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A COMPARATIVE STUDY ON THE EFFECT OF ROYAL JELLY ON BLOOD GLUCOSE AND SERUM LIPIDS IN STREPTOZOTOCIN INDUCED DIABETIC RATS

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ABSTRACT

Background: Royal jelly (RJ) originating from the pharyngeal glands of the honey bee was found to have various biological activities such as a hypotensive effect, insulin-like action and antitumor activity. **Objective:** This study was carried out to examine the effect of RJ on the blood glucose level alone and in combination with glibenclamide and metformin and to demonstrate the effect of RJ on serum lipids. **Material and methods:** a total number of 48 healthy male albino rats were subdivided into 8 equal groups: 1st group, Non diabetic control, 2nd group; was given RJ (300 mg/Kg), 3rd group diabetic control, 4th group diabetic given RJ 5th group was given Glibenclamide (0.6 mg/kg/B.W), 6th group was given metformin (100 mg/kg/B W), 7th group was given RJ with glibenclamide, 8th group was given RJ with metformin. **Results:** RJ significantly decreased blood glucose level and caused a further reduction when used in combination with oral hypoglycemic drugs and RJ also significantly reduced serum lipids. **Conclusion:** RJ has a significant inhibitory effect on blood glucose and serum lipids in streptozotocin induced diabetic rats.

KEYWORDS: diabetes, Streptozotocin, Royal jelly, glibenclamide, metformin, diabetic rats.

ABBREVIATIONS: CGRP: Calcitonin gene related peptide, FDR: Fructose Drinking Rats.

INTRODUCTION

Royal jelly (RJ) originating from the pharyngeal glands of the queen honey bee was found to have various biological activities such as a hypotensive effect, insulin-like action and antitumor activity.[1] Chemically, royal jelly comprises water (50% to 60%), proteins (18%), carbohydrates (15%), lipids (3% to 6%), mineral salts (1.5%) and vitamins[2] together with a large number of bioactive substances such as: 10-hydroxyl-2-decenoic acid[3] with immunomodulating properties[4], antibacterial protein[5], fatty acids[6] and several insulin like peptides.[1] Additionally, RJ has a healing effect for 5-fluorouracil-induced oral mucositis in hamsters.[7] RJ contains phenolic compounds with anti-oxidant activity.[8]

The royal jelly was found to have various biological activities such as a hypotensive effect, insulin-like action and antitumor activity.[9,1]

Guo et al.,[10] found that Royal jelly decreases total cholesterol (CH) and low density lipoproteins (LDL) by lowering small very low density lipoproteins (VLDL) and they concluded that RJ benefits lipoprotein metabolism in humans. They also believed that dietary Royal Jelly may help prevent life style related diseases in humans.

It was also found that RJ show significant reduction in total serum cholesterol[11] and He suggested that RJ may act on some important step of cholesterol biosynthesis, degradation, transport or uptake, common to many forms of hyperlipidemia. He also suggested that RJ may be effective in prevention of atherosclerosis.

RJ also was suggested to have health-promoting benefits, containing bioactive substances that improve not only insulin resistance, but also hypertension *via* indirectly vascular control dysfunction regulated by adrenergic and CGRPergic nerves in the hyperinsulinemic state. It was also reported that daily RJ intake would be effective to prevent the development of insulin resistance and hypertension.[12]

Kerem et al.,[8] reported that Royal Jelly may improve diabetic patients by decreasing hypercholesterolemia and insulin resistance.

Serum glucose levels were significantly lower (P = .041) after royal jelly administration in human healthy volunteers.[13] Substances originating from the pharyngeal glands of the honey bee with insulin-like activity are likely to have caused this effect and may thus

be, at least partially, responsible for the lowering impact of honey on blood glucose levels.[13]

However no studies up till now were carried out on rats to confirm the effect of Royal Jelly and combination of RJ with oral hypoglycemic drugs on serum glucose. So, this study was carried out to show the effect of Royal Jelly on blood glucose level in normal and Streptozotocin induced diabetic rats as well as to show the effect of RJ on serum lipids and the effect of combination with Glibenclamide and/or metformin on serum lipids.

