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# The physiological Effects of Royal Jelly Administration on Hypercholesterolemic Male Albino Rats

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**Abstract**

Hypercholesterolaemia disorder is characterized by very high level of cholesterol in the blood which is cardiovascular health risk. Chemical compound Medicines have serious side effects that cause an imbalance in the body's functions. Therefore, utilization of natural compounds could be an alternative concept in the treatment of diseases, as they have no side effects on human health. The present work aims to evaluate the physiological effects of royal jelly (RJ) on the hypercholestrolaemic male albino rats. Forty-two male albino rats were divided into six groups. Rats were treated with coconut oil, cholesterol (450 mg/kg b.w of cholesterol dissolved in 0.5 ml coconut oil) for four weeks, RJ treated group (300 mg/kg b.wt) for two weeks, RJ before and after cholesterol treated groups. Serum cholesterol showed significant increased levels of triglyceride (TG), total cholesterol (TC), low density lipoprotein cholesterol (LDL-C) and very low-density lipoprotein cholesterol (VLDL-C), and significant decrease in high density lipoprotein cholesterol (HDL-C), antioxidant enzymes, nitric oxide (NO) and vascular endothelium growth factor (VEGF) compared with those of control and other treated groups. Royal jelly administration reduced the high levels of LDL-C, VLDL-C, TG and TC because of cholesterolaemia. Also, RJ could improve the levels of HDL-C, serum antioxidant enzymes, NO and VEGF. Treatment with RJ before and after cholesterol could help to return serum NO, VEGF levels toward control values and causes significant decrease in LDL-C level and significant increase in HDL-C level compared to control group. In conclusion, RJ ameliorates the harmful effects of cholesterol administration on male albino rats and plays an important role in cardiovascular health protection.

**Keywords:** Hypercholestrolaemic rats, Royal jelly, Nitric oxide, Antioxidant enzymes, Lipid profile, Vascular endothelium growth factor.

## 1.Introduction

Hypercholestrolaemia is one of the greatest risk factors contributing to the prevalence of coronary heart disease. Hypercholestrolaemia and hyperlipidemia induce stress and play a key role in atherogensis. Atherosclerosis is a condition in which plaque formed inside arteries and it is one of the most chronic inflammatory diseases wide world which progresses through stimulating inflammatory cells, cytokines production and oxidized low density lipoprotein (LDL) [1]. Nowadays researches were directed to natural therapy to avoid the side effects of drugs which used as anti hypercholestrolaemia.

Honey is one of the important natural antioxidant and highly popular because of its potential role in contributing to human health. Honey, propolis, and RJ are functional foods with phenolic compounds

RJ contains considerable amounts of proteins, amino acids especially essential amino acids, hormone rich substance (testosterone), vitamins A, C, D, and E and mineral salts [٢]. Many studies shown that RJ has a number of physiological effects such as anti-inflammatory, antitumor, antiallergic, antioxidant activities and has a protective effect against lipid peroxidation caused by free radicals[3].

Therefore, this study is designed to investigate the physiological effects of RJ on hypercholestrolaemic male albino rat.

## 2.Material and methods

Cholesterol**,** white crystalline powder was obtained from Middle East Company for Medical and Scientific Apparatus Laboratory Equipment and Chemicals, Cairo, Egypt. It was dissolved by coconut oil. (Pyramid Company for New Industry).

Royal jelly **(**RJ) is a viscous jelly substance which is partially soluble in water with a density of 1.1 g/ml. Its color is whitish to yellow purshased by Sigma Pharm. Industries-Egypt.

### 2.1 Experimental animals

Forty-two male albino rats (*Rattus norvegicus*) weighting 100-150 g were obtained from Helwan Farm of Egyptian Organization for Vaccine and Biological Preparations. Rats were caged for 10 days before the beginning of the experiment in the laboratory, at 25±2 °C, 12 hr light/dark cycle and given food and water ad libitum**.**

### 2.2 Experimental design

Animals were divided randomly into six groups, 7 animals each. The 1st group was normal (untreated rats). The 2nd group (coconut oil group) rats were administered daily oral dose of coconut oil (0.5ml). The 3rd group (hypercholesterolaemic group) animals were administrated cholesterol orally with dose of 450 mg/kg b.wt, dissolved in 0.5 ml coconut oil [1]**.** The 4th group rats received RJ oral dose of 300 mg/kg/day [4]. The animals of 5th and 6th groups were received RJ before and after cholesterol administration respectively.

