Original Article

J Babol Univ Med Sci
Vol 17, Issu 2; Feb 2015. P:50-56

Comparison of the Analgesic Effects of Royal Jelly with Morphine and Aspirin in Rats Using the Formalin Test

A. Arzi (PhD)[1], Gh. Houshmand (PhD)[1], M. Goudarzi (PhD)*[2], H. Khadem Haghighian (PhD)[3], M.R. Rashidi Nooshabadi (Pharm D)[1]

1.Department of Pharmacology and Toxicology, School of Pharmacy, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, I.R.Iran

2.Student Research Committee, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, I.R.Iran

3.Department of Nutrition, School of Paramedicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, I.R.Iran

Received: Aug 3th 2014, Revised: Sep 24th 2014, Accepted: Nov 26th 2014.

ABSTRACT

BACKGROUND AND OBJECTIVE: The side effects of synthetic analgesics in clinical use as well as patients' growing interest in traditional medicine and natural products have drawn researchers' attention to studying the effects of natural pain relievers and comparing them with chemical and synthetic drugs. Royal jelly, which is produced by bees to feed the queen bee, exerts anti-inflammatory effects through inhibiting the production of pre-inflammatory cytokines by activated macrophages. This study aimed to compare the analgesic effects of morphine, aspirin and Royal Jelly as common painkillers.

METHODS: In this experimental study, 36 male Wistar rats were randomly classified into six groups of 6. The negative control group received the normal Saline of 5 kg/ml, one positive control group received morphine 2.5 mg/kg and the other received aspirin 300 mg/kg. The 3 treatment groups received Royal Jelly peritoneally with doses of 100, 200 and 400 mg/kg in single doses. Half an hour after the intraperitoneal injection, 50 micro liters of formalin 2.5% was subcutaneously injected into the bottom of the animals' right paw and the analgesic effects were studied using the Formalin test.

FINDINGS: Our findings indicate that as a dose-dependent analgesic, Royal Jelly is most effective at a dose of 200 mg/kg. The grade of acute pain in the groups of Saline, aspirin, morphine and Royal Jelly (100, 200 and 400 kg) was 2.61±0.09, 1.56±0.06, 1.05±0.14, 2.40±0.08, 1.65±0.04 and 1.53±0.05, respectively. The grade of chronic pain in the same groups was 2.34±0.09, 1.28±0.12, 0.33±0.12, 2.15±0.07, 1.21±0.03 and 1.12±0.05, respectively. The analgesic effect of royal jelly with the dosage of 200 kg/mg on acute pain was approximately equal to that of aspirin and less than that of morphine. However, Royal Jelly was less effective on chronic pain than morphine and it did not differ significantly with aspirin.

CONCLUSION: Given the prominent analgesic, antioxidant and anti-inflammatory qualities of royal jelly, this natural substance could be prescribed as a non-invasive method to reduce and relieve pain appropriately and applicably.

KEY WORDS: Royal Jelly, Pain, Formalin Test, Rat.

Please cite this article as follows:

Arzi A, Houshmand Gh, Goudarzi M, Khadem Haghighian H, Rashidi Nooshabadi MR. Comparison of the Analgesic Effects of Royal Jelly with Morphine and Aspirin in Rats Using the Formalin Test. J Babol Univ Med Sci. 2015; 17(2):50-6.

* **Corresponding Author; M. Goudarzi (PhD)**
Address: Department of Pharmacology and Toxicology, Faculty of Pharmacy, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, I.R.Iran.
Tel: +98 61 33738378
E-mail: gmahdi_787@yahoo.com

Introduction

Pain is the body's defense mechanism and it is sensed when a tissue is damaged. It will make the person react and try to remove the pain-inducing factor. Pain is an alarm going off only under certain circumstances. For instance, when intense stimulation is perceived from the outside (perceptible pain), or when the internal organs of the body are in abnormal condition (1). Searching for new analgesic compounds in the world started in 1960. As of today, there are two major categories of analgesics: Opioid and non-steroidal, anti-inflammatory drugs.