MATERIAL AND METHODS

Materials: Royal Jelly was obtained from the College of Agriculture and Veterinary medicine, Qassim university, KSA. A total number of 48 Male albino rats average weight (150-200 gm) were obtained from the Laboratory Animal Research Unit of College of medicine, Qassim University, KSA. Glibenclamide and metformin were obtained from sigma chemical co. and dissolved in dimethyl sulfoxide and distilled water, respectively before they were administered.

Methods

All animal experiments were done according to the National Research Council guide lines for the care and use of laboratory animals. Animals were housed at 25 ± 2°C under 12 hour cycles of dark and light and were allowed standard food and water. The rats were fasted for 18 hours before induction of diabetes.

Induction of diabetes

Diabetes was induced by a single intraperitoneal injection of freshly prepared streptozotocin (60 mg/kg) dissolved in 0.1 M citrate buffer (pH 4.5).[14] Another group of rats (control) were injected with the same volume of citrate buffer. Streptozotocin (STZ)-injected rats exhibited symptoms of diabetes mellitus such as Polyurea, polydipsia, polyphagia, and weight loss after 48 hours post STZ administration. Two days after the injection of STZ, fasting blood glucose concentration was measured with an Accu-Chek glucometer (Roche, Germany) using blood samples from the tail vein. Animals with blood glucose concentrations ≥ 14 mMol / L (250mg / dl) were considered diabetic and used in this study. Subsequently, fasting blood glucose was measured weekly in each rat. Using oral cannula, once each morning (9-10 pm) the rats were treated with Royal Jelly, glibenclamide and/or metformin for four weeks. RJ was diluted by adding distilled water and administered at dose of 300 mg/kg/d.[12] Each animal was administered RJ solution at a volume of 2ml/Kg once daily (9.00-10.00 am).

Animals were treated for 4 weeks as follows

1st group (6 rats): non diabetic control group are given distilled water.
2nd group (6 rats): non diabetic given RJ 300 mg/kg/d.
3rd group (6 rats): diabetic Control given distilled water (0.5 ml).
4th group (6 rats): diabetic given Royal Jelly (300 mg) [12].
5th group (6 rats): diabetic given Glibenclamide (0.6 mg/kg/body weight) [14].
6th group (6 rats): diabetic given metformin (100 mg/kg/body weight) [14].
7th group (6 rats): diabetic given RJ (300 mg) with glibenclamide.
8th group (6 rats): diabetic given RJ (300mg) with metformin.

At the end of the treatment period, the animals were fasted for at least 16 hours and sacrificed by decapitation. Blood samples were collected in centrifuge tubes without anticoagulants and allowed to clot. The clotted blood was then centrifuged at 3000 x g for 20 min. Serum was separated and then quickly stored at -80°C for biochemical analyses.

Statistical analysis

Statistical analysis was carried out using SPSS version 12. The data are expressed as mean ± SEM. Groups were compared by the Kruskal-Wallis H test followed by Mann-Whitney U test to identify significance of difference between two groups. *P* value < 0.05 was considered statistically significant.

Biochemical analyses

Serum glucose was determined by the glucose oxidase method as described by **Barham and Trinder.**[15] Serum insulin was determined using a rat insulin enzyme-linked immunosorbent assay kit (Crystal Chem, Chicago, IL) with rat antibody. Serum high density lipoprotein (HDL) was estimated by the method of **Warnick.**[16] Total cholesterol (CH) and triglycerides (TG) were estimated by the methods of **Siedel et al.**[17] and **Foster and Dunn**[18], respectively. Low density lipoprotein (LDL) and very low density lipoprotein (VLDL) were calculated by **Friedwald's formula.**[19]

RESULTS

Table1 and Figure 1: summarizes the results of serum glucose and serum insulin in different groups of animals at the end of the four-week treatment period. The serum glucose concentrations of the diabetic control rats were significantly higher (21.2 ± 0.9 mmol/L) than those of the non-diabetic control rats (5.6 ± 0.3 mmol/L). Treatment with glibenclamide, metformin, or Royal jelly significantly decreased the glucose levels (14.2 ± 2.4, 16.3 ± 2.5 or 15.3 ± 3.2 mmol/L, respectively) in diabetic rats. When Royal Jelly was added to glibenclamide or metformin, further reduction in glucose concentrations was found (10.4 ± 2.4 or 11.4 ± 2.5 mmol/L, respectively) in diabetic rats.

