Anticancer, antioxidant, and antihyperlipidemic effects of royal jelly

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Background/aim
Royal jelly (RJ) is a natural product obtained from honey bees and claimed to possess diverse health benefits. The aim of the present research was to search some of such health claims, including antioxidant, anticancer, and antihyperlipidemic effects, so as to support or negate such claims.
Materials and methods
RJ was tested for its antioxidant, anticancer, and antihyperlipidemic effects. The *in-vitro* antioxidant effect was screened using 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid), 2,2-diphenyl-1-picryl-hydrazyl-hydrate, and ferric reducing antioxidant power assays. The anticancer effect was carried out by applying MTT assay using human cancer cell line from breast (MCF-7, breast adenocarcinoma) and from liver (Huh-7, hepatocellular carcinoma). The *in-vivo* antihyperlipidemic effect was studied in a Triton X-100-induced hyperlipidemic rat model. The rats were divided into three groups; normal control, hyperlipidemic control, and hyperlipidemic group where rats were given 300 mg RJ/kg rat body weight as daily oral dose for 2 weeks before Triton injection and continued 3 days after the injection. Plasma triglycerides, total cholesterol (TC), high-density lipoprotein-cholesterol (HDL-C), low-density lipoprotein-cholesterol, malondialdehyde, and the activities of transaminases (alanine transaminase and aspartate transaminase) were analyzed in all rats. The ratio of TC/HDL-C was calculated as a cardiovascular risk factor. Livers of all rats were investigated for any histopathological changes.
Results
demonstrated *in-vitro* antioxidant activity with different degrees according to the assay type ranging from 0.43 to 5.634 μM Trolox eq/mg RJ. The anticancer effect showed IC50 of 51.133 and 107.332 μg/ml from RJ toward MCF-7 and Huh-7, respectively. The animal experiment demonstrated significant reduction in the activities of alanine transaminase and aspartate transaminase, levels of malondialdehyde, triglycerides, TC, low-density lipoprotein-cholesterol with concomitant elevation in HDL-C, and a decrease in TC/HDL-C, with improvement of liver histopathology in the group given RJ compared with the hyperlipidemic control group.
Conclusion
Within the extreme of the present research, RJ was efficient as antihyperlipidemic and hepatoprotective agent and has mild to moderate antioxidant activity according to the screened assays together with anticancer potential in cell lines, which was superior against MCF-7 compared with Huh-7.

Keywords:
Anticancer effect, human cell line, hyperlipidemia, *in-vitro* antioxidant activity, rats, royal jelly, Triton X-100

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Introduction

Cardiovascular diseases (CVDs) and malignancies are considered as the most common cause of morbidity and mortality in both developed and developing countries. Both inflammation and high oxidative stress are among the most important causes for developing such chronic diseases. It is well documented that hypercholesterolemia and high low-density lipoprotein-cholesterol (LDL-C) with decreased high-density lipoprotein-cholesterol (HDL-C) play a key role in atherogenesis as well as elevation of the risk of CVDs. Atherosclerosis is one of the most chronic inflammatory diseases worldwide which progresses through stimulating inflammatory cytokines production and oxidized LDL [1,2]. Natural therapy might be a safe approach to overcome such CVDs without the adverse effects seen during administration of anti-hypercholesterolemic

drugs. Moreover, natural antioxidants could afford protection from malignant tumors [3].

Natural antioxidants are highly popular owing to their potential role in human health. Royal jelly (RJ) has been reported as being among the most efficient natural functional food in this respect. RJ is a distinct product from hypopharyngeal and mandibular glands of young worker bees, which is thick and milky in nature [4] and is considered a functional food owing to its contents of functional ingredients. RJ contains amino acids; hormones; vitamins C, D, E, and A; minerals; proteins; lipids; carbohydrates; and other bioactive compounds like 10-hydroxyl-2-decenoic acid (10-HDA) [5,6]. The major RJ proteins comprise nine members; RJ 1-9. RJ protein-1 constitutes about 46% and the other eight members are represented by 54% of the proteins [7]. Such RJ proteins are covalently bound to oligosaccharides at the N-terminal residue [8] and possess numerous health benefits including antioxidant, anti-inflammatory, antitumor, and hypotensive effects and have protective effect against lipid peroxidation caused by reactive oxygen species [5,8–11]. RJ has been reported to reduce total cholesterol (TC) and increase HDL-C with simultaneous blood anti-coagulant effect [12]. It produces reduction in TC and total lipids in both blood and liver and inhibits atheroma formation in hyperlipidemic rabbits. In a dose of 50–100 mg, RJ showed reduction in serum lipids by 10% and TC by 14% in a clinical study [13]. In another study, RJ could lower TC and LDL-C but did not affect TG or HDL-C. It improved the levels of sex hormones such as dehydroepiandrosterone in human and thus reduced the risk of CVDs [2].

