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Effects of royal jelly consumption on clinical outcomes in patients with ischemic stroke: A triple-blind randomized controlled trial

Elham Karimi [a,b], Arman Arab [c,d,*], Fariborz Khorvash [e], Maryam Kazemi [f], Reza Amani [a,*]

[a] Department of Clinical Nutrition, School of Nutrition and Food Sciences, Isfahan University of Medical Sciences, Isfahan, Iran
[b] Research Development Center, Arash Women's Hospital, Tehran University of Medical Sciences, Tehran, Iran
[c] Division of Sleep Medicine, Harvard Medical School, Boston, MA, USA
[d] Medical Chronobiology Program, Division of Sleep and Circadian Disorders, Departments of Medicine and Neurology, Brigham and Women's Hospital, Boston, MA, USA
[e] Isfahan Neurosciences Research Center, Alzahra Hospital, Isfahan University of Medical Sciences, Isfahan, Iran
[f] Department of Nutrition, Harvard T.H. Chan School of Public Health, Boston, MA, USA

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ABSTRACT

The therapeutic impact of royal jelly (RJ) consumption in patients with ischemic stroke is unknown. Therefore, we conducted a randomized, triple-blind, placebo-controlled clinical trial to evaluate the role of RJ supplementation on clinical and biochemical outcomes in patients recovering from ischemic stroke. Out of 64 enrolled patients (45–80 yrs.) with ischemic stroke, 32 were randomized to the RJ group and 32 to the placebo (control) group. Groups completed a 12-week intervention. The intervention group received 1000 mg/d of RJ dragee after breakfast. We evaluated stroke-related disability, quality of life, and inflammatory and oxidative stress markers at baseline and post-intervention. At post-intervention, serum levels of erythrocyte sedimentation rate decreased in the RJ group compared to the control group (adjusted mean difference, −8.65 mm/h [95 % CI, −14.75 to −2.55]). Additionally, serum nitric oxide levels increased in the RJ group (adjusted mean difference, 10.18 nmol/mL [95 % CI, 0.51 to 19.86]) post-intervention compared to the control group. Furthermore, the RJ group exhibited a decreased oxidative status index (adjusted mean difference, −0.003 [95 % CI, −0.006 to −0.0001]) and reduced odds of stroke-related disability (adjusted odds ratio, 0.20 [95 % CI, 0.05 to 0.70]) compared to the placebo group. Moreover, RJ supplementation improved the quality-of-life measures in the RJ group (adjusted mean difference, 16.64 [95 % CI, 1.17 to 32.12]) compared to the control group. Our findings reflect the potential benefits of RJ consumption on clinical and biochemical outcomes of patients recovering from ischemic stroke. Importantly, we acknowledge the necessity of additional studies to verify the efficacy of RJ supplementation in patients recovering from strokes.

Trial registration: Iranian Registry of Clinical Trials (IRCT20180818040827N4), registered on October 9, 2021; https://www.irct.ir/trial/59275

1. Introduction

Ischemic stroke represents the third leading cause of long-term disability and the second leading cause of death worldwide (Feske, 2021; Johnson et al., 2016). It kills nearly 130,000 Americans each year, corresponding to the US $ 34 billion cost annually (Feske, 2021). Moreover, the increasing prevalence of obesity (Di Cesare et al., 2016) and diabetes mellitus (Danaei et al., 2011), along with aging, can also increase the incidence of stroke (Li L, Scott CA, Rothwell PM, Study OV, 2020). Ischemic stroke, the most encountered type of stroke, occurs when blood vessels in the brain or neck are blocked. The blockage is often due to blood clots forming inside the vessels, preventing blood from reaching brain tissue (Powers, 2020).

Ischemic stroke can cause a local and immediate inflammatory reaction, including the release of toxic inflammatory mediators, inflammatory cell infiltration, excitotoxicity, and oxidative stress. This reaction additionally leads to damage to nerve tissue and cell death. Nevertheless, these inflammatory responses are significant during the recovery of damaged neurons (Sakai & Shichita, 2019).

There is a growing interest in applying complementary and

* Corresponding authors.
E-mail addresses: aarab1@bwh.harvard.edu (A. Arab), r_amani@nutr.mui.ac.ir (R. Amani).

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alternative medicine among people with stroke owing to the chronic nature of the disease and the side effects of conventional treatments (Farooqui et al., 2022). It was estimated that over 50 % of stroke patients tend to use complementary and alternative therapies (Shin et al., 2008). Therefore, implementing affordable, effective, safe, and novel complementary therapies to modulate post-stroke-related outcomes should be prioritized.