The diabetic control rats had significantly ($p < 0.01$) reduced insulin level (0.26 ± 0.02 ng/ml) compared to non-diabetic rats (0.60 ± 0.11 ng/ml). Treatment of

diabetic rats with Royal Jelly, glibenclamide, metformin produced a significant increase in insulin levels (0.42±0.06, 0.40±0.05, 0.38±0.03 respectively) compared to diabetic controls (0.26±0.02)ng/ml.

Combination of RJ with glibenclamide and metformin also produced increase in insulin level in comparison with diabetic control (0.33±0.02, 0.35±0.03, 0.26±0.02 respectively) ng/ml.

Table 1: Effects of Royal Jelly, Glibenclamide and Metformin on serum glucose and serum insulin in Streptozotocin induced diabetic rats:

Group	Serum glucose (mMol/l)	Serum insulin(ng/ml)
1- NonD Control	5.6±0.3	0.60±0.11
2- NonD+Rj	5.5±0.2	0.59±0.12
3- D Control	21.2±0.9**	0.26±0.02**
4- D+ RJ	11.3±3.2#	0.42±0.06##
5- D+ GL	14.2±2.4*#	0.40±0.05##
6- D+ M	14.4±2.5#	0.38±0.03#
7- D+ GL+ RJ	9.4±2.4#	0.33±0.02#
8- D+ M+ RJ	10.4±2.5#	0.35±0.03*#

D: Diabetic, RJ: Royal Jelly, GL: Glibenclamide, M: Metformine.
* = significant (P < 0.05) compared to nondiabetic
** = highly significant (P< 0.01) compared to nondiabetic
# = significant (P < 0.05) compared to diabetic
= highly significant (P< 0.01) compared to diabetic
Data are expressed as mean ± standard Error of mean.

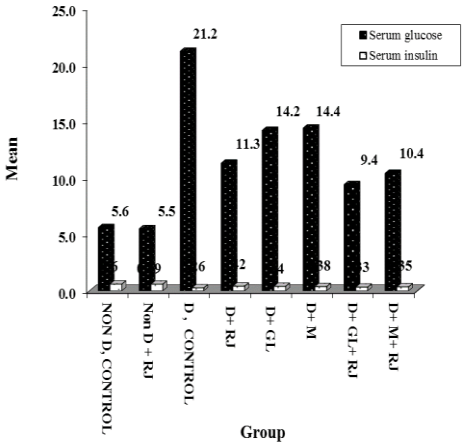

Figure (1): The mean Effects of Royal Jelly, Glibenclamide and Metformin on serum glucose and serum insulin in Streptozotocin induced diabetic rats.

Table (2) and Figure (2): shows the serum levels of triglycerides, total cholesterol, HDL, LDL and VLDL of control and streptozotocin-induced diabetic rats. Insignificantly increased levels of TG and VLDL with no much change in total cholesterol, HDL and LDL levels were observed in diabetic control rats compared to non-diabetic rats.

Administration of Royal jelly, glibenclamide or metformin significantly ($p < 0.05$) decreased the levels of TG in diabetic rats (0.26 ± 0.04, 0.32 ± 0.08, 0.22 ± 0.04 mg/dl respectively) compared to diabetic control rats (0.70 ± 0.21 mg/dl).

Administration of Royal jelly, glibenclamide or metformin significantly ($p < 0.05$) decreased the levels of VLDL in diabetic rats (0.15 ± 0.03, 0.16 ± 0.04, 0.14 ± 0.02 mg/dl respectively) compared to diabetic control rats (0.31 ± 0.11).

In addition, combination of glibenclamide or metformin with Royal jelly further reduced the levels of TG (0.29 ± 0.18, 0.18 ± 0.03mg/ml respectively) compared to diabetic control rats (0.70 ± 0.21 mg/dl).