Reduction in tumor size of renal cell carcinoma was ascribed to improvement of tumor necrosis factor-α and transforming growth factor-β levels in the serum during oral intake of RJ in patients [14]. It has been reported that RJ is capable of maintaining quality of life and suppresses drawbacks of anticancer therapy [15,16]. Numerous *in-vivo* and *in-vitro* research studies show RJ to possess anticancer effect in various malignancies [17–20], but the underlying mechanisms of action are not fully elucidated.

The aim of the present research was to search some of the previously reported health claims including antioxidant, anticancer, and antihyperlipidemic effects of RJ so as to support or negate such effects. The *in-vivo* antihyperlipidemic effect was assayed in Triton X-100-induced hyperlipidemic rats. It was taken into consideration to study the antioxidant effect *in-vivo* through assessing RJ effect on malondialdehyde (MDA) and *in-vitro* by applying three different assays. Moreover, the anticancer effect was assessed in human-derived cancer cells from both liver and breast.

Materials and methods

Main materials

RJ was obtained from a local market, Egypt. Triton X-100 was purchased from Loba Chemie Pvt Ltd, a manufacturer of laboratory reagents and fine chemicals (Mumbai, India). Human hepatocellular carcinoma cell lines (Huh-7) and breast cancer cell lines were obtained from Nawah-Scientific (Cairo, Egypt).

Study design

RJ was tested for its antioxidant, anticancer, and antihyperlipidemic effects, where the antioxidant activities were tested *in-vitro,* whereas the anticancer activity was screened using two types of human cell line (hepatocellular carcinoma and breast cancer). An animal experiment (*in-vivo* study) was designed to assess the antihyperlipidemic effect of RJ in rats.

Ethical consideration

The present study was conducted with the Code of Ethics of the World Medical Association, according to the principles expressed in the Declaration of Helsinki. The experiment was done in compliance with the public health guide for the care and use of laboratory animals and according to the Medical Research Ethics Committee of National Research Centre, Cairo, Egypt, with approval number 19/175.

In-vitro antioxidant activity of royal jelly

Three *in-vitro* antioxidant tests were applied to study the antioxidant activity of RJ including 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH), and ferric reducing antioxidant power (FRAP) assays.

2,2-diphenyl-1-picryl-hydrazyl-hydrate assay

Sample was prepared by mixing 642.7 mg from RJ with 10 ml of solution composed of dimethyl sulfoxide : distilled water, 50 : 50. The mixture was filtered, and the filtrate was tested. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), which is a strong antioxidant, was used as a standard and was prepared in different concentrations. The assay was carried out according to previous methods [21,22]. In brief, 100 μl of freshly prepared DPPH reagent (0.1%

in methanol) was added to 100 µl of the sample in a 96-well plate (n=6), and the reaction was incubated at room temperature for 30 min in dark. At the end of incubation time, the resulting reduction in DPPH color intensity was measured at 540 nm. The same procedure was applied on different Trolox concentrations. Data were represented as means±SD according to the following equation:

Percentage inhibition=[(average absorbance of blank-average absorbance of the test)/(average absorbance of blank)]× 100.

A calibration curve was established using different Trolox concentrations from which a linear regression equation was produced between Trolox concentration and % inhibition of the DPPH free radical. Then, the results were calculated by applying the calibration curve equation of standard Trolox through substitution of the % inhibition achieved by the sample as y according to the following equation: y=1.9422x–2.0985.

