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RESEARCH ARTICLE

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Hypocholesterolemic efficacy of royal jelly in healthy mild hypercholesterolemic adults

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ABSTRACT

Context: Royal jelly (RJ) has been reported for its health promoting factors such as antioxidant, anti-inflammatory and lipid lowering activities.

Objective: The present randomized, placebo-controlled study examines the hypolipidemic beneficial effect of RJ through evaluating anthropometric measurements, lipid profile and various hormone levels in mildly hypercholesterolemic participants.

Materials and methods: Forty subjects with mild hypercholesterolemia (180–200 mg/dL) were randomly selected and divided into two groups as experimental or placebo, who requested to intake nine capsules (350 mg/capsule) of RJ or placebo/day, respectively, for three months with one month of follow-up without any supplementation.

Results: No significant changes were noted in any of the anthropometric parameters like body weight, waist and body fat. The serum total cholesterol (TC; 207.05–183.15 mg/dL) and low-density lipoprotein cholesterol (LDL-c; 126.44–120.31 mg/dL) levels were reduced significantly ($p < 0.05$) after administration of RJ. However, triglyceride (TG) and high-density lipoprotein cholesterol (HDL-c) levels were not considerably altered. Moreover, three months of RJ consumption significantly ameliorated ($p < 0.05$) the concentration of sex hormones like dehydroepiandrosterone sulphate (DHEA-S; 1788.09–1992.31 ng/mL). Also, intake of RJ did not elicit any hepatic or renal damage.

Discussion and conclusion: Intervention with RJ for three months considerably lowered the TC and LDL-c levels through improving the levels of DHEA-S and thus alleviates the risk of cardiovascular disease (CVD).

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Introduction

Cholesterol is an essential substance with several physiological functions; however when its level elevates substantially in the blood (hypercholesterolemia) that leads to various deleterious conditions such as atherosclerosis and related cardiovascular diseases (CVD) (Roman et al. 2015). Hypercholesterolemia is considered as a major culprit for CVD, which contributes about one-third of total deaths globally (Katz et al. 2001). Even in Taiwan, the CVD incidence rate has enormously escalated in recent times (Chiu et al. 2015). At present, several lipid-lowering drugs are available to combat hypercholesterolemia including statins, bile acid sequestrants, fibrate and PSCK9 inhibitors, but those drugs are highly associated with several adverse events (Bao et al. 2012). Recently, researchers are concentrated more on natural dietary proteins, which might positively influence the blood lipid levels without any adverse effects (Matthan et al. 2007; Koury et al. 2014).

Royal jelly (RJ) is considered a functional food, widely used in many countries as a commercial food product/supplement as well as in the cosmetics industry (Ramadan & Al-Ghamdi 2012). RJ is a special secretory product, from the hypopharyngeal and mandibular glands of young worker bees (*Apis mellifera* Linn.), that is thick and milky in nature (Isidorova et al. 2009). RJ is composed of proteins (amino acids), lipids, carbohydrates (sugars), vitamins and many other bioactive substances such as 10-hydroxyl-2-decenoic acid (Nagai & Inoue 2004). The protein of RJ is comprised of many major RJ proteins (MRJPs) (Srisuparbh et al. 2003). The MRJP family has nine members; MRJPs 1–9 (Albert & Klaudiny 2004; Drapeau et al. 2006; Schonleben et al. 2007). Furusawa et al. (2008) pointed out total RJ proteins constituted about 46% of MRJP1, 11% of MRJP2, 13% of MRJP3 and the remaining 30% by other six types of MRJPs by using 2D SDS-PAGE technique. MPJPs are glycoproteins that are covalently bound to oligosaccharides at the N-terminal residue (Kashima et al. 2014). Ample amount of experiments has indicated that MRJPs of RJ are a major contributor for various physiological functions (Drapeau et al. 2006; Schonleben et al. 2007).

RJ shows numerous pharmacological activities including antioxidant, anti-inflammatory, antihypercholesterolemic, antitumor, as well as hypotensive and vasodilative dermaprotective properties (Nagai & Inoue 2004; Guo et al. 2007; Park et al. 2012;

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Kashima et al. 2014). Previous studies have indicated that the hypocholesterolemic effect of dietary proteins and peptides are associated with its bile acid binding capacity and its metabolism (Nagaoka et al. 2010; Kashima et al. 2014). Bile acid-binding proteins found in RJ have a hypocholesterolemic effect (Kashima et al. 2014). RJ has been already reported to alter lipoprotein metabolism in humans (Guo et al. 2007), however the reason or underlying mechanism for improving lipoprotein metabolism was not fully explored. The purpose of this study was to demonstrate that supplementation of protein enriched RJ can effectively regulate the blood lipid profile through improving the concentration of DHEA-S in healthy mild hypercholesterolemic subjects.