Combination of glibenclamide or metformin with Royal jelly further reduced the levels of VLDL (0.12 ± 0.08, 0.05 ± 0.01 mg/ml respectively) compared to diabetic control rats (0.31 ± 0.11).

However, Glibenclamide, glibenclamide with Royal jelly or metformin with Royal jelly significantly decreased total cholesterol (1.08 ± 0.08, 1.16 ± 0.15, 0.90 ± 0.15 mg/ml respectively) compared to diabetic control rats (1.51 ± 0.08 mg/dl).

Table (2): the effect of Royal Jelly, Glibenclamide and/or Metformin on serum lipids (mg/dl):

G	TG (mg/dl)	CH(mg/dl)	HDL(mg/dl)	LDL(mg/dl)	VLDL(mg/dl)
1. NON D, CONTROL	0.45 ± 0.03	1.50 ± 0.14	0.80 ± 0.07	0.41 ± 0.08	0.22 ± 0.01
2. Non D + RJ	0.50 ± 0.02	1.62 ± 0.11	0.86 ± 0.02	0.50 ± 0.08	0.22 ± 0.01
3. D, CONTROL	0.70 ± 0.21	1.51 ± 0.08	0.76 ± 0.09	0.40 ± 0.03	0.31 ± 0.11
4. D+ RJ	0.26 ± 0.04*#	1.43 ± 0.12	0.90 ± 0.09#	0.38 ± 0.04	0.15 ± 0.03#
5. D+ GL	0.32 ± 0.08#	1.08 ± 0.08*##	0.65 ± 0.09	0.30 ± 0.03	0.16 ± 0.04#

6. D+ M	0.22 ± 0.04*#	1.15 ± 0.07	0.81 ± 0.11	0.35 ± 0.03	0.14 ± 0.02*#
7. D+ GL+ RJ	0.29 ± 0.18**#	1.16 ± 0.15#	0.86 ± 0.08	0.40 ± 0.03	0.12 ± 0.08**#
8. D+ M+ RJ	0.18 ± 0.03**##	0.90 ± 0.15*#	0.68 ± 0.11	0.25 ± 0.03#	0.05 ± 0.01**#

D: Diabetic, RJ: Royal Jelly, GL: Glibenclamide, M: Metformin.
* = significant (P < 0.05) compared to nondiabetic
** = highly significant (P< 0.01) compared to nondiabetic
# = significant (P < 0.05) compared to diabetic
= highly significant (P< 0.01) compared to diabetic

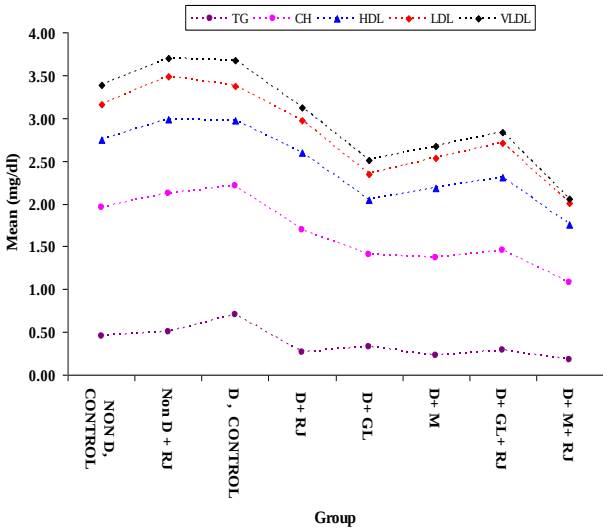

Figure (2): show the mean effect of Royal Jelly, Glibenclamide and⁄or Metformin on serum lipids (mg ⁄dl).

DISCUSSION

Royal jelly originating from the pharyngeal glands of the honey bee was found to have various biological activities such as a hypotensive effect, insulin-like action and antitumor activity.[4,20] RJ was also suggested to have health-promoting benefits, containing bioactive substances that improve not only insulin resistance, but also hypertension. It was also reported that daily RJ intake would be effective to prevent the development of insulin resistance and hypertension.[12] However no studies up till now were carried out on rats to demonstrate the effect of Royal Jelly alone and in combination with oral hypoglycemic drugs on serum glucose. Also we found that no study was done on the effect of RJ on serum lipids in rats. So, this study was carried out to show the effect of Royal Jelly on blood glucose and serum lipids in normal and Streptozotocin induced diabetic rats.