**2.3 Blood sampling**

At the end of the experimental period (5 weeks) rats were fasted overnight and seven animals of each group were weighted then anaesthetized by ether inhalation [5]. Blood samples were collected from dorsal aorta in dry tubes and centrifuged at 3000 rpm for 15 minutes. Sera were separated and stored at -20 °C until the biochemical analysis.

**2.4 Biochemical analysis**

By using Spinreact company kit at wavelength 546 nm, serum full name (TG) was determined spectrophotometerically [6]. Serum cholesterol was determined according to [7]. full name (HDL-C) was determined according to [8]. Concentration of full name (LDL-C) and full name (VLDL-C) were calculated according to [8] equation as follows:

**LDL- C = [Total C – (HDL- C + triglycerides/5)].**

**VLDL- C = triglycerides/5.**

Atherogenic index was calculated according to formula developed by [9] as follow:

**Atherogenic index= (TC-HDL- C)/HDL- C.**

Serum superoxide dismutase, SOD (u/mL) was determined using SOD Assay Kit-WST at 450 nm [10]. Serum glutathione peroxidase, GPx (u/L) was determined according to using OXLtek total glutathione peroxidase assay kit (Company) at 340 nm. Serum catalase, CAT (u/L) was determined OxiSelect TM Catalase Activity assay kit at 520 nm.

Quantitative colorimetric determination of serum NO concentration was determined using Assay kit (Company) Germany at 540nm.Serum VEGF concentration was determined at 520 nm by using Rat-VEGF Assay Kit-IBL (Immuno-Biological Laboratories), Japan .

The values of measured and calculated parameter were expressed as the mean of 7 individual values ± standard deviation "SD". Statistical analysis carried out using one-way analysis of variance (ANOVA) followed by Duncan test [11] by using SPSS (version 20) program produced by IBM Software, Inc. Chicago, USA.

**3.Results and discussion**

Rats treated with cholesterol showed significant increases in serum levels of TG, TC, LDL-C, VLDL-C and atherogenic index and significant decrease in HDL-C level compared with those of control and other treated groups. RJ administration caused significant reductions in serum levels of LDL-C, VLDL-C, TG, TC and atherogenic index and induced significant elevations in HDL-C level as compared to control group. Treatments with RJ before and after cholesterol caused significant decreases in LDL-C level and atherogenic index and significant increases in HDL-C level. A significant increase was noticed in HDL-C level in coconut oil treated group when compared to animals of control and other treated groups Table (1).

Antioxidant enzymes; SOD, GPx and CAT activities showed significant differences among all treated groups and control one. Cholesterol and cholesterol that followed by RJ induced significant depression in the activities of all antioxidants compared to control and other treated groups, while RJ administration before cholesterol showed significant reduction in GPx and CAT activates Table (2). The administration of RJ caused significant increase of SOD, GPx and CAT activities when compared to control and other treated groups.

Also, NO level recorded a significant variation among control and all treated groups sera. Cholesterol treated group showed the least NO level as compared to control and other treated groups. Inversely, the highest value of serum NO level was found in RJ treated group in comparison to control. Data showed non-significant change of NO level when RJ was administered before or after cholesterol treatment where NO levels were at the control value Table (3).

Values of serum VEGF recorded no significant change among control, RJ and coconut oil groups Table (3). Animals of cholesterol treated group showed significant reduction in serum VEGF level as compared to those of control and other treated groups. The measurement showed no change with RJ administration before or after cholesterol treatment.

Royal Jelly contains many complex substances as water, lipid, proteins, mineral salts, carbohydrates, vitamins, enzymes, natural antibiotics and hormones. For this reason, RJ nowadays used in pharmacological activities as antioxidant, hypocholesterolemic, hepato-protective, hypotensive, neurotrophic, antitumor, antiallergic etc. [12].

The present study revealed increased seral TC, TG, LDL-C and VLDL-C levels. where a decrease of HDL-C level by cholesterol administration. This may be due to cholesterol lead to down regulation of LDL-receptors which involved in cholesterol incorporation in the liver thus LDL cannot influx into cells and its serum levels raised [13]. Royal jelly administration caused significant reductions in serum LDL-C, VLDL-C, TG and TC levels whereas, elevations of HDL-C level was noticed. These results may be due to that RJ decreased gene expression of squalene epoxidase enzyme (SQLE), "key enzyme in cholesterol biosynthesis" and also cause decrease in sterol regulatory element binding protein (SREB)-1, which may be a transcriptional factor of SQLE. On the other hand, RJ increased gene expression of low-density lipoprotein receptor

(LDLR) [14] or may be a result of lowering very low-density lipoprotein (VLDL) levels due to RJ strongest hydroxyl radical scavenging quality [3].