Materials and methods

Chemicals

Potassium dihydrogen phosphate (KH_2PO_4) and trichloroacetic acid (TCA) were bought from Merck KGaA, Darmstadt, Germany. Hydrogen peroxide (H_2O_2), hydrochloric acid (HCl), β-nicotinamide-adenine dinucleotide phosphate reduced form (β-NADPH), sodium carbonate (Na_2CO_3), sodium chloride (NaCl) and CKJS II-7170 were acquired from Wako Chemicals, Richmond, VA. Cholesterol reagent, triglyceride (TG) reagent, HDL cholesterol reagent, LDL cholesterol reagent, glucose reagent, albumin reagent and coat-A-Count TSH IRMA were purchased from Tonyar Biotech. Inc., Taipei, Taiwan.

Composition of RJ and placebo capsule

Experimental RJ capsules and placebo capsules were provided by the Bee Touched, Yunlin County, Taiwan. Each RJ capsule (350 mg), contained protein 50 mg, fat 10 mg, carbohydrate 70 mg and sodium 0.19 mg. The placebo capsule contained only corn starch. Both experimental RJ and placebo capsules appeared similar to each other in terms of colour, flavour, size and shape to make sure that they cannot be distinguished.

Participants and experimental grouping

Totally 40 mild hypercholesterolemic (TC 180–200 mg/dL) healthy volunteers were chosen and randomly divided into two groups as an experimental group with 20 participants, including 11 males and nine females and the placebo group with 20 participants, including 10 males and 10 females. The exclusion criteria for this study included subjects with liver, kidney or heart disease, uncontrolled diabetes, mental illness or depression, pregnancy, breast-feeding woman and uterine myoma, as well as with the history of stroke. All participants refrained from taking antibiotics and other nutritional supplements or other drugs pertaining to metabolic syndrome. The entire study consist of two weeks of adoption period followed with three months of administration period (intervention), during which subjects in the experimental or placebo groups were prescribed to intake nine capsules (350 mg/capsule) of RJ or placebo every day respectively; with one month of follow-up without any supplementation. A questionnaire was asked to each participant at the beginning of the study (especially dietary pattern and mental health) and after three months, to explore the opinions on the basic physical conditions (any adverse events). A physical examination was carried out at the beginning of the study and every month through the clinical trial. All the participants were requested to sign a consent form before their enrolment in this study.

This randomized, placebo-controlled and single-blind design clinical trial was carried out in Chung Shan Medical University Hospital from April 2013 to July 2014. This trial was conducted in accordance with the Declaration of Helsinki, 2008 and subsequent revisions and approved by the Institutional Review Board (IRB) of Chung Shan Medical University Hospital, Taichung, Taiwan (IRB No. CS13070). During the baseline (initial), 1st, 2nd, 3rd and 4th months (follow-up), fasting blood samples were collected for biochemical assays, and anthropometric measurements (body weight, waist circumference, and body fat mass) were performed. During the intervention, three subjects were withdrawn owing to a family situation and thus end up with 19 subjects in the placebo and 18 subjects in the experimental group ($n = 37$). A diagrammatic representation of current study is illustrated in Figure 1.

Blood collection and biochemical analysis

Blood samples were collected from overnight fasted subjects in EDTA coated tube for plasma and another tube without anticoagulant for serum preparation. Plasma was separated by