Long-term use of opioids is likely to generate dependence and tolerance. Similarly, non-steroidal, anti-inflammatory drugs can bring about undesirable changes like gastrointestinal discomfort in the form of nausea and diarrhea, occasional bleeding and ulcers and allergic reactions like angioedema, rash and bronchospasm. Other possible side effects of such drugs are headaches, vertigo, dizziness and tinnitus along with other hearing disorders (2, 3). For these reasons, researchers are constantly looking for replacement drugs with fewer side effects to reduce pain. Recently, special attention has been drawn to herbs and natural ingredients as pain relievers of high performance and less restrictive effects. It is regarded as a new, constructive approach in the field of analgesics.

Several studies find the Formalin test to be the leading method of evaluation in case of acute and especially chronic pain. Acute pain is correlated with non-inflammatory pain (neurogenic) and it might enter the chronic phase due to the inflammation caused by the formalin injection. Subcutaneous formalin injection tends to inflict a biphasic pain one of the features of which is pain assessment. It is suspected that such neurotransmitters as substance P, bradykinin, serotonin and glutamate play a key role in the infliction of both acute and chronic pain caused by Formaldehyde (4). Royal Jelly is a milk-white substance of viscosity with a pungent odor. It is secreted by young worker bees and it is the main material the larvae in the hive live on. Royal jelly contains 15-12% protein, 12-10% carbohydrates, 3-7% lipids, minerals and water-soluble B vitamins (5-7). Royal jelly has strong antimicrobial agents against bacteria and fungi (8, 9). Furthermore, it is known to have remarkable healing effects on atherosclerosis, warts, diabetic foot ulcers and collagen (10-12). For another thing, the enzymatic hydrolysis mixture of royal jelly is proven to have exceptional antioxidant activities against reactive oxygen species like the superoxide anion and hydroxyl radicals (13, 14). Through inhibiting the production of cytokines by activated macrophages, royal jelly does anti-inflammatory activities (15). Since the analgesic effects of royal jelly in the Formalin test have scarcely been studied, we intended to review the pain relieving properties of this substance using the Formalin test.

Methods

In this experimental study, we used Wistar male rats weighing 200±20 g. The animals were purchased from the Laboratory Animal Center of Medical University of Jundishapur, Ahvaz, Iran. They were kept in polycarbonate cages at 20±2 °C within a cycle of 12 hours of light and 12 hours of darkness. The animals were provided with tap water and sufficient compact food. The royal jelly for the experiment was obtained from the beekeepers of Ardebil region and it was converted into the lyophilized form using the Freeze Dryer machine. Afterwards, the dried product was preserved in a dark glass container in a cool and dry place. The animals were randomly divided into six groups of 6 rats that are as follows:

1. The negative control group received Saline (5ml/kg); 2. The positive control group 1 received morphine (2.5 mg/kg) (16); 3. The positive control group 2 received aspirin (300 mg/kg) (17); 4. Treatment group 1 received royal jelly (100mg/kg); 5.

Treatment group 2 received royal jelly (200mg/kg); 6. Treatment group 3 received royal jelly (400mg/kg)

According to the pilots done before starting the work and the conducted dose-response surveys, the minimum effective dose of the lyophilized royal jelly that could be responsive to the pain caused by the formalin was calculated as much as 100 mg/kg. The other two treatment groups received multiple doses of the calculated basis. Saline was used as solvent in order to prepare a solution for the injection of morphine, aspirin and royal jelly at the desired concentration.

About half an hour after the intrapritoneal injection of the desired compound to the groups, 50 micro liters of formalin 2.5% was subcutaneously injected into the bottom of the animals' right paw. Afterwards, the rats were immediately placed into the containers with transparent walls and were carefully graded. If the animal could walk without any difficulty and the injected foot was able to carry his weight well, the animal was proven to have no pain and the score was zero. If the animal could not put the injected foot on the glass easily and tried to place his weight on the other foot, it was decided that he was feeling pain and the score was 1.