2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) assay

The sample and Trolox standard were prepared as under DPPH. ABTS assay was carried out according to the method of Arnao *et al*. [23], with minor modifications to be carried out in microplates. In brief, 192 mg of ABTS was dissolved in distilled water and transferred to a 50-ml volumetric flask and then the volume was completed with distilled water. Exactly, 1 ml of the previous solution was added to 17 µl of 140 mM potassium persulfate, and the mixture was left in the dark for 24 h. After that, 1 ml of the reaction mixture was completed to 50 ml with methanol to obtain the final ABTS dilution used in the assay. Then, 190 µl of the freshly prepared ABTS reagent was mixed with 10 µl of the sample in a 96-well plate (n=6), and the reaction was incubated at room temperature for 30 min in dark. At the end of incubation time, the decrease in ABTS color intensity was measured at 734 nm in a microplate reader. The same procedure was applied on different Trolox standard concentrations. Data were represented as means±SD according to the same equation of % inhibition as under DPPH. Results of the samples were presented as µM Trolox equivalent (TE)/mg sample using the linear regression equation extracted from the calibration curve (linear dose-inhibition curve of Trolox).

Ferric reducing antioxidant power assay

The sample and Trolox standard were prepared as under DPPH. The assay was carried out according to the previously described method [24], with minor modifications to be carried out in microplates. A freshly prepared TPTZ reagent [300 mM acetate buffer (pH=3.6), 10 mM tripyridyl triazine in 40 mM hydrochloric acid, and 20 mM ferric chloride, in a ratio of 10 : 1 : 1 v/v/v, respectively] was made. Overall, 190 µl from the freshly prepared TPTZ reagent was mixed with 10 µl of the sample in a 96-well plate (n=3), and the reaction was incubated at room temperature for 30 min in dark. The same procedure was applied for the different standard concentrations. At the end of the incubation period, the resulting blue color was measured at 593 nm in a microplate reader. Data were represented as means ±SD. The linearity of Trolox in the FRAP assay was assessed as follows: the ferric reducing ability of the samples is presented as µM TE/mg sample using the linear regression equation extracted from the calibration curve (linear dose-response curve of Trolox).

Anticancer activity of royal jelly against hepatocellular carcinoma and breast cancer human cell lines

Yellow MTT [3-(4, 5-Dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide, a tetrazole] is reduced to purple formazan in the mitochondria of living cells. This reduction takes place only when mitochondrial reductase enzymes are active, and therefore conversion can be directly related to the number of viable (living) cells. The cytotoxicity test was determined in human hepatocellular carcinoma cell line (Huh-7) and breast cancer cell line (MCF-7). Cells were grown in Dulbecco's modified eagle medium supplemented with 10% fetal bovine serum, 100 U/ml of penicillin, and 100 mg/ml of streptomycin. Cultures were maintained in a humidified atmosphere with 5% CO_2 at 37°C. The RJ sample was diluted in Dulbecco's modified eagle medium complete media at 37°C to give a stock solution. Ten concentrations of two-fold serial dilutions were performed. Confluent monolayers of cells were grown in 96-well microtiter plates for 24 h. Cells were incubated with various concentrations of the test samples in triplicate at 37°C in a CO_2 environment for 72 h. After that, 20 µl of 5 mg/ml MTT was gently added to each well and incubated at 37°C for 4 h. Then media were carefully removed, and 150 µl of MTT solvent was added. Cells were covered with tinfoil and agitated on an orbital shaker for 15 min Finally, the absorbance was measured at 570 nm in a microplate reader. The relation between surviving fractions of cancer cells and RJ doses was plotted. The concentrations of RJ that reduced survival of the exposed cancer cells by 50% (IC50) were obtained from the curves [25–27].

In-vivo hyperlipidemic experiment

Rats

Male Wistar rats of body weight ranging from 215 to 225 g were obtained from the Animal House Unit, National Research Centre, Egypt. Rats were kept in stainless steel cages at room temperature, with 12 h light/dark cycle. Food and water were given *ad libitum*.

Preparation of royal jelly to be administered to rats

An emulsion from RJ was prepared by adding tween 80 as 10% from RJ weight and then diluted with water. RJ was given orally to rats in a dose of 300 mg/kg rat body weight according to a previous study [28]. A vehicle was prepared consisting of water and tween 80 having the same concentration as in the RJ emulsion (vehicle 1) to be given to control groups.