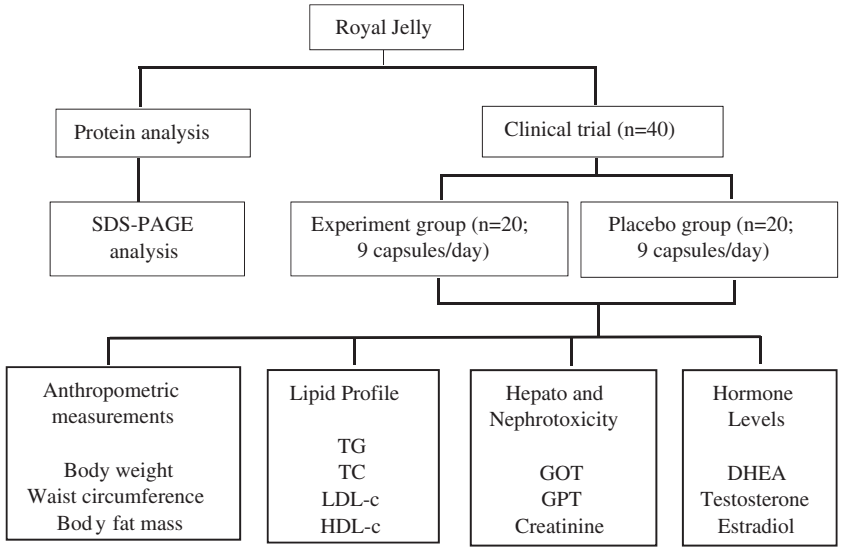


Figure 1. Diagrammatic representation of current study.

centrifuging at 1500×*g* (Supercentrifuge, 1K15, Sigma, Taufkirchen, Germany) and used for determining the TG, TC, high-density lipoprotein cholesterol (HDL-c), low-density lipoprotein cholesterol (LDL-c), proteins, hepatic marker enzymes like glutamic oxaloacetic transaminase (GOT) and glutamic pyruvate transaminase (GPT) as well as total protein by the Lowry method. Serum samples were used to determine the levels of renal marker enzymes like creatinine (Cre) and to measure the concentration of sex hormones including testosterone (T), DHEA, estradiol (E2) by Versa Max ELISA kit method (Molecular Devices, Sunnyvale, CA). Total protein in RJ was evaluated by BCA assay kit. Royal jelly protein analysis was done by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) technique.

Statistical analysis

All results were explicated as a mean ± standard deviations (SD). Paired *t*-test was utilized to compare the difference within the experimental group and placebo group with the help of software – Statistical package for the social sciences (SPSS) version 23.0 (IBM Inc., Chicago, IL). All statistical outcomes with *p* values less than 0.05 ($p < 0.05$) were recognized as statistically significant.

Results

Analysis of proteins in RJ by SDS-PAGE

The total protein present in the experimental RJ samples was found to be 142.85 ± 0.35 mg/g by biochemical method (BCA kit). However, the SDS-PAGE technique was employed to analyse the different types of proteins (MRJP) in the RJ experimental samples (Figure 2). Lane 1 (M) showed the protein ladder-molecular weights range of 10–130 kDa. Lanes 2 and 3 (experimental samples 1 and 2) showed the two major RJ proteins (MRJP1 and MRJP2), with prominent protein band near the molecular weight 40 kDa and 55 kDa. However, the streaky band was also noted between 35 and 40 kDa. The SDS-PAGE gel was picturized and quantified using the software Image J (Bethesda, MD), which revealed the presence of MRJP1 (19.21 mg/g) and MRJP2 (41.68 mg/g).

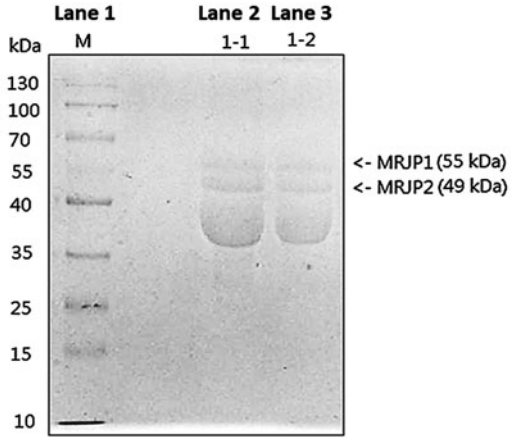


Figure 2. SDS-PAGE analysis of experimental RJ samples. Lane 1 (M) represents Molecular Protein Ladder (10–130 kDa). Lane 2 (1-1) represents RJ sample 1 and lane 3 (1-2) represents RJ sample 2.

Subjects characteristics

Continuous use of RJ or placebo for three months did not show any substantial differences in body weight, waist circumference and body fat as compared with the initial (Table 1). Thus, showcasing that RJ consumption did not induce any physiological changes or any discomfort to any of the subjects during the experimental period, which were also endorsed by continuous monitoring of health status of each subject.

