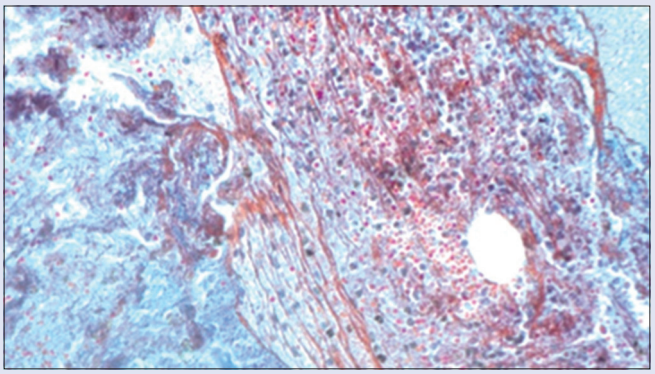

**Figure 3:** A photomicrograph of a section of the spleen of a rat from control +ve group showing diffuse necrosis and mild hyalinization of septa (arrow) (H and E, ×100)

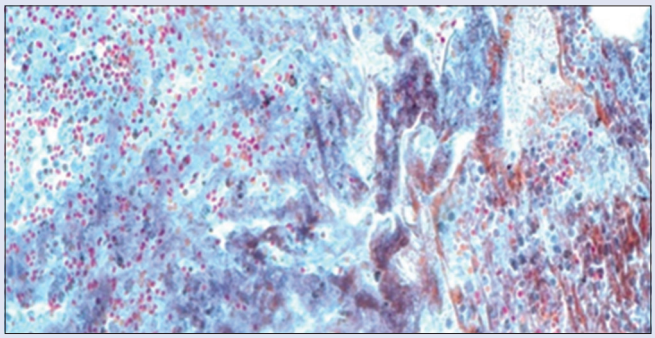

**Figure 4:** A photomicrograph of a section of the spleen of a rat from control +ve group showing diffuse necrosis and extravagated red blood cells (H and E, ×100)

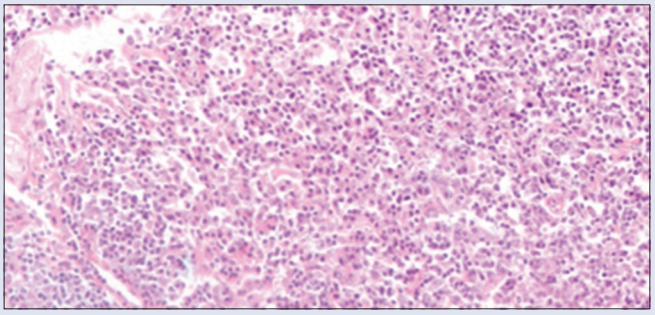

**Figure 5:** A photomicrograph of a section of the spleen of a rat from treated group with low dose showing normal splenic structure (H and E, ×100)

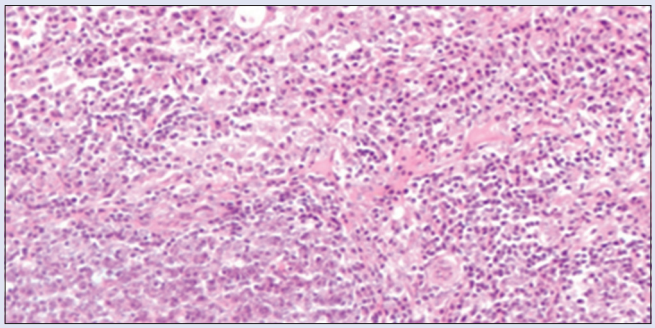

**Figure 6:** A photomicrograph of a section of the spleen of a rat from treated group with high-dose group showing normal splenic structure (H and E, ×100)

RJ may have hypoglycemic functions, Münstedt *et al.* reported that single doses of RJ decreased blood glucose levels in healthy individuals.[13] In addition, findings of *in vivo* and *in vitro* studies indicated that RJ has hypotensive,[14] antihypercholesterolemic,[15] anti-inflammatory,[16] antitumor,[17] and antioxidant effects.[18] It has insulin-like activity, which may improve insulin resistance.[19,20] Results from our study reveal that BWG% increased significantly in diabetic rats with the rise in the amount of RJ administered. For instance, diabetic rats in group two without RJ had the BWG% 3.3 ± 1.98. However, rats administered with 50 mg/kg and 100 mg/kg of RJ had the BWG% of 4.86 ± 1.22 and 7.42 ± 1.53*, respectively.