Preparation of Triton X-100

Triton X-100 was prepared by dissolving in saline (1 g triton was completed to 15 ml by saline) and was given to rats as 100 mg Triton/kg rat body weight as intraperitoneal injection according to a previous study [29]. Saline was given intraperitoneally to the normal control (NC) rats as vehicle 2.

Composition of the diet

Rats were maintained on a balanced diet throughout the experiment. The diet constituted 62.5 carbohydrates, 25% protein, 4% fat, 4% crude fibers, 1% vitamin mixture, and 3.5% mineral mixture [30].

Design of animal experiment

Rats were divided into three groups with eight rats each. The first group served as NC. Rats of the second and third groups were treated with one intraperitoneal dose of Triton X-100 (100 mg/kg rat body weight) after an overnight fast from which one group (second group) served as hyperlipidemic control (HC), whereas the rats of the third group were given RJ for 2 weeks before Triton injection and 3 days after injection as daily oral dose of 300 mg/kg rat body weight. Rats of the NC and HC groups were given daily oral dose of vehicle 1 (Tween 80 in distilled water). Rats of the NC group were given intraperitoneal saline as vehicle 2. All rats were fed on a balanced diet throughout the experiment. Body weights of all rats were measured at the start and the end of the experiment, which continued for 18 days.

Blood sampling and biochemical analysis

Blood samples were obtained from fasted anesthetized rats, 3 days after triton injection, and received in heparinized test tubes. Blood samples were centrifuged to obtain the plasma. The determined biochemical parameters were plasma triglycerides (TGs), TC, HDL-C, and LDL-C, which constitute plasma lipid profile using previously described methods [31–34]. MDA was assayed as a biomarker of lipid peroxidation by the colorimetric method of Satoh [35]. The activities of alanine transaminase (ALT) and aspartate transaminase (AST) were assessed as biomarkers of liver function by the method adopted previously [36]. All kits used were purchased from Spectrum Diagnostics, Cairo, Egypt, except for MDA, which was obtained from Bio Diagnostic Co., Cairo, Egypt. The ratio of TC/HDL-C was calculated as an indicator of CVD risks [37]. Livers were obtained from dissected rats and kept in 10% formalin for histopathological examination [38].

Statistical analysis

Data from *in-vivo* experiment were expressed as means ±SE. Data were statistically analyzed by one-way analysis of variance followed by the Tukey multiple comparison tests using the SPSS statistical program, version produced by SPSS Inc., Chicago, USA. Differences were considered significant at P value less than or equal to 0.05. Data from *in-vitro* antioxidant activity were expressed as means±SD.

Results

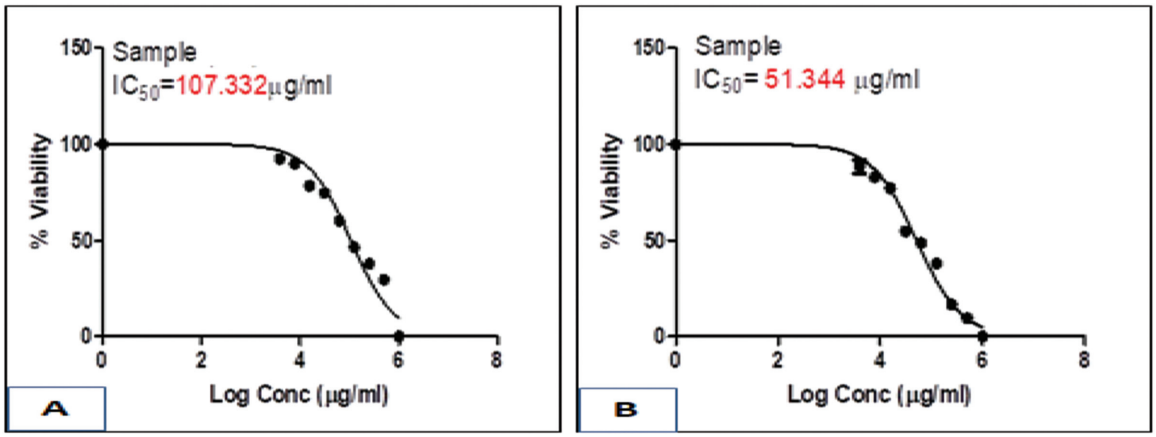
The antioxidant activities of RJ as related to Trolox, a strong antioxidant compound, are compiled in Table 1. It could be noticed that the antioxidant activity of RJ was 5.634±0.296, 2.05±0.17, and 0.43±0.012 μM Trolox eq/mg RJ when assessed using ABTS, FRAP, and DPPH assays, respectively. The percentage inhibitions of ABTS and DPPH by RJ were 52 and 25.1%, respectively.