Changes in hepatic and renal markers

Analysis of liver and kidney functions were conducted before and after the experimental period in healthy mild hypercholesterolemic subjects (Table 2). No considerable changes were noted in the levels of GOT, GPT and Cre in the experimental or placebo group.

Changes in the levels of sex hormones

Studies have proved that the levels of E2, T and DHEA-S were significantly lower in CVD subjects than non-CVD subjects. Therefore, the levels of E2, T and DHEA-S were assessed to check its link between CVD after RJ consumption (Table 3). The results showed that after supplementation with RJ, the contents of DHEA-S were greatly enhanced ($p < 0.05$), but no notable changes were noted during follow-up period. Meanwhile, the levels of E2 and T contents were unchanged on neither experimental nor placebo group.

Serum lipoprotein metabolism

The experimental group showed that the administration of RJ, significantly reduced ($p < 0.05$) the TC and LDL-c levels by 11.5% and 4.8% on equivalence with initial period (Table 4). However, TC and LDL-c levels inclined after the follow-up period and indicated that RJ cessation might alter the lipid profile especially TC. Meanwhile, the levels of TG and HDL-c were unaltered upon treatment with RJ. In the placebo group, the levels of TC, LDL-c, TG and HDL-c were not changed.

Discussion

Royal Jelly is a popular functional food, owing to its several health promoting properties like antioxidant, anti-inflammatory and hypolipidemic activities. Studies have indicated that MRJPs of RJ are major contributors for various physiological functions (Drapeau et al. 2006; Schonleben et al. 2007). RJ has been already reported to alter lipoprotein metabolism in humans (Guo et al. 2007), however the link between lipid metabolism and DHEA-S pertain to MRJP were yet to be explored. Hence, the aim of this current study was to investigate, whether the consumption of protein enriched RJ can effectively regulate the blood lipid profile by ameliorating the concentration of DHEA-S in healthy mild hypercholesterolemic subjects.

The protein estimation by spectrometric method showed the increased presence of protein in the experimental group. SDS-PAGE analysis showed the two major RJ proteins (MRJP1 and MRJP2), with prominent protein band at 49 kDa and 55 kDa. Also, quantification of our experimental sample showed that MRJP2 was higher than MRJP1. Our results corresponded with

Table 1. Anthropometric parameters in healthy mild hypercholesterolemic subjects after treatments.

	Weight (kg)		Waist (cm)		Body fat (%)	
	Placebo ($n = 20$)	Experiment ($n = 20$)	Placebo ($n = 20$)	Experiment ($n = 20$)	Placebo ($n = 20$)	Experiment ($n = 20$)
Initial	69.88 ± 14.59	70.68 ± 31.62	82.02 ± 10.99	83.35 ± 12.79	25.08 ± 5.54	26.65 ± 4.92
Month 1	69.07 ± 14.48	64.53 ± 15.26	81.95 ± 11.41	81.82 ± 13.99	25.48 ± 5.49	25.67 ± 5.10
Month 2	69.04 ± 14.13	64.66 ± 15.47	81.65 ± 11.62	81.75 ± 14.68	24.71 ± 5.85	25.69 ± 5.45
Month 3	69.26 ± 13.96	64.55 ± 15.65	80.62 ± 11.87	84.00 ± 12.65	24.14 ± 5.68	25.59 ± 4.70
F/U	68.41 ± 13.98	68.89 ± 31.62	81.07 ± 11.73	84.40 ± 12.29	24.50 ± 5.28	24.88 ± 5.69

Values are expressed as mean ± SD.

Table 2. The lipid profile on healthy mild hypercholesterolemic subjects after treatments.

