



## Does Supplementation with Royal Jelly Improve Oxidative Stress and Insulin Resistance in Type 2 Diabetic Patients?

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### Abstract

**Background:** Animal studies have shown antioxidant effects of Royal Jelly (RJ) and its effect on insulin resistance as the most common complication of Type 2 diabetes. This study was conducted to determine the effect of RJ intake on serum total antioxidant capacity, Malondialdehyde and insulin resistance in T2DM.

**Methods:** In this randomized controlled trial, forty-six type 2 diabetic patients, aged 25-65 years, with BMI of 20-30 kg/m<sup>2</sup>, and HbA1c of 6-8% were included. The patients were randomly assigned to receive 1000 mg of RJ supplement or placebo, 3 times daily for 8 weeks. HOMA-IR, anthropometric measurements, fasting blood glucose, serum insulin, total antioxidant capacity and malondialdehyde level were measured.

**Results:** In comparison with placebo, HOMA-IR decreased ( $P=0.015$ ) while serum total antioxidant capacity increased significantly in RJ group ( $P=0.016$ ). No significant difference was detected for serum insulin and MDA in two groups.

**Conclusions:** RJ intake may have favorable effects on serum TAC and HOMA-IR in diabetic patients.

**Keywords:** Royal Jelly, Type 2 diabetes mellitus, Insulin resistance, Total antioxidant capacity

### Introduction

According to recent estimates, developing countries in Asia and in the Middle East will have the largest increase in the prevalence of diabetes by 2030 (1). The prevalence of type 2 diabetes (T2D) is reported to be more than 14% in Tehran, Iran, with an estimated incidence of new cases in about 1% of population per year (2). There is a strong genetic predisposition but the risk is greatly increased when accompanied with lifestyle factors such as overweight or obesity, high blood pressure, insufficient physical activity, poor diet and visceral fat. There are some characteristics such as hyperglycemia, insulin resistance, hyperlipidemia, oxidative stress and inflammation attributed to DM (3).

Among these characteristics, hyperglycemia may lead to oxidative stress which can cause cellular damage, pancreatic beta cell dysfunction and insulin resistance (4). Oxidative stress (OS) plays a critical role in development and progression of diabetes and complications such as atherosclerosis, coronary heart and renal diseases (3). Vitamin E and C supplementation may improve oxidative stress and glycemic control in T2DM patients (5-8).

Royal jelly (RJ) is a bee product, secreted from the hypopharyngeal and mandibular glands of worker bees. RJ produced by honey bees is known to contain three major nutrients including amino

acids, vitamins and minerals (3). Additionally, RJ has various biological activities such as a hypotensive effect, insulin-like action and antitumor activity (9). Therefore, it is possible that RJ may have some effects on insulin resistance, which is considered to be the major cause of DM. In a study, supplementation of rats with different doses of royal jelly (10, 30, and 300 mg/kg) led to a decrease in systolic blood pressure and significantly decreased serum levels of insulin and the Homeostasis Model Assessment ratio, an index of insulin resistance (10). Only one human study reported that 1000 mg royal jelly for 8 weeks reduced Malondialdehyde, glucose and HbA<sub>1c</sub> but this dose was not sufficient to influence antioxidant status, on the other hand this study had some limitation especially it was done only in women and insulin resistant as the most important deleterious factor in diabetes, was not assessed (11).

Although several animal studies have showed the effects of royal jelly on insulin resistance or oxidative stress, to our knowledge, human studies with respect to insulin resistance as a main cause of diabetes in both sexes is lacking. We hypothesized that RJ supplementation can improve insulin resistance and oxidative stress in type 2 diabetic patients.

## **Materials and Methods**

### *Study design and participants*

The Randomized controlled Trial (RCT) was conducted in Endocrine and Metabolism Institute of Iran University of Medical Sciences, Tehran, Iran. A randomized double blind placebo-controlled trial aiming to compare royal jelly effect on insulin resistance (HOMA-IR), total antioxidant capacity (TAC) and malondialdehyde (MDA) level in type 2 diabetic patients was designed. The inclusion criteria were: (a) ages 25-65 years, (b) BMI of 20-30 kg/m<sup>2</sup>, (c) Iranian ethnicity, (d) diagnosis of type 2 diabetes for 5-10 years (e) HbA<sub>1c</sub> of 6-8%, and (f) receiving oral hypoglycemic drugs. The exclusion criteria were: (a) serum triglyceride level (TG) >400 mg/dl, serum cholesterol level > 240 mg/dl (b) lactating or pregnancy, (c) diagnosis of