If the animal could not place the injected foot on a surface and tried to place his entire weight on the other foot, the score was 2. Finally, if the rat kept licking, shaking or biting the injected foot continuously, the score was 3 (18). The numbers of these quantitative data were counted as 12 five-minute blocks and it was recorded within each time period based on the pain score. Data registration continued for 60 minutes after the formalin injection. The following formula was applied for calculating the mean pain score in each block:

Pain score= 0T0+1T1+2T2+3T3/300

In the calculation of the mean pain scores, T0, T1, T2 and T3 are the number of every 15-second intervals during which the rat shows the behaviors of zero, 1, 2 and 3, respectively over a period of five minutes. In all the groups, the acute phase of pain was considered between zero and 5 minutes while the chronic phase accounted for 16 to 60 minutes. The Kruskal-Wallis test was used for the statistical analysis of the obtained results between the control and the experimental groups and p<0/05 was considered significant.

Results

In comparison with the Saline group, the royal jelly doses of 200 and 400 mg/kg significantly reduced the pain response scores during the first and second phase of the Formalin test (p<0.05). The acute pain scores in the groups of Saline, aspirin, morphine and royal jelly 100, 200 and 400 mg/kg were 2.61±0.09, 1.56±0.06, 1.05±0.14, 2.40±0.08, 1.65±0.04 and 1.53±0.05, respectively. On the other hand, the chronic pain scores were 2.34±0.09, 0.12±1.28, 0.33±0.12, 2.15±0.07, 1.21±0.03 and 1.12±0.05, respectively. Since no significant differences were observed between the dosages of 200 and 400 mg/kg, the former was selected as the most effective (fig 1).

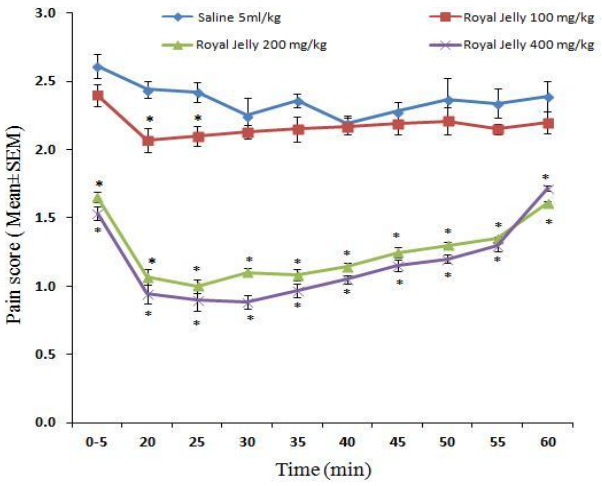

Figure 1. Comparison of analgesic effects of different doses of royal jelly intrapritoneally injected to Saline group (pain phase I and II)

***Difference with Saline group is significant (p<0.05)**

The pain response score in the group receiving 100 mg/kg of royal jelly was lower than the Saline group at all times. However, the decrease was statistically significant only at minute 20 and 25 after the injection ($p<0.05$). On the other hand, compared with 100 mg/kg, doses of 200 and 400 mg/kg of royal jelly significantly reduced the pain response score during both the first and the second phase of the Formalin test (fig 2 and 3).