Royal jelly (RJ) is a functional food produced by nurse bees to feed queen bees and young worker larvae (Li et al., 2010). Owing to its complex nutritional composition (carbohydrates, lipids, amino acids, proteins, vitamins, minerals, nucleotides, polyphenols, and minor heterocyclic compounds), RJ has many pharmacological properties (e.g., anti-inflammatory, antioxidant, immunomodulatory, wound healing, neuroprotective, and anti-aging) (Ahmad et al., 2020; Melliou & Chinou, 2014; Ramadan & Al-Ghamdi, 2012).

The RJ was shown to help protect the brain from oxidative injury (Cihan & Arsav, 2011). Moreover, it may reduce the number of apoptotic cells in an animal model of a traumatic spinal cord injury by increasing the endogenous non-enzymatic and enzymatic antioxidative system and decreasing lipid peroxidation (Aslan et al., 2012; Hadi et al., 2018; Hadi et al., 2021). Accordingly, RJ can improve brain function by reducing malondialdehyde (MDA) and increasing superoxide dismutase (SOD) levels in the brain tissue (Zhang et al., 2019). Likewise, previous reports have suggested neuroprotective properties for RJ by reducing brain tissue damage and death (Mohamed et al., 2015).

Evidence suggests a potential role of RJ in patients with ischemic stroke; however, a lack of clinical trials examining the beneficial impacts of RJ on post-stroke clinical outcomes is notable. To address this knowledge gap, we evaluated the effects of RJ supplementation on stroke-related disability, quality of life, and the biochemical profile of inflammation and oxidative stress in patients recovering from acute ischemic stroke. We hypothesized that 12 weeks of RJ supplementation would improve post-stroke-related outcomes of patients with ischemic stroke.

2. Methods

2.1. Study design and participants

The detailed protocol for the present study has been published elsewhere (Karimi et al., 2023). In brief, the current study is a randomized, triple-blind, placebo-controlled clinical trial carried out between November 2021 and December 2022 in AL Zahra Hospital, a referral hospital affiliated with Isfahan University of Medical Sciences, Isfahan, Iran.

Patients aged 45–80 years with a diagnosis of acute ischemic stroke by a neurologist (F·K.) and a National Institutes of Health Stroke Scale (NIHSS) score between 5 and 20 were eligible for inclusion in the study. Patients were excluded if they: (1) had a previous stroke with a modified Rankin Scale (mRS) score $\geq$ 1, any malignancies, cardiovascular diseases, acute liver or kidney diseases, asthma, dermatitis, allergies to honey or honey products, or other neurological conditions; (2) were pregnant or breastfeeding; (3) were on Warfarin therapy; and (4) had consumed multivitamins, antioxidant supplements, or followed any specific diet in the 12 weeks preceding enrollment. Additionally, patients experiencing recurrent stroke, death, or any adverse effects from the RJ supplements were excluded from the study.

2.2. Ethics

The protocol for the present study was approved by the Medical Research Ethics Committee at Isfahan University of Medical Sciences (Registration number: IR.MUI.RESEARCH.REC.1400.291; registration date: 09/10/2021). Also, the protocol was registered with the Iranian Registry of Clinical Trials (Registration number: IRCT20180818040827N4; registration date: 16/10/2021). The study protocols complied with the principles outlined in the Declaration of Helsinki (Goodyear et al., 2007), and the reporting of results corroborated with the Consolidated Standards of Reporting Trials (CONSORT) guidelines (Schulz et al., 2010). All participants provided written informed consent before enrollment, and their participation was voluntary.

2.3. Randomization and masking

Enrolled patients were allocated into the intervention or control group in a ratio of 1:1. An independent statistician carried out the randomization process using a permuted block randomization method with a block size of six, employing computer-generated random numbers (STATA software, version 16 [Stata Corp, College Station, TX, USA]). The first investigator (E.K.) received randomization codes, which were 6-digit numbers, in opaque, sealed envelopes. The envelopes were opened sequentially at the time of the participants' admission. To maintain the integrity of the study, participants, researchers, statistical advisors, and all individuals interacting with the patients remained blind to the treatment allocation.

2.4. Sample size

The sample size was calculated, with the primary outcome being the modified Rankin Scale (mRS) using Stata software version 17 (Stata Corp, College Station, TX, USA). Under the assumption of a maximum type 1 error rate of 0.05 and a statistical power of 80 % and given an mRS standard deviation of 0.8 with an effect size of 0.6, it was estimated that each group would require the enrollment of 32 patients. This calculation also considered a 10 % dropout rate to accommodate potential participant attrition (Hashemilar et al., 2020).