	LDL-c (mg/dL)		HDL-c (mg/dL)		TC (mg/dL)		TG (mg/dL)	
	Placebo ($n = 20$)	Experiment ($n = 20$)	Placebo ($n = 20$)	Experiment ($n = 20$)	Placebo ($n = 20$)	Experiment ($n = 20$)	Placebo ($n = 20$)	Experiment ($n = 20$)
Initial	102.77 ± 30.16	126.44 ± 26.37	56.31 ± 13.24	52.33 ± 7.96	176.35 ± 31.54	207.05 ± 28.50	84.00 ± 81.38	131.45 ± 57.81
Month 1	99.89 ± 21.75	124.49 ± 28.33	54.22 ± 14.15	49.59 ± 10.05	167.55 ± 28.81	193.65 ± 25.45[a]	88.25 ± 79.69	154.65 ± 190.03
Month 2	96.67 ± 22.08	122.10 ± 25.26	54.19 ± 14.61	50.32 ± 7.64	171.3 ± 25.12	190.15 ± 23.38[a]	89.10 ± 76.07	129.25 ± 84.72
Month 3	96.92 ± 19.11	120.31 ± 21.72[a]	54.55 ± 14.93	53.39 ± 8.42	172.45 ± 25.02	183.15 ± 24.86[a]	95.45 ± 98.11	145.00 ± 114.23
F/U	98.4 ± 25.54	121.47 ± 33.71	53.98 ± 15.16	52.53 ± 6.34	170.55 ± 29.61	197.85 ± 35.59	90.70 ± 108.63	145.25 ± 100.45

Values are expressed as mean ± SD.
[a]Significant difference as compared with the initial ($p < 0.05$).

Table 3. The changes in renal and hepatic markers on healthy mild hypercholesterolemic subjects after treatments.

	GPT (IU/L)		GOT (IU/L)		Cre (mg/dL)	
	Placebo ($n = 20$)	Experiment ($n = 20$)	Placebo ($n = 20$)	Experiment ($n = 20$)	Placebo ($n = 20$)	Experiment ($n = 20$)
Initial	25.05 ± 10.04	25.15 ± 19.21	20.00 ± 4.77	21.15 ± 6.50	0.78 ± 0.18	0.79 ± 0.17
Month 1	25.55 ± 11.60	25.75 ± 19.18	20.25 ± 4.03	21.85 ± 7.31	0.78 ± 0.18	0.78 ± 0.20
Month 2	26.20 ± 9.92	26.00 ± 20.67	20.05 ± 3.94	21.45 ± 7.90	0.81 ± 0.20	0.81 ± 0.19
Month 3	25.95 ± 8.35	26.60 ± 20.41	19.95 ± 4.81	22.80 ± 8.55	0.79 ± 0.20	0.80 ± 0.17
F/U	25.55 ± 9.98	26.55 ± 19.48	19.85 ± 6.11	22.55 ± 7.81	0.82 ± 0.19	0.81 ± 0.19

Values are expressed as mean ± SD.

Table 4. The changes in blood hormone in healthy mild hypercholesterolemic subjects after treatments.

	DHEA (ng/mL)		Testosterone (ng/mL)		Estradiol (ng/mL)	
	Placebo ($n = 20$)	Experiment ($n = 20$)	Placebo ($n = 20$)	Experiment ($n = 20$)	Placebo ($n = 20$)	Experiment ($n = 20$)
Initial	1894.00 ± 150.48	1788.09 ± 106.06	1.89 ± 0.69	1.96 ± 0.62	125.40 ± 12.51	68.85 ± 6.69
Month 1	1883.10 ± 102.50	1947.03 ± 168.19	1.85 ± 0.78	1.90 ± 0.56	133.65 ± 7.28	66.70 ± 4.47
Month 2	1857.40 ± 122.41	2050.72 ± 109.30[a]	1.78 ± 0.85	1.91 ± 0.60	137.90 ± 15.93	69.15 ± 5.34
Month 3	1886.85 ± 133.66	1992.31 ± 102.09[a]	1.75 ± 0.41	1.87 ± 0.54	127.90 ± 8.28	71.60 ± 6.18
F/U	1901.40 ± 124.21	1841.54 ± 259.36	1.72 ± 0.75	1.85 ± 0.49	128.80 ± 8.57	72.10 ± 10.35

Values are expressed as mean ± SD.
[a]Significant difference as compared with the initial ($p < 0.05$).

the results of Kashima et al. (2014). Hence, we speculated that the presence of protein in RJ, specifically MRJP1 and MRJP2, might be the primary contributor for the hypocholesterolemic property.

Three months intervention with experimental RJ or placebo did not exhibit any concomitant differences in body weight, waist circumference and body fat. Thus, the results showed that RJ consumption could not induce any physiological changes and discomfort. The subjects enrolled in this study were healthy with normal postural status. Similar results are shown by Guo et al. (2007), who demonstrate that intake of 6 g of RJ does not show any substantial changes in the levels of body weight, fat and waist.