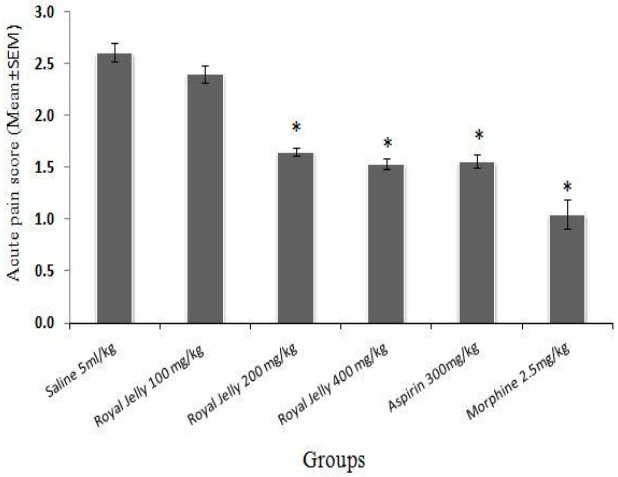

Figure 2. Comparison of analgesic effects of different doses of royal jelly intrapritoneally injected to positive control groups (morphine and aspirin) with normal Saline group (phase I – time 0 to 5 minutes)

***Difference with Saline group is significant ($p<0.05$)**

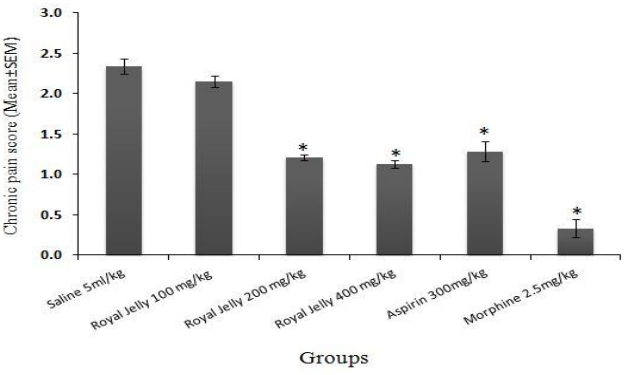

Figure 3. Comparison of analgesic effects of different doses of royal jelly intrapritoneally injected to positive control groups (morphine and aspirin) with normal Saline group (phase II – time 15 to 60 minutes)

***Difference with Saline group is significant ($p<0.05$)**

Furthermore, it was found that analgesically, 200 mg/kg of royal jelly was less effective than morphine during the first phase while it was equally effective as aspirin (fig 4). There were no significant differences in the analgesic effects of the royal jelly-receiving group (200 mg/kg) and the aspirin-receiving group in the first phase.

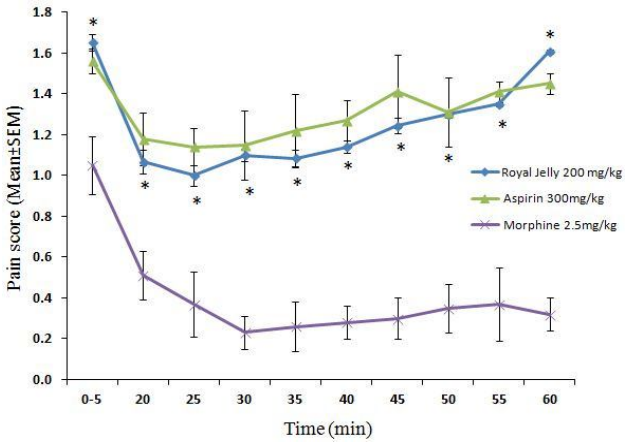

Figure 4. Analgesic comparison of the most effective dose of royal jelly (200 mg/kg) with positive control groups (morphine and aspirin) (phase I and II)

***Difference with morphine is significant ($p<0.05$).**

Discussion

In the present study, royal jelly at doses of 200 and 400 mg/kg exerted noticeable analgesic effects similar to aspirin during the acute and chronic phases of pain**.** Through inhibiting the production of pro-inflammatory cytokines by activated macrophages, royal jelly does anti-inflammatory activities (19). Such anti-inflammatory properties could explain the impact of royal jelly on the latency as well as its ability to reduce the pain scores in the current study. As the major structural components of cell membranes, fats and proteins might be damaged by free radicals and lose their integrity giving rise to the production of inflammatory cytokines which are a major cause of pain (20). In a study conducted by Bincoletto and colleagues (2005),

royal jelly was discovered to strengthen the body's immune system as well as heighten the survival rate. It is suspected that increased survival rate in their study might have been a result of increased Prostaglandin E2 after treatment (21).