heart, liver and renal failure, cancer, acute myocardial infarction, stroke, or serious injuries, (d) receiving vitamin or mineral supplements at least 3 months before the beginning of the study, (e) smoking/ alcohol consumption (f) taking oral contraceptive or lipid lowering drugs, (g) insulin infusion, (h) allergy to royal jelly and (i) any other conditions not suitable for trial as evaluated by the physician. The study was approved by the Bioethics Committee of Iran University of Medical Sciences ([IRCT201103012709N18](#)). Written consent forms were signed and handed back from all participants before participation. Forty-six patients were randomly assigned to receive Royal jelly supplements (Natural life. Frengrove Co. Australia) (Group A) 1000 mg, 3 times daily or exactly the same placebo (glycerin) (Pars Minoos Inc. Tehran Iran) (Group B) 1000 mg, 3 times daily for 8 weeks.

Participants were instructed to maintain an isocaloric diet, continue their previous eating habits, and not to change their routine physical activities during the study period. Throughout the study period, subjects were directed to continue taking the same dose of any prescribed hypoglycemic agents unless hypoglycemia occurred, in which case they were directed to reduce their dose immediately. Daily food intake was obtained by a 24 hour dietary recall questionnaire and physical activity level by IPAQ questionnaire in three days (two regular days and one holiday) at the beginning and end of the study. This dietary intake data was analyzed by nutritionist 4 (N4) software.

Participants were randomly assigned to one of the two groups via computer-generated numbers. Both active and placebo treatments were contained in the same opaque capsules. Products were administered by a blinded research assistant.

### *Outcome measures*

Homeostasis model assessment for insulin resistance (HOMA-IR) (fasting glucose (mg/dl) × fasting insulin (μmol/ml) /405) was used as the major outcome measurement. At baseline and after 8 weeks of treatment the TAC, MDA and fasting insulin level were measured for both groups. Serum TAC was measured by FRAP method (12), and serum MDA was measured by spectroscopy (13).

Fasting blood sugar was measured by an enzymatic method (Pars Azmon Co. kit, Tehran, Iran) using Liasys autoanalyzer while insulin was measured by IRMA method (Immunotech Co. kit). Nutritional data were obtained via 24 hour diet recall. Height was measured with a wall-mounted stadiometer to the nearest 0.1 cm, weight was measured on a calibrated balance beam scale to the nearest 0.1 kg, and BMI was calculated according to the formula:  $BMI = \text{weight} / \text{height}^2$  (kg/m<sup>2</sup>). Demographic data was collected during the initial anthropometric assessment.

### Statistical Analysis

In designing the study, we considered power of 90% with a two-sided test with  $\alpha = 0.05$  (type I error) to detect a 5% difference in serum glucose between the two groups. On the basis of SDs, reported in similar studies (14), the number of subjects needed to treat to detect this difference was 20/group. Given an anticipated dropout rate of 25 percent, we set the enrollment target at 25 subjects. All data were expressed by means  $\pm$  SD. The level of significance was determined at  $P < 0.05$ . Statistical analyses were performed with PC SPSS 16 (Chicago, IL, USA). Normal distribution of the variables was checked by Kolmogorov Smirnov Test; student's *t* test was used to test whether the differences between the mean values of the items studied in both groups were significant. The mean differences in both groups of participants were compared before and 8 weeks after the intervention were evaluated by paired *t*-test.

## Results

### Demographics and anthropometric measurements at baseline

Among the 50 type 2 diabetic patients screened at our outpatient clinic, four patients withdrew because they did not want to continue the supplementation program. Then, 46 patients were allocated equally into Groups A and B. The mean and standard deviation of demographic and nutrients intake data are shown in Table 1 and 2. There were no significant differences in age, body mass index (BMI), diabetes duration (Table 1), energy and nutrient intake (Table 2), in each group and between two groups before and after the intervention. The level of physical activity and lipid lowering drugs was not different between two groups at baseline.

### Laboratory Analysis Measurements

As shown in Table 3, there was no significant difference in serum glucose, TAC, MDA and HOMA-IR between two groups before the intervention. However, after 8 weeks of RJ supplementation, serum TAC ( $P = 0.016$ ) increased compared with the initial values and serum glucose ( $P = 0.006$ ) and HOMA-IR ( $P = 0.015$ ) decreased at the end of study in RJ group compared with placebo. There was also significant decrease in mean differences of HOMA-IR between two groups ( $P = 0.023$ ) as shown in Table 3. There was no significant difference after RJ supplementation compared to before values in serum glucose, insulin levels, TAC, MDA and HOMA-IR.