Both hepatic and renal function markers were not significantly altered after the intervention with either experimental RJ or placebo. El-Nekeety et al. (2007) found that rats receiving a low dose (100 mg/kg body weight) of RJ did not show a significant difference in the activities of GOT and GPT as compared with control group. The outcome of the present finding justified that intake of RJ for three months continuously did not damage either hepatic or renal organ and thus proved as a safe drug without any adverse events.

Studies have demonstrated that E2, T and DHEA-S exert various health promoting activities like anti-aging (longevity), anti-atherogenic, hypocholesterolemic, hypotensive, immunomodulatory as well as enhance cognitive effects (Jedrzejuk et al. 2003; Corona et al. 2011). Previous studies have proved that E2, T and DHEA-S contents were significantly lower in CVD subjects than non-CVD subjects (Shufelt et al. 2011). Therefore, the levels of E2, T and DHEA-S were assessed to check its link between CVD after RJ consumption. Supplementation with RJ notably improved the content of DHEA-S, but no considerable changes

during the follow-up period. Some experiments also inferred that MRJP and 10-hydroxy-2-decenoic acid (10-HDA) have estrogenic activity in different models (Mishima et al. 2005; Moutsatsou et al. 2010). Hence, RJ can exert and can increase the level of DHEA-S owing to its estrogenic activity. Our results were in congruence with the observation of Morita et al. (2012). Meanwhile, the levels of E2 and T contents remained unchanged in either experimental nor placebo group.

A small number of trials have proven that increased levels of LDL-c with decreased levels of HDL-c might elevate the risk of CVD (Chiu et al. 2015). Hence, it is necessary to evaluate the levels of TC, LDL-C and HDL-c. Previous studies have found that dietary protein influences the concentration of cholesterol in the blood (Teede et al. 2001; Madani et al. 2003). The administration of RJ concomitantly reduced the TC and LDL-c levels owing to the presence of MRJP1 and 2. Nagaoka et al. (2010) pointed out that MRJP1 has a high bile acid binding capacity, which hampers the micelle formation and thereby suppresses the cholesterol absorption that results in decreased cholesterol and LDL-c levels. A recent study also indicates that the amount of cholesterol 7-α-hydroxylase (CYP7A1) (the key enzyme in the metabolism of cholesterol in the liver) is significantly upregulated in hepatic cells upon administration of MRJP1 in a rat model (Kashima et al. 2014). Some experiments also inferred that MRJP and 10-HDA have estrogenic activity in different models (Mishima et al. 2005; Moutsatsou et al. 2010). To support our results, during the follow-up (no RJ treatment) period, the levels of TC and LDL-c levels started to increase. Meanwhile, the levels of TG and HDL-c were unaltered upon treatment with RJ. Few clinical trials have also shown a decrease in the levels of TC, LDL-c and small VLDL after the intake of RJ (Guo et al. 2007; Morita et al. 2012).

Studies show that DHEA-S can influence the activity of glucose 6-phosphate dehydrogenase and glycerol 3-phosphate dehydrogenase (a key enzyme in HMP pathway) and thereby halt the production of NADPH, which in turn inhibit the synthesis of fatty acid, phospholipids and cholesterol (Porsova-Dutoit et al. 2000). Similarly, Tchernof et al. (1997) also indicated that elevated levels of DHEA-S might alter the lipid metabolism through attenuating the levels of TC and LDL-c. As mentioned earlier, RJ supplementation substantially increased the levels of DHEA-S. Hence we hypothesized that the intake of protein enriched RJ especially MRJP could effectively regulate the blood lipid profile by ameliorating the concentration of DHEA-S in healthy mild hypercholesterolemic subjects.

Conclusions

The preliminary protein analysis of RJ by SDS-PAGE revealed the presence of MRJP1 and MRJP2. Daily ingestion of nine RJ capsules for three months could significantly increase the concentration of DHEA-S and thereby considerably lower the serum TC and LDL-c levels. The outcome of the present finding showed that RJ could act as a good hypocholesterolemic agent and thus effectively attenuated the risk of CVD. In the future, the investigation about the exact mechanism behind the hypocholesterolemic activity of RJ in correlation with MRJP1 is required.

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Disclosure statement

The authors declare that there is no conflict of interest to disclose.

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