Furthermore, AMP N-Oxide is a natural substance specifically found in royal jelly. It is capable of preventing the proliferation of PC12 cells and hindering the destruction of neurons eventually causing the pain to recede (13, 22). In a study conducted in Russia on patients with gastric ulcer, it was revealed that administrating 100 mg/kg of royal jelly on a daily basis could improve the quality of life and reduce the pain in the subjects (23).

By contrast, this amount (100 mg/kg) of single, intrapritoneal dose of royal jelly did not appear to have any noticeable analgesic effects in our experiment. Moreover, there were no significant differences with the normal Saline. The mechanism of pain in people with stomach ulcers is probably a direct result of the reduced production of pro-inflammatory cytokines and an increase in the Prostaglandin E2 (24). In addition, recent research has it that such fatty acids as Dihydroxy-decanoic acid which are isolated from royal jelly could help T cells to modulate the immune response in rats by reducing the production of IL-2 and increasing the production of IL-10 (25).

In animal studies, it has been indicated that through inhibiting oxidative stress and improving ATP, royal jelly is capable of enhancing antioxidant effects as well as improving the Hyperinsulinemia and insulin resistance (26). The antioxidant activity of royal jelly becomes possible by its connecting to free radicals like free oxygen and ionic superoxide dismutase and neutralizing them (27).

The Formalin test has been established as the standard test for measuring the response to pain-inducing stimuli. Regarding the prominent analgesic, antioxidant and anti-inflammatory properties of royal jelly, prescribing this natural substance as a non-invasive method could help relieve pain appropriately and applicably.

Acknowledgments

Hereby, we would like to extend our deepest gratitude to the Student Research Committee of Jundishapur University of Medical Sciences, Ahvaz for their financial support of this research.

References

1. Gyton AC, Hall JE. Tex book of medical physiology. 9th ed, Philadelphia:WB.Saunders; 1996. p.609-62.

2.Waldhoer M, Bartlett SE, Whistler JL. Opioid receptors. Annu Rev Biochem. 2004;73(1):953-90.

3.Macrae F, Mackenzie L, Mccoll K, Williams D. Strategies against NSAID-induced gastrointestinal side effects. Part 1. Pharmaceut J. 2004;272:187-9.

4.Shibata M, Ohkubo T, Takahashi H, Inoki R. Modified formalin test: characteristic biphasic pain response. Pain. 1989;38(3):347-52.

5.Bogdanov S. Royal jelly, bee brood: composition, health, medicine: a review. Bee Product Sci. 2011:1-35. Available at: http://www.bee-hexagon.net/files/file/fileE/Health/RJBookReview.pdf.

6.Melampy RM, Breese Jones D. Chemical composition and vitamin content of royal jelly. Exp Biol Med (Maywood). 1939;41(2):382-8. Available at: http://ebm.sagepub.com/content/41/2/382.abstract.

7.Albert S, Bhattacharya D, Klaudiny J, Schmitzová J, Simúth J. The family of major royal jelly proteins and its evolution. J Mol Evol. 1999;49(2):290-7.

8.Kamakura M, Mitani N, Fukuda T, Fukushima M. Antifatigue effect of fresh royal jelly in mice.