**Table 1:** Baseline characteristics of participants

Variable	RJ (n=23)	Placebo (n=23)
Gender (male/female)	10/13	12/11
Age (yr)	51.78 ( $\pm 9.65$ )	53.13 ( $\pm 7.45$ )
BMI (kg/m <sup>2</sup> )	27.79 $\pm$ 2.65 <sup>b</sup>	28.01 $\pm$ 3.81 <sup>b</sup>
	29.91 $\pm$ 2.53 <sup>a</sup>	27.97 $\pm$ 3.61 <sup>a</sup>
Duration of diabetes (years)	6.15 $\pm$ 1.19	6.74 $\pm$ 1.68

Data are expressed as means  $\pm$  SD. Differences between groups were evaluated by independent *t*-test  
<sup>a</sup> after study, <sup>b</sup> before study

**Table2:** Comparison of dietary intake of some nutrients between two groups of study

Dietary intake	RJ group n=23	Placebo group n=23	P-value*
<b>Total Energy: (Kcal)</b>			
Before intervention	1685.69±217.79	1770.04±184.28	0.289
After intervention	1716.95±213.37	1730.65±246.94	0.306
<b>Total protein (g/day)</b>			
Before intervention	65.34±8.93	69.32±10.26	0.102
After intervention	69.32±10.26	65.25±12.10	0.083
<b>Total carbohydrate (g/day)</b>			
Before intervention	237.49±30.93	248.84±28.91	0.566
After intervention	240.37±29.87	242.29±34.57	0.310
<b>Total fat (g/day)</b>			
Before intervention	53.81±10.76	57.56±9.72	0.236
After intervention	55.84±9.05	53.65±7.65	0.083
<b>SFA<sup>1</sup> (g/day)</b>			
Before intervention	24.04±5.15	25.08±4.14	0.114
After intervention	22.04±4.85	24.30±4.95	0.550
<b>MUFA<sup>2</sup> (g/day)</b>			
Before intervention	13.78±3.69	13.56±4.81	0.084
After intervention	14.73±3.76	12.95±2.89	0.301
<b>PUFA<sup>3</sup> (g/day)</b>			
Before intervention	15.78±3.46	17.82±3.91	0.498
After intervention	16.56±4.31	16.06±3.69	0.144
<b>Vitamin C (mg)</b>			
Before intervention	77.53±18.48	74.22±21.13	0.874
After intervention	78.28±18.81	80.27±18.51	0.107
<b>Vitamin E (mg)</b>			
Before intervention	18.09±5.09	17.93±7.05	0.632
After intervention	17.67±5.59	14.43±5.81	0.117

Data are expressed as means ±SD. \*Differences between groups were evaluated by independent *t*-test/<sup>1</sup>SFA:Saturated Fatty Acid, <sup>2</sup> MUFA: Monounsaturated Fatty Acid, <sup>3</sup> PUFA: Polyunsaturated Fatty Acid

**Table3:** Effects of 8 weeks RJ supplementation on serum glucose, insulin, TAC, MDA and HOMA-IR in type 2 diabetic patients

Variable	group	Before mean±SD	After mean±SD	Mean difference	P value*
Serum glucose (mg/dl)	R J	128.43±44.4	119±30.9	9.43±26	0.096
	Placebo	145.73±48.4	149.73±40.2	4±30.4	0.535
	P value**	0.213	0.006*	0.115	
Insulin (µIU/ml)	R J	8.19±4.1	6.74±2.2	-1.45±4.1	0.107
	Placebo	7.40±6.2	8.56±4.9	1.16 ±5.1	0.285
	P value**	0.61	0.115	0.063	
HOMA-IR index	R J	2.64±1.7	1.98±0.8	-0.65±1.6	0.067
	Placebo	2.57±2	3.13 ±1.9	0.56 ±1.8	0.164
	P value**	0.9	0.015*	0.023*	
TAC (µmol/L)	R J	858.13±213.8	907.63±207.0	49.5±130.8	0.091
	Placebo	784.78±260.4	756.69±196.5	28.08 ±282.5	0.638
	P value**	0.309	0.016*	0.247	
MDA (nmol/ml)	R J	3.01±0.6	3.24±0.9	0.23±1.3	0.418
	Placebo	3.04±0.8	3.59±0.9	0.44±1	0.054
	P value**	0.898	0.217	0.546	

Data expressed as a mean ± standard deviation. Before, after and mean differences and P values are mentioned in the table.-/ (P value \*)Comparison in each group between before and after values by paired t-test / (P value\*\*) Comparison between two groups of before values and also after 8 weeks values of two groups and also comparison of mean differences by independent *t*-test

## Discussion

To our knowledge, this is the first RCT conducted to assess the effect of royal jelly supplementation on HOMA-IR, oxidative stress and MDA in both sexes of human subjects. Oxidative stress is associated with type 2 diabetes, and there is compelling biochemical evidence suggesting that ROS may even play a role, even if only secondary, in the pathogenesis of type 2 diabetes (15).