2.5. The intervention of the experimental and the control groups

A total of 64 eligible patients with ischemic stroke were allocated to the intervention ($n = 32$) or the control (n = 32) groups to receive either RJ or a placebo for 12 weeks. The intervention group received 1000 mg of RJ dragee daily after breakfast. Each dragee contained 53 % honey powder, 17 % filler, and 30 % RJ powder (21 mg of 10-hydroxy-2-decenoic acid [10-HAD], corresponding to 1000 mg of fresh RJ). The placebo contained 47 % filler and 53 % honey powder. The RJ and placebo provided to participants were comparable in flavor, shape, color, and size and were manufactured by Kooze-asal Arya Ravis, a knowledge-based company located in Isfahan, Iran. Participants were instructed to maintain their usual dietary intake and physical activity levels throughout the study duration. Additionally, they communicated weekly with the leading investigator (E.K.) during the study to improve their compliance.

2.6. Baseline assessment

Information on education, biological sex, age, smoking status, time since stroke, history of stroke, and medication was collected by reviewing participants' medical records and a direct, in-person interview with the first investigator (E.K.). A neurologist (F·K.) computed the NIHSS at baseline to examine stroke severity. The overall score ranged between 1 and 42, with higher scores indicating more severe conditions (Kazemnejad-Leili et al., 2016). Mental health aspects, specifically depression, anxiety, and stress, were assessed using a validated 21-item version of the Depression, Anxiety, and Stress Scale (DASS-21) questionnaire (Sahebi et al., 2005). The cognitive function was measured using the mini-mental state examination (MMSE) test (Ansari et al., 2010). The fatigue status of participants was evaluated using the Fatigue Severity Scale (FSS) questionnaire (Johansson et al., 2014). The appetite status of patients was assessed using the Simplified Nutritional Appetite Questionnaire (SNAQ) (Mohammadi et al., 2019). All aforementioned

questionnaires were filled out through a face-to-face interview with the first investigator (E.K.).

2.7. Study endpoints

All reported outcomes were considered primary and evaluated at baseline and post-intervention stages using a blinded approach.

Venous blood specimens were obtained for each participant following a 12-h fasting period at baseline and after the 12-week intervention. Post-collection, the specimens were subjected to centrifugation at a velocity of 3500 rpm, after which the resultant serum was aliquoted and preserved at a temperature of −80 °C for subsequent analyses. The erythrocyte sedimentation rate (ESR) was ascertained employing non-hemolyzed EDTA-anticoagulated whole blood, utilizing the Westergren methodology with the assistance of an ESR analyzer (Model: Sedimex; Manufacturer: Parsian Teb Zaman Co., Tehran, Iran). The catalytic activity of Glutathione Peroxidase (GPx) was quantified via its efficacy in catalyzing the transformation of hydrogen peroxide into dihydrogen oxide (water). Concentrations of Nitric oxide (NO) were determined utilizing the Griess reaction, whereas levels of Malondialdehyde (MDA) were assessed through the Thiobarbituric Acid Reactive Substances (TBARS) assay. The Total Oxidative Status (TOS) was gauged employing a modification of the Ferrous Oxidation-xylenol Orange (FOX) technique, Total Antioxidant Capacity (TAC) was quantified via the CUPric Reducing Antioxidant Capacity (CUPRAC) assay, and Superoxide Dismutase (SOD) using the activity of Mn-SOD. The measurements were carried out using commercial kits (Kiazist Life Sciences, Iran). Also, C-reactive protein (CRP) and serum levels of uric acid (UA) were assayed using standard commercial kits (biorexfars, Shiraz, Iran). The oxidative status index (OSI) was calculated using a formula: "[(TOS, nmoL/mL))/(TAC, nmoL equivalent of Trolox/mL)/100] (Eren et al., 2015). All intra- and inter-assay coefficients of variation were < 10 %.

A specialized neurologist (F.K.) assessed the stroke-related disability using the mRS at baseline and post-intervention. The mRS is a valid and reliable instrument for evaluating the level of disability or dependence in daily activities resulting from stroke. It employs a scoring system that extends from 0, indicating the absence of symptoms, to 6, denoting death (Karimi et al., 2023; Quinn et al., 2009).

The stroke-specific quality of life scale (SS-QOL), a validated instrument, was used to assess patients' quality of life at baseline and after 12 weeks (Mahmoodi et al., 2015). The questionnaire comprises 49 items, yielding an overall score range of 49 to 245. The questionnaire was filled out through a face-to-face interview with the first investigator (E.K.). Higher scores on the scale reflect a better quality of life (Karimi et al., 2023; Williams et al., 1999).