**Table (1)** Serum lipid profile of hypercholesterolemic male albino rats treated with cholesterol (450 mg/kg b.wt. dissolved in 0.5 ml coconut oil for 3 weeks) before and after royal jelly treatments (300 mg/kg b.wt. for 2 weeks)

| | Groups | | | | | |
|---|---|---|---|---|---|---|
| **Parameter** | **Control** | **Coconut oil** | **Cholesterol** | **Royal Jelly** | **Royal Jelly then cholesterol** | **Cholesterol then Royal Jelly** |
| Triglycerides (mg/dl) | 105.85±0.57$^{e}$ | 112.48±0.55$^{b}$ | 130.48±0.50$^{a}$ | 91.23±0.96$^{f}$ | 107.38±0.48$^{d}$ | 108.38±0.48$^{c}$ |
| Total cholesterol (mg/dl) | 70.83±0.62$^{c}$ | 81.23±0.26$^{b}$ | 100.63±0.75$^{a}$ | 68.45±0.78$^{e}$ | 70.05±0.10$^{d}$ | 70.95±0.10$^{c}$ |
| High density lipoprotein (mg/dl) | 25.63±0.48$^{d}$ | 50.20±0.22$^{a}$ | 23.30±0.41$^{e}$ | 42.89±0.61$^{b}$ | 39.53±0.61$^{c}$ | 40.13±1.31$^{c}$ |
| Low density lipoprotein (mg/dl) | 24.03±0.67$^{b}$ | 8.53±0.39$^{c}$ | 51.23±1.09$^{a}$ | 7.31±0.49$^{d}$ | 9.05±0.58$^{c}$ | 9.15±1.39$^{c}$ |
| Very low density lipoprotein (mg/dl) | 21.17±0.11$^{e}$ | 22.49±0.11$^{b}$ | 26.09±0.11$^{a}$ | 18.25±0.53$^{f}$ | 21.48±0.095$^{d}$ | 21.68±0.096$^{c}$ |
| Atherogenic index | 1.76±0.29$^{b}$ | 0.62±0.18$^{c}$ | 3.32±0.83$^{a}$ | 0.6±0.28$^{c}$ | 0.77±0.02$^{d}$ | 0.79±0.01$^{d}$ |

Number of animals in each group =7.

In the same row:
Similar letters mean non-significant difference.
Different letters mean significant difference

**Table (2)** Serum antioxidant enzymes of hypercholesterolemic male albino rats treated with cholesterol (450 mg/kg b.wt. dissolved in 0.5 ml coconut oil for 3 weeks) before and after royal jelly treatments (300 mg/kg b.wt. for 2 weeks)

| | Groups | | | | | |
|---|---|---|---|---|---|---|
| **Parameter** | **Control** | **Coconut oil** | **Cholesterol** | **Royal Jelly** | **Royal Jelly then cholesterol** | **Cholesterol then Royal Jelly** |
| Superoxide dismutase (u/ml) | 0.04±0.01$^{b}$ | 0.05±0.01$^{a}$ | 0.03±0.00$^{c}$ | 0.05±0.00$^{a}$ | 0.04±0.00$^{b}$ | 0.03±0.00$^{c}$ |
| Glutathione peroxidase (nmol/min/ml) | 218.26±9.75$^{b}$ | 209.75±8.45$^{b}$ | 141.74±6.86$^{d}$ | 239.76±3.73$^{a}$ | 168.75±4.71$^{c}$ | 155.70±3.57$^{c}$ |
| Catalase (u/ml) | 72.52±3.65$^{e}$ | 71.05±3.03$^{b}$ | 51.16±2.45$^{e}$ | 80.35±1.66$^{a}$ | 61.64±3.39$^{c}$ | 57.54±1.79$^{d}$ |

Number of animals in each group =7.
Data were presented as mean ±SD.