Table 1. Antioxidant activity of royal jelly using 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid), 2,2-diphenyl-1-picryl-hydrazyl-hydrate, and ferric reducing antioxidant power

Assay type	Mean±SD
ABTS scavenging activity	
μM Trolox eq/mg RJ	5.634±0.296
Percentage inhibition of ABTS	52.0%
DPPH scavenging activity	
μM Trolox eq/mg RJ	0.43±0.012
Percentage inhibition of DPPH	25.10
FRAP	
μM Trolox eq/mg RJ	2.05±0.17

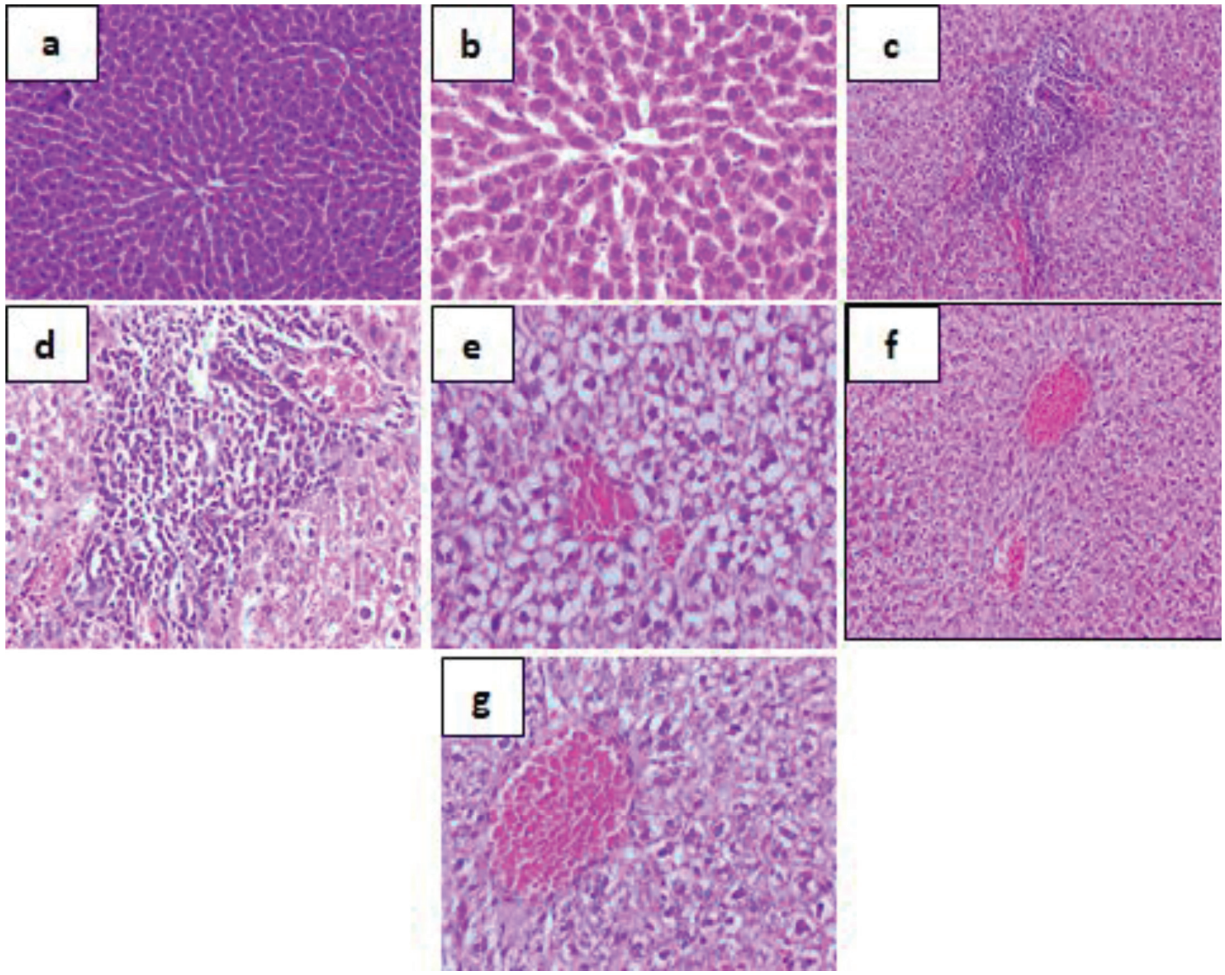
ABTS, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid); DPPH, 2,2-diphenyl-1-picryl-hydrazyl-hydrate; FRAP, ferric reducing antioxidant power; RJ, royal jelly.