In this study, RJ intake reduced serum glucose and HOMA-IR while it increased serum TAC significantly after 8 weeks of intervention in comparison to placebo group but no significant difference was shown before and after RJ supplementation in serum glucose, insulin level, TAC, MDA and HOMA-IR. This is due to small sample size.

RJ significantly affects serum glucose levels in healthy subjects (16). RJ reduces the index of insulin resistance (HOMA-IR) but not blood glucose levels in rats (17). RJ contains biologically active substances which cause insulin-like activity (18).

Insulin resistance associates with changes in oxidative stress levels and RJ has protective effects against oxidative stress due to its antioxidant peptides. Thus, RJ can ameliorate insulin resistance via antioxidant effect (17).

Antioxidant peptides derived from RJ proteins have the strong antioxidant activities (19). For example, in one study, supplementation with RJ for 16 weeks significantly reduced 8-hydroxy-2-deoxyguanosine as a marker of oxidative stress and the average life span was extended by about 25% compared to the control group (18). In another study, 29 antioxidative peptides were isolated from RJ protein hydrolysate. The 12 antioxidative peptides showed strong hydroxyl radical scavenging activity (19).

Supplementation with RJ was reported to have beneficial effects against genotoxicity and oxidative damages induced by cadmium in albino mice. RJ had a protective effect on the chromosome aberration, micronucleus, and oxidative stress, and this effect was connected with dose. The protective effect of RJ on toxicity induced by Cadmium may be explained by the therapeutic properties

and antioxidant capacity of RJ, which is a product of honeybees (20).

In another study, yeast cells were cultivated with different concentrations of RJ. In the RJ treated cells, lower ATP pool size did not cause growth decline, which might be due to decreased cell requirements for energy, since decreased intracellular oxidant level was observed in a dose dependent manner indicating lower energy consumption for induction of endogenous antioxidant defending and repair systems. This was also confirmed by 2-D gels (electrophoresis), where down-regulation of enzymatic antioxidant system Cu-Zn superoxide dismutase was observed at 6 h (2.2-fold). Decreased cell requirement for energy in RJ treated cells was observed also by glucose consumption rate measured at 6 h, which was for 14.5% lower compared with controls (21). The protecting effects of RJ against oxidative status may be related to scavenging abilities of the superoxide radical (20).

However the decreased serum glucose in our study was consistent with Pourmoradian et al. study (11), but increase of TAC and decrease of insulin resistance which was occurred in our study was not shown in that study. On the contrary, MDA had no significant difference in our study. Of course, participants of Pourmoradian's study were only women and baseline values of MDA were higher than of our study, so these might be responsible for MDA decreasing. To our knowledge, this is the first randomized, controlled trial on RJ supplementation in both sexes of DM patients but it is limited in that it had a small sample size and short duration. Therefore, larger sample size and longer duration are needed before reaching conclusive results. However, administration of RJ in diseases accompanied with oxidative stress history is suggested. Moreover, the effect of 10-hydroxydecanoic acid as an unsaturated fatty acid in royal jelly, on HOMA-IR, oxidative stress and MDA needs further investigation.

## Conclusion

RJ intake increased serum TAC and decreased HOMA-IR in diabetic patients. So, administration

of RJ in diseases accompanied with oxidative stress history may be useful.