However, the outcomes for Group IV that fed on 100 mg/kg of RJ demonstrated reduced S/b.wt 1 and L/b.wt 2 0.41 ± 0.01 and 2.05 ± 0.05, respectively, in comparison to Group III parameters that fed on 50 m/kg of RJ at 0.44 ± 0.02 and 2.85 ± 0.14, respectively [Table 2]. In this respect, the use of RJ led to a reduction in the spleen and liver weight to the body weight ratio for diabetic rats.

Nondiabetic rats had the lowest glucose level at 101.9 ± 8.1** mg/dl. While diabetic rats with no RJ had the highest glucose level at 237.6 ± 8.02 mg/dl. Nonetheless, when it comes to biological effects of RJ on the glucose level, Group IV rats which fed on 100 mg/kg of RJ had a lower glucose level at 181.35 ± 7.46** in comparison to Group III rats that fed on 50 mg/kg of RJ that has the glucose level of 222.43 ± 12.14* [Table 3]. Based on these results, we

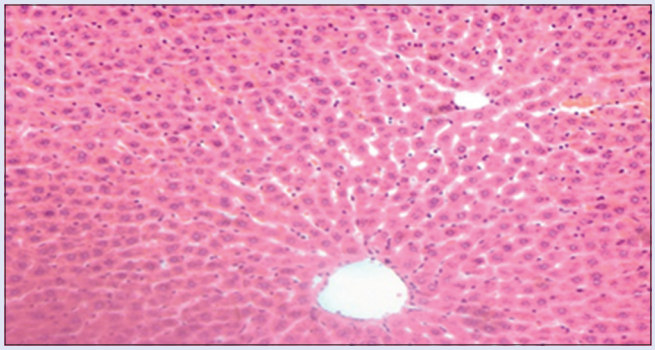

**Figure 7:** A photomicrograph of a section of the liver of a rat from control –ve group showing normal hepatic structure (H and E, ×100)

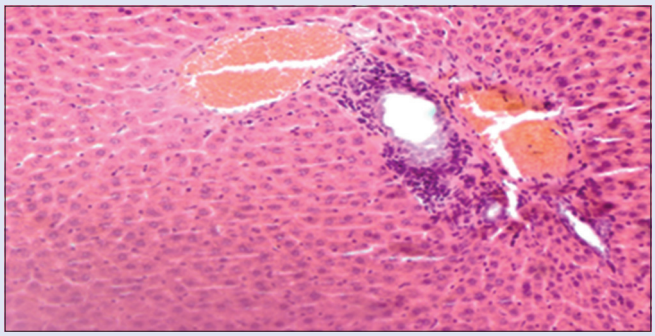

**Figure 9:** A photomicrograph of a section of the liver of a rat from control +ve group showing piecemeal necrosis and interface inflammation (H and E, ×100)

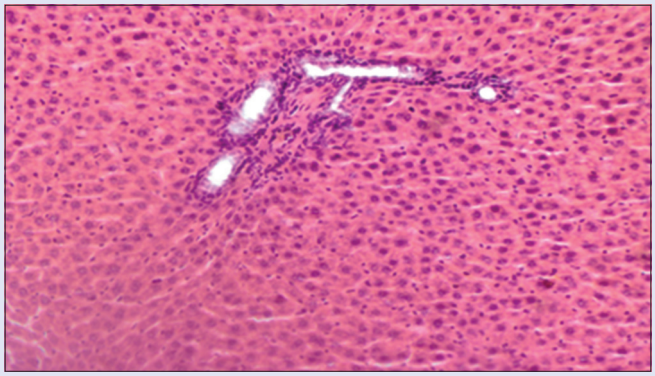

**Figure 11:** A photomicrograph of a section of the liver of a rat from treated group with low-dose group showing unremarkable pathological changes (H and E, ×100)

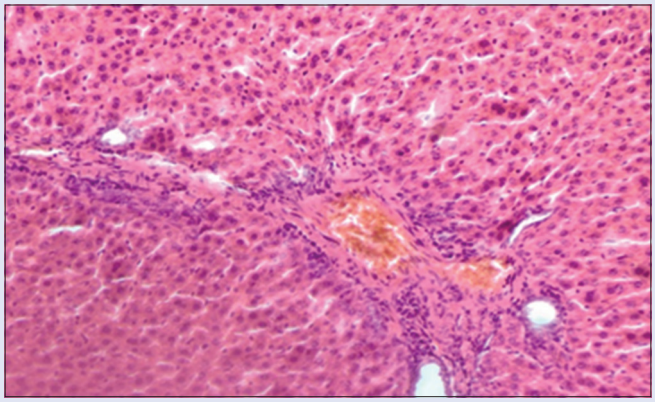

**Figure 8:** A photomicrograph of a section of the liver of a rat from control +ve group showing portal congestion and periportal inflammation (H and E, ×100)