Figure 1.



Percentage viability of Huh-7 hepatocellular carcinoma against different log concentrations of royal jelly (A). Percentage viability of MCF-7 breast cancer against different log concentrations of royal jelly (B).

Figure 2.



Liver histopathology of the experimental groups: (a and b) livers of the normal control group show normal structure (×100 and ×200, respectively). (c, d, and e) Livers of the hyperlipidemic control group demonstrated that blood vessels were severely congested with severe necrosis of hepatic cells, fat deposition, strong vacuolar degeneration, and inflammatory cells infiltration along with severe leukocytic cell infiltration (×100, ×200, and ×200, respectively). (f and g) Livers of the hyperlipidemic rats treated by royal jelly exhibited moderate congestion of blood vessels and fatty change, whereas only moderate vacuolar degeneration was observed along with mild necrosis and mild inflammatory cell infiltration with absence of cell degeneration (×100 and ×200, respectively).

The anticancer activities designated by IC50 of RJ toward MCF-7 and Huh-7 are shown in Table 2 and Fig. 1 (A&B). It can be noticed that IC50 values were 51.133 and 107.332 μg/ml from RJ toward MCF-7 and Huh-7, respectively.

The different biochemical and body weight parameters of the *in-vivo* experiment are demonstrated in Table 3. The results showed that Triton injection induced significant increase in plasma TC, TGs, LDL-C, and TC/HDL-C as could be seen in the HC group compared with the NC group. Moreover, a significant reduction in HDL-C was noticed in the HC group compared with the NC group. RJ administration resulted in significant reduction in TC, TGs, LDL-C, and TC/HDL-C along with a significant increase in HDL-C compared with the HC group. The levels of plasma TC, TGs, and TC/HDL-C in the RJ-treated group matched with those of the NC group, whereas LDL-C and HDL-C were still different significantly from the NC group.

Table 2. Anticancer activity of royal jelly as determined by IC50

Cancer cell line type	IC50 of RJ (μg/ml)
Huh-7	107.332
MCF-7	51.133

IC50, the concentrations of RJ which reduced survival of the exposed cancer cells by 50%; Huh-7, human hepatocellular carcinoma; MCF-7, human breast cancer; RJ, royal jelly.

Plasma levels of MDA and the activities of AST and ALT (Table 3) were significantly high in the HC group compared with those in the NC group. RJ treatment produced reduction in MDA, ALT, and AST compared with the HC group. The only normalized parameter was MDA, whereas AST and ALT were still significantly higher than those of the control. Concerning body weight parameters (Table 3), the final body weight and body weight gain of Triton-treated rats of the HC group and the group given RJ showed significant reduction compared with the NC group. Such parameters showed some sort of reduction when the group treated with RJ was compared with the HC group but insignificantly.