2.8. Statistical analysis

We reported continuous variables as mean ± standard deviation (SD) and categorical variables as counts (percentages). The normal distribution of continuous variables was assessed using histograms, Q-Q plots, and skewness statistics, and those with extreme deviation from normality underwent a logarithmic transformation. Baseline characteristics between the intervention groups were compared using the independent sample *t*-test for continuous data and the Chi-square test for categorical data. The continuous endpoints were analyzed using a multivariable mixed-effect linear model after controlling for baseline measurements of each endpoint, treatment allocation, and potential confounders. To find the best set of confounders, we used stepwise selection methods. During the trial, nine patients (four in the RJ group and

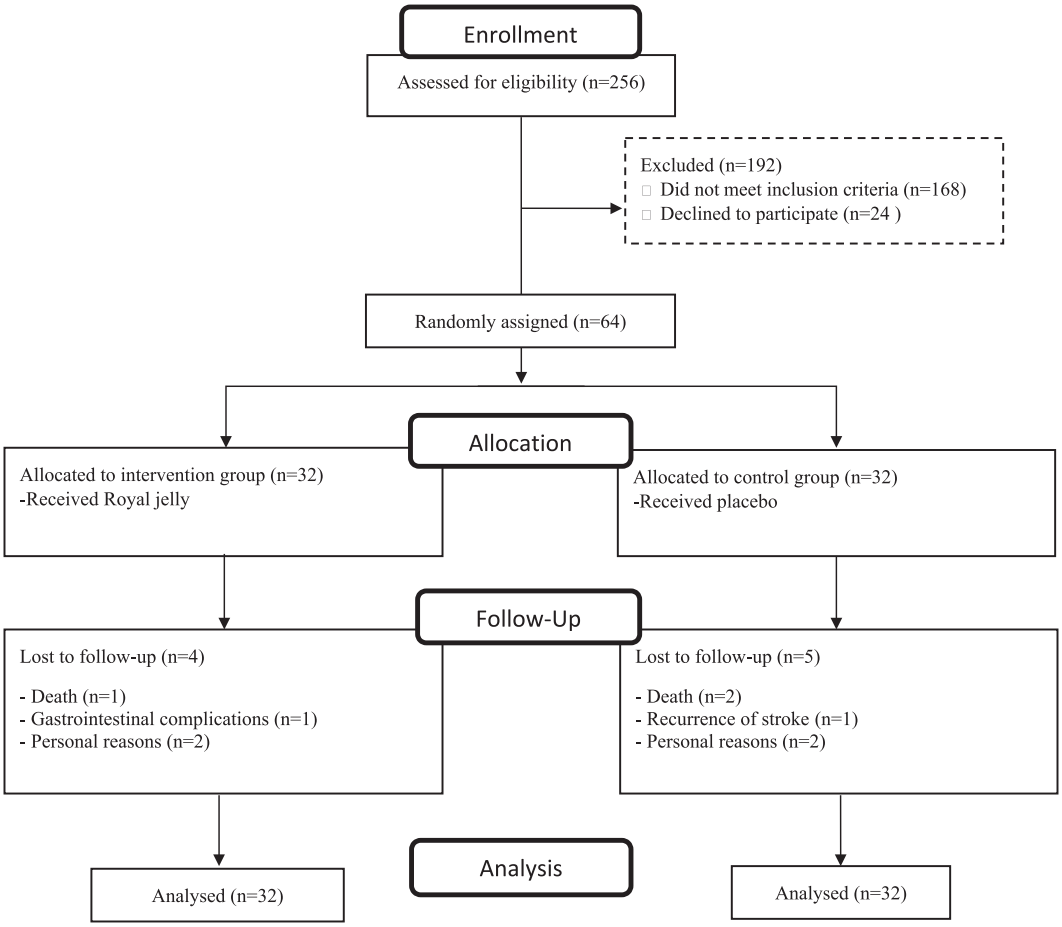

Fig. 1. CONSORT flow diagram of study participants.

five in the placebo group) did not complete the final endpoints, resulting in incomplete observations (Fig. 1). To address this, we employed multiple imputations based on chained equations (White et al., 2011). This method fills in missing values across multiple variables iteratively using a sequence of univariate imputation models with a fully conditional specification of prediction equations. The estimated treatment differences from the multivariable model are reported as adjusted mean differences and corresponding 95 % confidence intervals (CI). An intention-to-treat (ITT) approach was implemented to analyze the data. Hence, information on all randomized people was analyzed. Reported *P*-values are two-sided, with statistical significance set at a threshold of <0.05. All statistical analyses were conducted using Stata software version 17 (Stata Corp, College Station, TX, USA).

3. Results

Sixty-four eligible patients with ischemic stroke were enrolled in the current study, of which four in the RJ group and five in the placebo group were lost to follow-up (Fig. 1). Briefly, patients in the RJ group were lost to follow-up due to personal reasons ($n = 2$), gastrointestinal complications ($n = 1$), and death (n = 1). Similarly, patients of the placebo group were lost due to personal reasons (n = 2), recurrent stroke (n = 1), and death (n = 2). No adverse events were observed in the RJ or placebo groups during the study. The baseline characteristics of the study population stratified by the allocated intervention are shown in Table 1. The RJ and placebo groups were comparable in terms of baseline demographic and clinical characteristics.