In the same row:
Similar letters mean non-significant difference.
Different letters mean significant difference

**Table (3)** Serum nitric oxide and vascular endothelial growth factor of hypercholesterolemic male albino rats treated with cholesterol (450 mg/kg b.wt. dissolved in 0.5 ml coconut oil for 3 weeks) before and after royal jelly treatments (300 mg/kg b.wt. for 2 weeks)

| | Groups | | | | | |
|---|---|---|---|---|---|---|
| **Parameter** | **Control** | **Coconut oil** | **Cholesterol** | **Royal Jelly** | **Royal Jelly then cholesterol** | **Cholesterol then Royal Jelly** |
| Nitric oxide (Mmol/) | 21.79±1.23$^{b}$ | 19.10±2.01$^{c}$ | 13.89±1.11$^{e}$ | 24.75±1.26$^{a}$ | 16.51±0.18$^{d}$ | 15.75±0.28$^{d}$ |
| Vascular endothelial growth factor (pg/mL) | 447.91±6.08$^{a}$ | 478.38±4.27$^{a}$ | 305.59±1.36$^{c}$ | 488.50±4.97$^{a}$ | 386.33±4.84$^{b}$ | 353.70±6.66$^{bc}$ |

Number of animals in each group =7.
Data were presented as mean ±SD.

In the same row:
Similar letters mean non-significant difference.
Different letters mean significant difference

Atherogenic index is a predictor of the development of atherosclerosis and underscores the progression of cardiovascular disease [15].

The results of the current study indicated an increase in the atherogenic index as a result of cholesterol administration while royal jelly

treatments caused significant reduction in atherogenic index. These may be due to MRJP1 (Major RJ protein 1) has hypocholesterolemic effects in rats. The cholesterol-lowering action induced by MRJP1 occurs because MRJP1 interacts with bile acids induces a significant increase in fecal bile acids excretion and a tendency to increase in fecal cholesterol excretion and also enhances the hepatic cholesterol catabolism and decreases the micellar solubility of cholesterol so decrease the level of cholesterol and atherogenic index [16].

Cholesterol induced a decreased in the levels of glutathione and catalase and increased level of superoxide dismutase enzymes which caused damage to the oxidative defense system of cells. This respectively leads to the release of reactive oxygen species (ROS) such as hydrogen peroxide, superoxide anions, and hydroxyl radicals which are generated under normal cellular conditions and are immediately detoxified by major scavenger enzymes. However, excessive ROS production causes antioxidant imbalance and leads to lipid peroxidation and antioxidant depletion [1].

The present work revealed that the treatment by RJ and RJ before cholesterol caused increased the levels of SOD, GPx and CAT[17].

In the current study, cholesterol treated rats showed depression of serum NO which may be due to hypercholesterolemia is associated with endothelial cell dysfunction, a near-complete abrogation in vascular nitric oxide bioavailability, elevated oxidant stress and the creation of a strongly pro-inflammatory condition [18]. The present work showed that the highest value of serum NO was found in RJ [19].

Vascular endothelial growth factor (VEGF) is a key regulator of pathogenic angiogenesis in diseases such as cancer and diabetic retinopathy. It has a crucial role in vascular development both in physiological and pathological processes. It is one of the key molecules shown to enhance angiogenesis and is thus considered as a potent therapeutic agent to stimulate blood vessel formation in myocardial and limb ischemia. Treatment with cholesterol reduced serum VEGF level. This may be due to deposition of lipids into vessel walls and chronically increased cholesterol levels trigger a number of vascular events, such as oxidative stress, endothelial dysfunction, blood–brain barrier disturbances and vascular inflammation which decrease the level of VEGF in the serum. In the present study royal jelly administration before or after cholesterol increased the level of VEGF in the serum.

Major efforts have been made in human patients with atherosclerosis to stimulate angiogenesis by delivery of vascular endothelial growth factor (VEGF) or of viral vectors or plasmids containing the VEGF transgene and increase the level of VEGF is necessary for prevention of coronary heart or peripheral occlusive artery disease, so using of royal jelly is necessary in enhance angiogenesis and stimulate blood vessel formation in myocardial and limb ischemia by increase the level of VEGF in the serum.

## 4. Conclusion

Administration of RJ in rats improved the disturbance in lipid profile, antioxidant enzymes, nitric oxide and vascular endothelium growth factor which caused by cholesterol treatment. In addition, RJ could also be used as a prophylactic agent (food protector) to prevent the increase of total cholesterol and its harmful effects.

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