J Nutr Sci Vitaminol(Tokyo). 2001;47(6): 394-401.
9.Fontana R, Mendes MA, de Souza BM, Konno K, César LMM, Malaspina O, et al. Jelleines: a family of antimicrobial peptides from the Royal Jelly of honeybees (Apis mellifera). Peptides. 2004;25(6):919-28.
10.Koya-Miyata S, Okamoto I, Ushio S, Iwaki K, Ikeda M, Kurimoto M. Identification of a collagen production-promoting factor from an extract of royal jelly and its possible mechanism. Biosci Biotechnol Biochem. 2004;68(4):767-73.
11.Abdelatif M, Yakoot M, Etmaan M. Safety and efficacy of a new honey ointment on diabetic foot ulcers: a prospective pilot study. J Wound Care. 2008;17(3):108-10.
12.Madar J, Malý E, Neubauer E, Moscovic F. Effect of bee royal jelly (gelee royale) on the cholesterol level, total lipids in the serum and on the fibrinolytic activity of plasma of elderly arteriosclerotic patients. Z Alternsforsch. 1965;18(2):103-8.
13.Nagai T, Inoue R, Suzuki N, Nagashima T. Antioxidant properties of enzymatic hydrolysates from royal jelly. J Med Food. 2006;9(3):363-7.
14.Nagai T, Sakai M, Inoue R, Inoue H, Suzuki N. Antioxidative activities of some commercially honeys, royal jelly, and propolis. Food Chem. 2001;75(2):237-40.
15.Kohno K, Okamoto I, Sano O, Arai N, Iwaki K, Ikeda M, et al. Royal jelly inhibits the production of proinflammatory cytokines by activated macrophages. Biosci Biotechnol Biochem. 2004;68(1):138-45.
16.Jourdan D, Alloui A, Eschalier A. Pharmacological validation of an automated method of pain scoring in the formalin test in rats. J Pharmacol Toxicol Methods. 1999;42(3): 163-70.
17.Arzi A, Sarkaki A, Aghel N, Nazari Z, Saeidnejad S. Study of analgesic effect of hydroalcoholic extract of cinammom. Jundishapur Sci Med J. 2011;10(3):272-9.
18.Rosland JH, Tjølsen A, Mæhle B, Hole K. The formalin test in mice: effect of formalin concentration. Pain. 1990;42(2):235-42.
19.Mihajlovic D, Vucevic D, Chinou I, Colic M. Royal jelly fatty acids modulate proliferation and cytokine production by human peripheral blood mononuclear cells. Eur Food Res Technol. 2014;238(5):881-7.
20.Cortese M, Sinclair C, Pulendran B. Translating glycolytic metabolism to innate immunity in dendritic cells. Cell Metab. 2014; 19(5):737-9.
21.Bincoletto C, Eberlin S, Figueiredo CA, Luengo MB, Queiroz ML. Effects produced by Royal Jelly on haematopoiesis: relation with host resistance against Ehrlich ascites tumour challenge. Int Immunopharmacol. 2005;5(4): 679-88.
22.Zamami Y, Takatori S, Goda M, Koyama T, Iwatani Y, Jin X, et al. Royal jelly ameliorates insulin resistance in fructose-drinking rats. Biol Pharm Bull. 2008;31(11):2103-7.
23.Bogdanov S. Bee venom: composition, health, medicine: A review. Bee Product Sci. 2012:1-20. available at: http://www.bee-hexagon.net/files/file/fileE/Health/VenomBookReview.pdf.
24.Liu Y, Chen LY, Sokolowska M, Eberlein M, Alsaaty S, Martinez-Anton A, et al. The fish oil ingredient, docosahexaenoic acid, activates cytosolic phospholipase A2 via GPR120 receptor to produce prostaglandin E2 and plays an anti-inflammatory role in macrophages. Immunology. 2014;143(1):81-95.
25.Vucevic D, Melliou E, Vasilijic S, Gasic S, Ivanovski P, Chinou I, et al. Fatty acids isolated from royal jelly modulate dendritic cell-mediated immune response in vitro. Int Immunopharmacol. 2007;7(9):1211-20.
26.El-Nekeety AA, El-Kholy W, Abbas NF, Ebaid A, Amra HA, Abdel-Wahhab MA. Efficacy of royal jelly against the oxidative stress of fumonisin in rats. Toxicon. 2007;50(2):256-69.

27.Silici S, Ekmekcioglu O, Kanbur M, Deniz K. The protective effect of royal jelly against cisplatin-induced renal oxidative stress in rats. World J Urol. 2011;29(1):127-32.