In the 12th week post-intervention, patients in the RJ group had significantly lower ESR than those in the placebo group (15.01 ± 15.19 mm/h *v.* 20.99 ± 16.59 mm/h). The ESR mean difference between the groups, adjusted for baseline values of anxiety, stress, appetite, history of stroke, NIHSS, age, quality of life, ESR, TAC, and TOS, was −8.65 mm/h (95 % CI, −14.75 to −2.55; $P = 0.006$). Serum NO concentrations were higher post-intervention in the RJ group, with an adjusted mean difference of 10.18 nmol/mL (95 % CI, 0.51 to 19.86; $P = 0.039$), controlled for the baseline values of depression, anxiety, stress, fatigue, history of stroke, NIHSS, smoking, age, CRP, NO. Patients in the RJ group had decreased OSI values at week 12 post-intervention compared to those in the placebo group (0.003 ± 0.005 *v.* 0.005 ± 0.007). After adjustment for baseline values of OSI, CRP, age, smoking, NIHSS, history of stroke, fatigue, stress, anxiety, and depression, the adjusted mean difference of OSI was −0.003 (95 % CI, −0.006 to −0.0001; $P = 0.038$). Patients of the intervention group tended to have higher serum levels of TAC post-intervention (2174.20 ± 543.97 nmol equivalent of Trolox/mL *v.* 2032.99 ± 536.45 nmol equivalent of Trolox/mL), with a marginally significant adjusted mean difference of 263.16 nmol equivalent of Trolox/mL (95 % CI, −9.24 to 535.56; $P = 0.058$).

At week 12 post-intervention, no significant efficacy was detected for RJ supplementation compared to placebo, with an adjusted mean difference of −0.39 mg/L (95 % CI, −6.63 to 5.84) for CRP; −0.44 mg/dL (95 % CI, −2.01 to 1.11) for serum uric acid, 4.69 U/mL (95 % CI, −7.52 to 16.92) SOD, 2.61 U/mL (95 % CI, −1.31 to 6.54) GPx, 1.14 nmol/mL (95 % CI, −2.61 to 4.90) MDA, and − 5.44 nmol/mL (95 % CI, −13.16 to 2.26) for TOS (Table 2).

After 12 weeks of intervention, patients in the RJ group reported decreased stroke-related disability scores, as measured by mRS, compared to the controls (2.01 ± 1.38 *v.* 3.36 ± 1.62). Following adjustment for baseline values of mRS, age, NIHSS, smoking, time since stroke, and history of stroke, the intervention group had lower odds of stroke-related disability, with an adjusted OR of 0.20 (95 % CI, 0.05 to 0.70). Moreover, those in the RJ group showed higher quality of life than those in the control group (165.12 ± 40.64 *v.* 153.39 ± 43.36), with an adjusted mean difference of 16.64 (95 % CI, 1.17 to 32.12) (Table 3).

Table 1
Baseline characteristics of the study population.

Measure	Royal jelly ($n = 32$)	Placebo (n = 32)
Age (year)	65.56 ± 11.54	65.03 ± 10.84
Female	15 (46.9)	17 (53.1)
Diploma or lower education	24 (75.0)	18 (56.3)
Current smoker	10 (31.3)	4 (12.5)
History of stroke	8 (25.0)	6 (18.8)
Time since stroke (day)	4.68 ± 2.40	5.84 ± 4.94
NIHSS	8.09 ± 3.20	9.46 ± 4.25
MMSE	14.12 ± 8.25	12.53 ± 9.23
Stress	21.31 ± 10.71	20.87 ± 11.61
Anxiety	12.87 ± 9.10	12.53 ± 9.98
Depression	16.56 ± 11.65	16.18 ± 11.39
Fatigue	34.06 ± 14.70	33.96 ± 13.67
Appetite	16.12 ± 2.72	16.15 ± 2.61
FPG (mg/dL)	152.43 ± 72.34	125.00 ± 52.55
Total cholesterol (mg/dL)	165.62 ± 40.22	145.78 ± 37.58
Medications used		
Antihyperlipidemic	18 (56.3)	25 (78.1)
Antihyperglycemic	12 (37.5)	9 (28.1)
Anticoagulant	27 (84.4)	26 (81.3)
Antihypertensive	22 (68.8)	25 (78.1)

Data are presented as mean ± SD or number (%).
Abbreviations: NIHSS, National Institutes of Health Stroke Scale; MMSE, Mini–Mental State Examination; FPG, Fasting Plasma Glucose.

4. Discussion

The current triple-blind, placebo-controlled, randomized clinical trial was conducted to assess the effects of RJ supplementation in patients with acute ischemic stroke on stroke-related disability, quality of life, and the biochemical profile of inflammation and oxidative stress, including serum levels of CRP, ESR, Uric Acid, SOD, GPx, MDA, TAC, TOS, NO, and OSI. We found that 12 weeks of RJ consumption compared to placebo resulted in improved stroke-related disability, quality of life, ESR, NO, and OSI.