## Ethical considerations

Ethical issues (Including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

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## References

- Hadaegh F, Fahimfar N, Khalili D, Sheikholeslami F, Azizi F (2010). New and known type2 diabetes as coronary heart disease equivalent: Results from 7.6 year follow up in a middle-east population. *Cardiovasc Diabetol*, 9:84.
- Harati H, Hadaegh F, Saadat N, Azizi F (2009). Population-based incidence of Type 2 diabetes and its associated risk factors: Results from a six-year cohort study in Iran. *BMC Public Health*, 9:186.
- Mesallamy H E, Suwailem S, Hamdy N (2007). Evaluation of C-reactive protein, endothelin-1, adhesion molecule (s), and lipids as inflammatory markers in type 2 diabetes mellitus patients. *Mediators of Inflammation*, 73: 635-637.
- Ceriello A, Motz E (2004). Is oxidative stress the pathogenic mechanism underlying insulin resistance, diabetes, and cardiovascular disease? The common soil hypothesis revisited. *Arterioscler Thromb Vasc Biol*, 24 (5):816-23.
- Johansen JS, Harris AK, Rychly DJ, Ergul A (2005). Oxidative stress and the use of antioxidants in diabetes: linking basic science to clinical practice. *Cardiovasc Diabetol*, 4 (1):5.
- Neri S, Signorelli SS, Torrisi B, Pulvirenti D, Mauceri B, Abate G, Ignaccolo L, Bordonaro F, Cilio D, Calvagno S, Leotta C (2005). Effects of antioxidant supplementation on postprandial oxidative stress and endothelial dysfunction: a single-blind, 15-day clinical trial in patients with untreated type 2 diabetes, subjects with impaired glucose tolerance, and healthy controls. *Clin Ther*, 27:1764-1773.
- Vega-Lopez S, Devaraj S, Jialal I (2004). Oxidative stress and antioxidant supplementation in the management of diabetic cardiovascular disease. *J Investig Med*, 52:24-32.
- Lee DH, Falsom AR, Harnack L, Halliwell B, Jacobs Jr DR (2004). Does supplemental vitamin C increase cardiovascular disease risk in women with diabetes? *Am J Clin Nutr*, 80:1194-1200.
- Tokunaga K. H, Yoshida C, Suzuki K. M, Maruyama H, Futamura Y, Araki Y, Mishima S (2004). Antihypertensive effect of peptides from royal jelly in spontaneously hypertensive rats. *Biol Pharm Bull*, 27 (2): 189-192.
- Nomura M, Maruo N, Zamami Y, Takatori S, Doi S, Kawasaki H (2007). Effect of long-term treatment with royal jelly on insulin resistance in Otsuka Long-Evans Tokushima Fatty (OLETF) rats. *Yakugaku Zasshi*, 127 (11):1877-82.
- Pourmoradian S, Mahdavi R, Mobasseri M, Faramarzi E, Mobasseri M (2014). Effects of Royal Jelly Supplementation on Glycemic Control and Oxidative Stress Factors in Type 2 Diabetic Female: A Randomized Clinical Trial. *Chin J Integr Med*, 20 (5):347-352.
- Benzie F.F, Steain J.J (1999). Ferric Reducing/Antioxidant Assay: direct measure antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. *Methods in Enzymology*, 299:15-23.
- Rubaba K, Zabun N, Mohammad S.I, Maizbha U.A, AGM M, Hasanuzzaman Sh, Abdullah A.M, Abul H (2011). Serum MDA and Vitamin C level in Conversion Disorder Patients. *J Pharm Sci*, 10 (1): 59-64.
- Guo H, Saiga A, Sato M, Miyazawa I, Shibata M, Takahata Y, Morimatsu F (2007) Royal jelly supplementation improves lipoprotein metabolism in humans. *J Nutr Sci Vitaminol*, 53 (4):345-8.

15. Opara EC (2004). Role of oxidative stress in the etiology of type 2 diabetes and the effect of antioxidant supplementation on glycemic control. *J Investig Med*, 52 (1): 19-23.
16. Munstedt K, Bargello M, Hauenschild A (2009). RJ reduces the serum glucose levels in healthy subjects. *J Med Food*, 12 (5):1170-2.
17. Zamami Y, Takatori S, Goda M, Koyama T, Iwatani Y, Jin X, Takai-Doi S, Kawasaki H (2008). RJ ameliorates insulin resistance in fructose-drinking rats. *Biol Pharm Bull*, 31 (11): 2103-07.
18. Kramer (1982). Purification of Insulin like peptides from insect haemolymph and RJ. *Insect Biochem*, 12 (1): 91-8.
19. Guo H, Kouzuma Y, Yunekura M (2009). Structures and properties of antioxidative peptides derived from royal jelly protein. *Food Chemistr*, 113: 234-45.
20. Çavuşoğlu K, Yapar K, Yalçın E (2009). Royal jelly (honey bee) is a potential antioxidant against cadmium-induced genotoxicity and oxidative stress in albino mice. *J Med Food*, 12 (6):1286-92.
21. Jamnik P, Gorano D, Raspor P (2007). Antioxidative action of RJ in the yeast cell. *Exp Gerontol*, 42 (7): 594-60.