The liver histopathology of the different groups is presented in Fig. 2 and Table 4. Livers of the NC

Table 3. Plasma lipids, malondialdehyde, and alanine transaminase and aspartate transaminase activities and body weight parameters of the experimental groups of the hyperlipidemic experiment

Parameters	Groups Normal control group	Hyperlipidemic control group	Royal jelly group
Plasma lipid profile			
TG (mg/dl)	62.04±3.65[a]	83.48±7.45[b]	66.30±6.44[a]
TC (mg/dl)	87.11±2.58[a]	112.73± 3.27[c]	96.29±6.59[a]
HDL-C (mg/dl)	50.75±4.3[b]	20.03±3.78[a]	37.70±3.8[c]
LDL-C (mg/dl)	23.952±4.87[a]	76.004±5.18[c]	45.33±5.81[b]
TC/HDL-C	1.716±0.05[a]	5.628±0.11[c]	2.554±0.1[a]
Plasma MDA (nm/ml)	6.4±0.29[a]	10.3±0.1[b]	6.8±0.05[a]
Plasma ALT (IU/l)	42.43±0.38[a]	74.5± 0.66[c]	62.6±0.49[b]
Plasma AST (IU/l)	56.35±0.18[a]	81.26±0.17[c]	72.34±0.2[b]
Weight parameters			
Initial body weight (g)	220±7.7[a]	219.6±3.2[a]	219.6±4.2[a]
Final body weight (g)	292±25.02[b]	249.2±7.7[a]	233±5.5[a]
Body weight gain (g)	72±17.7[b]	29.6±5.01[a]	13.4±1.6[a]

All values are expressed as means±SE.
ALT, alanine transaminase; AST, aspartate transaminase; HDL-C, high-density lipoprotein-cholesterol; LDL-C, low-density lipoprotein-cholesterol; MDA, malondialdehyde; TC, total cholesterol; TG, triglycerides.
Different superscript letters (a, b, c) within the same row indicate significant difference at *P* value less than 0.05 using analysis of variance test.

Table 4. Liver histopathological changes of different experimental groups

Changes	Groups Normal control group	Hyperlipidemic control group	Royal jelly treated group
Degeneration	–	+++	+
Vacuolar degeneration	–	+++	++
Fat deposition	–	+++	++
Necrosis and Inflammatory cells infiltration	–	+++	+
Congestion	–	+++	++

–, within normal limits; +, mild change; ++, moderate changes; +++, severe changes.

group showed normal structure appearance. Livers of the HC group demonstrated that blood vessels were severely congested with severe necrosis of hepatic cells, fat deposition, strong vacuolar degeneration, and inflammatory cells infiltration along with severe leukocytic cell infiltration. Livers of hyperlipidemic rats treated by RJ exhibited moderate congestion of blood vessels and fatty changes, whereas only moderate vacuolar degeneration was observed along with mild necrosis and mild inflammatory cell infiltration with absence of cell degeneration.

Discussion

RJ is considered as a functional food owing to the different claimed health-promotion effects, including antioxidant, anti-inflammatory, and antihyperlipidemic effects. RJ contains lipids, sugar, proteins, and water; ~90% of RJ lipids are free fatty acids commonly 8–12 carbons in either hydroxyl or dicarboxylic forms [39]. The major component of RJ is 10-HDA reported to have a vital role in prevention of various biological activities including inflammation and oxidative stress [40]. The content of 10-HDA was demonstrated to be 0.8–6.5%, and such compound is not present in any other natural sources even in other bee products; therefore, it is considered as an exceptional compound [41]. Moreover, other studies showed that RJ proteins are other major contributors of the health benefits of RJ [42,43]. The present research deals with studying health effects of RJ, including *in-vitro* antioxidant, anticancer, antihyperlipidemic, hepatoprotective, and cardioprotective activities.

The reduction in LDL-C and elevation of HDL-C after treatment with RJ might be attributed to alteration in lipoproteins on RJ administration reported previously [9]. Moreover, the reduction in TC and LDL-C as could be seen from the present study agreed with a previous work [2], which was ascribed to the presence of RJ proteins 1 and 2. Previously, dietary protein was shown to influence blood cholesterol [44]. Major RJ protein-1 was reported to have high bile acid capacity that results in inhibition of cholesterol absorption leading to reduction in blood TC and LDL-C [45]. RJ protein-1 also upregulates one of the key enzymes in cholesterol metabolism in the liver, which is cholesterol 7-α-hydroxylase, thereby influences blood cholesterol [8]. Some clinical studies demonstrated reduction in TC and LDL-C on administration of RJ [9,46]. RJ might also decrease TC and LDL by lowering small very LDL [8]. The cholesterol-lowering action of RJ was also reported to be owing to major RJ protein-1 that interacts with bile acids inducing increase in bile acid and cholesterol excretion with concomitant enhancement of hepatic cholesterol catabolism [47]. It was reported that RJ administration reduced gene expression of squalene epoxidase, another key enzyme in cholesterol synthesis [48].