The current document suggests that RJ supplementation can improve biochemical measures of inflammation and oxidative stress. The mean percent change of ESR was −49.69 % in the intervention and − 33.80 % in the control groups. Our investigation corroborates a previous open-label study on the beneficial role of RJ on ESR among 20 children with systemic lupus erythematosus (Zahran et al., 2016). The mean percent change for CRP was −57.72 % in the RJ group and − 55.09 % in the placebo group without reaching a significant level. Previous reports on CRP were contradictory; some were in agreement with our results (Fujisue et al., 2022), and others did not confirm our findings (Mobasseri et al., 2014; Petelin, Kenig, & Kopinč, 2019). Moreover, we found an increase of 10.35 % and a reduction of −25 % in TAC and OSI, respectively, which can be interpreted as an improvement in oxidative stress. RJ supplementation among healthy adults (Petelin, Kenig, & Kopinč, 2019) and type 2 diabetes patients (Shidfar et al., 2015) was beneficial regarding TAC. However, another investigation in women with type 2 diabetes failed to show significant results (Pourmoradian et al., 2014). We could not find significant results for SOD, GPx, MDA, and TOS; while previous documents were mixed regarding the efficacy of RJ supplementation on these biomarkers (Pourmoradian et al., 2014; Shidfar et al., 2015). The occurrence of non-significant outcomes, however, can be contextualized by variables including the dosage of RJ administered, the duration of supplementation, the scale of the sample size, and particularly the demographics of our study cohort. Consequently, it is conjectured that augmentations in the sample size, prolongation of the supplementation period, or escalation of RJ dosage could potentially unveil significant enhancements in additional biomarkers.

There are two main mechanisms regarding RJ's antioxidant activities. Firstly, RJ can enhance the expression of genes associated with glutathione peroxidases and glutathione-S-transferase while simultaneously reducing the activity of cytochrome P450 4A14 enzymes and genes for enzymes that facilitate the peroxidation of endogenous lipids

Table 2
The oxidative stress and inflammatory indices pre-and post-intervention.

Measure		Royal jelly (n = 32)	Placebo (n = 32)	Mean Difference (95 % CI)	*p-value*
CRP (mg/L)	Pre	18.71 ± 28.64	16.28 ± 27.53	2.43 (−11.60, 16.47)	0.730
	Post	7.91 ± 15.39	7.31 ± 13.53	**−0.39 (−6.63, 5.84)** [a]	0.900[1]
ESR (mm/h)	Pre	29.84 ± 27.01	31.71 ± 25.24	−1.87 (−14.94, 11.19)	0.775
	Post	15.01 ± 15.19	20.99 ± 16.59	**−8.65 (−14.75, −2.55)** [a]	0.006[1]
Uric acid (mg/dL)	Pre	4.82 ± 1.77	5.05 ± 1.60	−0.22 (−1.07, 0.62)	0.597
	Post	4.10 ± 1.55	4.09 ± 1.51	**−0.44 (−2.01, 1.11)** [b]	0.576[1]
SOD (U/mL)	Pre	51.48 ± 18.07	50.78 ± 20.52	0.69 (−8.96, 10.36)	0.886
	Post	53.31 ± 21.62	51.12 ± 26.36	**4.69 (−7.52, 16.92)** [c]	0.450[1]
GPx (U/mL)	Pre	6.51 ± 5.12	6.75 ± 6.01	−0.24 (−3.03, 2.55)	0.863
	Post	9.01 ± 8.81	7.63 ± 9.23	**2.61 (−1.31, 6.54)** [c]	0.193[1]
MDA (nmol/mL)	Pre	25.08 ± 8.63	22.01 ± 8.83	3.07 (−1.28, 7.44)	0.163
	Post	28.63 ± 8.31	25.46 ± 8.41	**1.14 (−2.61, 4.90)** [c]	0.550[1]
TAC (nmol equivalent of Trolox/mL)	Pre	1970.34 ± 249.17	1978.16 ± 767.16	−7.81 (−292.85, 277.21)	0.956
	Post	2174.20 ± 543.97	2032.99 ± 536.45	**263.16 (−9.24, 535.56)** [c]	0.058[1]
TOS (nmol/mL)	Pre	7.53 ± 7.86	8.04 ± 11.57	−0.50 (−5.45, 4.43)	0.838
	Post	9.11 ± 12.54	11.75 ± 17.17	**−5.44 (−13.16, 2.26)** [c]	0.164[1]
NO (nmol/mL)	Pre	29.62 ± 19.29	34.25 ± 20.43	−4.63 (−14.56, 5.29)	0.354
	Post	46.84 ± 22.50	46.43 ± 20.29	**10.18 (0.51, 19.86)** [c]	0.039[1]
OSI	Pre	0.004 ± 0.004	0.004 ± 0.006	−0.0004 (−0.003, 0.002)	0.760
	Post	0.003 ± 0.005	0.005 ± 0.007	**−0.003 (−0.006, −0.0001)** [c]	0.038[1]