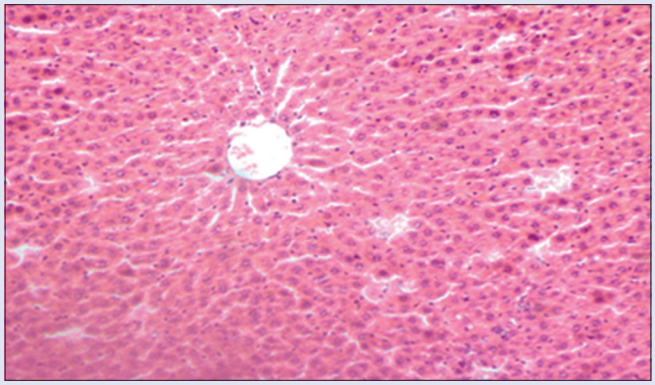

**Figure 10:** A photomicrograph of a section of the liver of a rat from control +ve group showing hydropic degeneration (H and E, ×100)

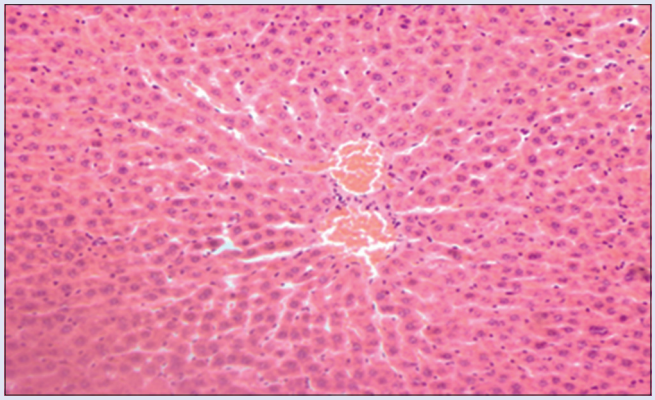

**Figure 12:** A photomicrograph of a section of the liver of a rat from treated group with high-dose group showing unremarkable pathological changes except congested central vein (H and E, ×100)

can, therefore, deduce that RJ lowers the glucose level in diabetic rats and that the higher the quantity of RJ consumed, the lower the glucose level [Table 4].

Mobasseri *et al.*, 2015 who reported that RJ does not appear to have significant immediate effects on glycemic factors in patients with type 2 diabetes. However, further studies with larger sample sizes and different doses of RJ are needed to achieve more precise results.[21]

The data obtained also indicated that RJ enhances the immunity indices of diabetic rats. For instance, rats in group one had a higher immunity in comparison to rats in group two. In the same breadth, rats in Group 3 and 4, respectively, had enhanced immunity indices in comparison to rats from Group 2. While the use of RJ enhances the immunity indices, rats from Group III had a higher immunity compared to rats from Group IV [Table 5].

Ultimately, RJ is essential to the body when it comes to generating more immune cells, fighting bacteria, and healing wounds speedily. This occurs following the combination of unique proteins to RJ. The typical

protein that boosts the immunity is the royalisin. Royalisin and major RJ proteins (MJRPs), a complex protein, provides bees the ability to fight diseases, destroy bacteria, and allergies in nature. The MJRPs contains about 80%–90% of protein composition in RJ, and they are distinct to honeybees.[22] This is one of the reasons why RJ is an essential nutrient. In test tubes as well as in animals, MJRPs have demonstrated to be strong in triggering the production of a number immunity cells and minimizing allergies. The queen bee has the highest resistance and lifecycle, which is a result of the dense concentration of proteins in the RJ. Moreover, there are not only a few but also intriguing studies on RJ's ability to increase wound healing.[23]

Nevertheless, evidence in this field is limited, though encouraging. Studies have not been carried out with many patients or in good conditions; however, RJ has significantly influenced the ability of wound healing

## CONCLUSION

Histopathology tissues indicated moderate-to-extreme cellular transformations upon treated rats. The histopathological variations in treated rat cells were found to be evident. For this reason, there is likelihood that RJ may have an effect on splenic tissue repair in diabetic rats.


### Financial support and sponsorship

Nil.


### Conflicts of interest

There are no conflicts of interest.

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