In this study we found that royal jelly reduced significantly blood glucose level when given to diabetic rats. This finding was found to be in agree with study done by **Münstedt et al.,**[13], who found that Royal jelly decreased blood glucose level in human healthy volunteers. Also, this study is supported by **Erejuwa et al.,**[14], who found that honey (royal jelly one of its components) decreased significantly blood glucose level in streptozotocin induced diabetic rats.

Our study was also supported by **Tokunaga et al.,**[9] who found Serum glucose levels were significantly lower after royal jelly administration in human healthy volunteers. **Münstedt et al.,**[13] suggested that Royal Jelly was likely to have caused this effect and may thus be, at least partially, responsible for the lowering impact of honey on blood glucose levels.

We also found that Royal Jelly added to glibenclamide or metformin caused a significant reduction in glucose concentrations. This finding confirms the hypoglycemic effect of royal jelly and is supported by **Tokunaga et al.,**[1] who found that royal jelly improved glucose tolerance in healthy volunteers given royal jelly for six months. This effect is very beneficial as we can use RJ in combination with oral hypoglycemic drugs to lower blood glucose level as well as valuable antioxidant in diabetic patients.

We also found that RJ elevated serum insulin levels significantly in diabetic rats. These findings are in agreement with other studies found that RJ can reduce blood sugar level via insulin-like peptides and other compounds (like chromium, sulphur, vitamins B3 and H).[21]

Our study is also supported by **Batchelder,**[22] who found that RJ is also capable to sustain the optimal blood level of sugars by taking part in the oxidation of glucose to obtain energy, through the insulinic effect of insulin-like peptides found in it. **O'Connor**,[21] reported that the insulin found in RJ very closely resembles the insulin found in mammals**.**

In this study, we also found that Administration of Royal jelly, glibenclamide or metformin significantly ($p < 0.05$) decreased the levels of triglycerides (TG) and VLDL (Very low density lipoproteins) in diabetic rats compared to diabetic control rats. Besides, combination of glibenclamide or metformin with Royal jelly further reduced the levels of TG and VLDL in diabetic rats. Our findings are supported by **Guo et al.,**[10] **who** found that Royal jelly decreases total cholesterol (CH) and low density lipoproteins (LDL) by lowering small very low density lipoproteins (VLDL) and they concluded that RJ benefits lipoprotein metabolism in humans. Our study is also supported by **J.Vittek**,[11] who reported that RJ show significant reduction in total serum cholesterol and **He** suggested that RJ may act on some important step of cholesterol biosynthesis, degradation, transport or uptake, common to many forms of hyperlipidemia.

In this study, we also found that glibenclamide, glibenclamide with Royal jelly or metformin with Royal jelly significantly decreased total cholesterol in diabetic rats. This finding in agreement with **Gue et al.,**[10] who found that serum total cholesterol and LDL-C in the RJ group decreased significantly more than those in the control group. We found also that RJ increased insignificantly serum HDL in nondiabetic rats and increased it significantly in diabetic rats and this effect is of high clinical importance in diabetics. These findings were found to be in agreement with **J.Vittek**[11] who suggested that RJ may be effective in prevention of Atherosclerosis.

In conclusion, this study demonstrated, for the first time, that Royal Jelly significantly lowered blood glucose level in rats whether used alone or in combination with oral hypoglycemic drugs. This study also confirmed that Royal jelly combined with Glibenclamide and/or metformin caused further reduction in blood glucose. This study also proved the beneficial lowering effect of Royal jelly on serum lipids which is a common complication in diabetic persons. So, RJ can be used safely in diabetic hyperlipidemic patients. These results can be used in clinical medicine by using Royal Jelly as adjuvant with oral hypoglycemic drugs; however, further studies are needed to confirm this combined effect of royal jelly on humans.

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