Data are presented as mean ± SD.
Bold values are presented as adjusted mean difference (95 % CI).
Abbreviations: CRP, C-Reactive Protein; ESR, Erythrocyte Sedimentation Rate; SOD, Superoxide Dismutase; GPx, Glutathione Peroxidase; MDA, Malondialdehyde; TAC, Total Antioxidant Capacity; TOS, Total Oxidative Stress; NO, Nitric Oxide; OSI, Oxidative Stress Index; NIHSS, National Institutes of Health Stroke Scale.
[1] Calculated by multivariable mixed-effect linear model.
[a] Adjusted for baseline values, quality of life, age, TAC, TOS, NIHSS, history of stroke, appetite, stress, and anxiety.
[b] Adjusted for baseline values, age, fasting plasma glucose, total cholesterol, and antihyperglycemic agents.
[c] Adjusted for baseline values, CRP, age, smoking, NIHSS, history of stroke, fatigue, stress, anxiety, and depression.

Table 3
Stroke severity and quality of life measures pre- and post-intervention.

Measure		Royal jelly (*n* = 32)	Placebo (n = 32)	Mean Difference (95 % CI)	*p-value*
Modified Rankin Scale	Pre	3.15 ± 1.46	3.84 ± 1.29	−0.68 (−1.37, 0.01)	0.051
	Post	2.01 ± 1.38	3.36 ± 1.62	0.20 (0.05, 0.70) *[a]	0.012[1]
Quality of life	Pre	138.37 ± 47.12	131.21 ± 42.87	7.15 (−15.35, 29.67)	0.528
	Post	165.12 ± 40.64	153.39 ± 43.36	16.64 (1.17, 32.12) ǂ[b]	0.035[1]

Data are presented as mean ± SD.
Abbreviations: NIHSS, National Institutes of Health Stroke Scale; MMSE, Mini–Mental State Examination.
[1] Calculated by multivariable mixed-effect linear model.
[*] Adjusted odds ratio (95 % CI).
[ǂ] Adjusted mean difference (95 % CI).
[a] Adjusted for baseline values, age, NIHSS, smoking, time since stroke, and history of stroke.
[b] Adjusted for baseline values, age, NIHSS, history of stroke, sex, education, MMSE, stress, anxiety, and depression.

(Kanbur et al., 2009). Secondly, three tyrosyl dipeptides (Tyr-Tyr, Tyr-Arg, and Tyr-Lys) of RJ have shown high antioxidant activity in vitro and scavenge the free radicals by the hydroxyl group of their hydrogen atom (Guo et al., 2009). We hypothesize that the observed amelioration in selected inflammation and oxidative stress markers in the RJ group can be explained through the aforementioned molecular mechanisms. Further studies examining the biological mechanisms by which RJ impacts oxidative stress and inflammation could offer valuable insights into its effects, including investigations into genetic, molecular, and cellular pathways.

We found a 58.14 % increase in NO levels after RJ consumption vs. 35.56 % in the placebo group. Previous in vitro and in vivo (Bouamama et al., 2021; Liang et al., 2018; Pan et al., 2019) studies corroborate our findings. NO is produced and released by the endothelium of cerebral vessels and is vital for maintaining proper cerebral perfusion (Cyr et al., 2020). The NO inhibits the endothelial synthesis of endothelin, a potent vasoconstrictor and mitogen, thereby preventing the adhesion and infiltration of monocytes (Di Pietro et al., 2020). It also protects against undue thrombus formation by impeding platelet aggregation and adhesion. Furthermore, NO restrains medial hypertrophy and remodeling and acts as a potent vasodilator (Cyr et al., 2020; McCarty, 2000). A previous systematic review also concluded that the administration of NO in experimental models of ischemic stroke may reduce cerebral infarct volume (Willmot, Gray, et al., 2005a). NO produced by the endothelial isoform of nitric oxide synthase (eNOS) is beneficial in ischemic stroke, whereas that derived from an inducible or neuronal isoform is detrimental (Willmot, Gibson, et al., 2005b). Surprisingly, RJ was proposed to increase NO levels by upregulating eNOS (Pan et al., 2019). However, we did not assess the effect of RJ consumption on the activity of different isoforms of NOS. Therefore, further studies are suggested to investigate the genetic and molecular pathways.