The present study showed reduction in rats′ body weight gain on administration of RJ but insignificantly, which did not agree with the study of Chiu *et al*. [2] in humans, which showed that RJ did not cause any differences in body weight and body fat when given as a 3-month intervention. It was reported that RJ produced satiety in overweight adults [49] which agreed with the present result, where reduction in body weight gain was seen. It was also reported that patients with normal hepatic function did not show any change of the parameters that reflected liver function [2]. The present work demonstrated that RJ produced reduction in AST and ALT activities (that showed elevation due to Triton injection) pointing to improvement of liver function. However, El-Nekeety *et al*. [50] reported no improvement in ALT and AST on administration of low RJ dose (100 mg/kg) in rats with high oxidative stress. Liver inflammation, degeneration, necrosis, congestion, and fat deposition induced by Triton in the present study were reduced on the administration of RJ, which supported the improvement in liver function represented by ALT and AST. The reduction in liver fat deposition on administration of RJ might result in an improvement in lipid profile in the present study. Some studies also inferred that both 10-HDA and RJ proteins have estrogenic activity [51,52], which might have a role as being cardioprotective that may be reflected in the reduction of the atherogenic ratio (TC/HDL-C) in the present study. El-Shafey *et al*. [28] reported cardiovascular health protection on RJ administration through elevation of HDL-C and reduction of LDL-C, TC, and very LDL-C. In addition, the same authors showed reduction in oxidative stress on treatment with RJ, which go in line with the present study that showed reduction in MDA and improvement in lipid parameters. Hypercholesterolemia produced reduction in the antioxidant enzymes (glutathione and catalase) causing damage to the oxidative defense system of the cell. Such changes lead to reactive oxygen species that lead to high oxidative stress [1] as was the case on injection of Triton in the HC group. Moreover, RJ administration demonstrated improvement in antioxidant capacity and lipid profile and reduction

of inflammation in overweight adults [49]. Another study reported that RJ administration exhibited increase in antioxidant enzymes [53], thereby reduced oxidative stress. Such reported improvement in oxidative stress and inflammation might explain the inhibition of inflammation and degeneration of hepatic cell by RJ as shown from the liver histopathological changes in the current study. The *in-vitro* antioxidant activities of RJ as shown in the present study supported the *in-vivo* reduction of MDA in triton-treated rats given RJ in the current work and agreed with the results of the aforementioned studies.

Regulation of inflammation and immunity was reported during the treatment of RJ [49,54]. Fascinatingly, carcinogenesis and malignancy are strongly related to inflammation and disorder of the immune system [55]. Inflammatory cytokines are reported to be correlated with malignancies [56]. RJ was reported to regulate such cytokines [57]. An anticancer effect owing to 10-HAD was reported, which mediated through the regulation of inflammatory function and oxidative stress [20,58]; however, Nakaya *et al*. [17] showed 10-HDA to have no antiproliferative effect. The present study showed RJ to possess anticancer activity against hepatocellular carcinoma Huh-7 type and against breast cancer MCF-7. The value of IC50 of RJ showed that RJ was more efficient in reducing MCF-7 than Huh-7. The proposed anticancer mechanism of action might be related to an effect on inflammatory cytokines and immune system; however, more investigations are needed in this respect.

Based on the abovementioned results and discussion, RJ might be used as complementary to hypolipidemic and chemotherapeutic drugs. It might be also used within the daily meals as functional food for protection from hyperlipidemia and cancer.

Conclusion

The outcome of the present finding is that RJ possesses hepatoprotective, antihyperlipidemic, antioxidant, and anticancer activities.

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Conflicts of interest

There are no conflicts of interest

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