Accordingly, we found a lower stroke-related disability, as shown by lower mRS scores, following RJ consumption compared to those in the placebo group. In an animal model of ischemic stroke, RJ consumption was associated with lower morbidity through increasing the expression of T-box 1 transcription factor (T-bet) and GATA Binding Protein 3 (GATA3) (Kazemi Arababadi et al., 2022). Moreover, improved stroke-related disability may also be justified through improvements in inflammation and oxidative stress (Gu et al., 2022; Whiteley et al., 2009). Likewise, increased serum levels of NO may also impact stroke-related disability (Jiang et al., 2020).

Impaired quality of life is a common phenomenon after a stroke (Nichols-Larsen et al., 2005). Our study revealed a 19.33 % enhancement in the post-stroke quality of life scores following the consumption of RJ. Supplementation with RJ has been reported to improve the quality of life in patients with Multiple Sclerosis (Oshvandi et al., 2020). Moreover, the administration of vaginal RJ was effective among post-menopausal women to increase their quality of life (Seyyedi et al.,

2016). It was shown that stroke-related disability correlates with the post-stroke quality of life (Bruno et al., 2011). Furthermore, the link between oxidative stress and quality of life has been indicated across various study populations (Choghakhori et al., 2017; Fuchs-Tarlovsky et al., 2011; Lopez-Jornet et al., 2014). Therefore, it is plausible to speculate that the observed improvement in the quality of life of stroke patients may result from the reduction in oxidative stress, inflammation, and stroke-related disability.

In the current study, RJ consumption for 12 weeks in a dose of 1000 mg was safe, and patients reported no adverse events except for one individual with a complaint of gastrointestinal discomfort. In previous studies, there were three reports on RJ consumption and respiratory distress among Asthmatic patients (Bullock, 1994; Peacock et al., 1995; Thien et al., 1996) and one report of hemorrhagic colitis associated with RJ (et al., 1997). Moreover, RJ was reported to interact with Warfarin (Lee & Fermo, 2006). There is limited research on the safety of RJ consumption among patients with ischemic stroke. Based on current evidence regarding the safety of RJ, we excluded those with asthma, dermatitis, and allergies to honey or honey products. Moreover, those who taking Warfarin were excluded. Therefore, eligible individuals for RJ supplementation should be chosen cautiously with respect to the co-existing disease and medication regimens.

4.1. Strength and limitation

The current study is among the first investigations on RJ supplementation among patients with ischemic stroke. Furthermore, our study employed a triple-blind methodology, accounting for many potential confounding variables. However, some limitations warrant consideration. Our findings on the positive impacts of RJ in stroke patients are preliminary and warrant confirmation through further research with a larger sample size. Our work represented a single-center study, which may have impacted its generalizability. We asked participants not to change their physical activity level; however, we didn't assess or control the effect of physical activity, physical therapy, and/or stroke rehabilitation.

4.2. Suggestions for future studies

Future multi-center studies should include larger and more diverse populations to enhance the generalizability of the findings. Conducting long-term studies can provide insights into the chronic effects of RJ on post-stroke-related outcomes. This approach can help to understand the sustained impact and potential long-term benefits or risks. Future research should explore different dosages and RJ administration schedules to determine the most effective regimen. This includes investigating the optimal treatment duration and administration timing relative to stroke events. Investigating the underlying biological mechanisms through which RJ influences oxidative stress and inflammation can provide a deeper understanding of its effects. This could involve exploring genetic, molecular, and cellular pathways.

5. Conclusion

Supplementation with RJ for 12 weeks has favorable effects in reducing stroke-related disability, improving patients' quality of life, and reducing inflammatory and oxidative stress outcomes such as ESR, OSI, and NO in patients with acute ischemic stroke. However, we observed no significant improvements in the serum levels of CRP, uric acid, SOD, GPx, MDA, TAC, and TOS. Importantly, we acknowledge that our observations derive from preliminary data, necessitating additional studies to verify the efficacy of RJ supplementation in patients recovering from strokes.

Ethics approval and consent to participate

The Research Ethics Committee of Isfahan University of Medical Sciences approved the study protocol on October 9, 2021, under the reference number IR.MUI.RESEARCH.REC.1400.291. The study protocol was also registered with the Iranian Registry of Clinical Trials on October 16, 2021, with the identifier IRCT20180818040827N4—all study protocols aligned with the principles of the Declaration of Helsinki (Goodyear et al., 2007). The research required the acquisition of written informed consent forms from all patients before participation, ensuring informed and voluntary engagement in the study.

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Consent for publication

Not applicable.

CRediT authorship contribution statement

Elham Karimi: Writing – review & editing, Writing – original draft, Methodology, Investigation, Data curation, Conceptualization. **Arman Arab:** Writing – review & editing, Writing – original draft, Methodology, Formal analysis, Data curation. **Fariborz Khorvash:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Maryam Kazemi:** Writing – review & editing. **Reza Amani:** Writing – review & editing, Supervision, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Data will be made available on request.

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