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Long-term administration of Greek Royal Jelly improves spatial memory and influences the concentration of brain neurotransmitters in naturally aged Wistar male rats

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ABSTRACT

Ethnopharmacological relevance: Royal Jelly (RJ) is a bee-derived product that has been traditionally used in the European and Asian systems of medicine for longevity. RJ has various pharmacological activities that may prevent aging e.g., anti-inflammatory, anti-oxidative, anti-hypercholesterolemic and anti-hyperglycemic properties.
Aim of the study: To evaluate the behavioral and neurochemical effects of long-term oral, previously chemically analyzed, Greek RJ administration to aged rats.
Materials and methods: RJ powder was given to 18-month old male Wistar rats (50 and 100 mg of powder/kg b.w./day) by gastric gavage for 2 months. The spatial memory was assessed in the water maze and next the level of neurotransmitters, their metabolites and utilization in the selected brain regions were estimated.
Results: The improvement of memory in rats pretreated with the smaller dose of RJ was observed compared with controls. In biochemical examination mainly the depletion of dopamine and serotonin in the prefrontal cortex along with an increase in their metabolite concentration and turnover were seen.
Conclusion: Better cognitive performance in the old animals using a non-toxic, natural food product in the view of the process of the aging of human population is noteworthy. Our results contribute towards validation of the traditional use of RJ in promoting a better quality of life in old age.

1. Introduction

Royal Jelly (RJ) is a secretion of hypopharyngeal and mandibular glands of worker bees *Apis mellifera.* It is used to feed temporarily the brood of workers and drones but it is a sole food of the queen bee for both her larval and adult life. RJ is a yellowish, creamy and acidic material with a slightly pungent odor and taste. It is a specific substance that contains big amount of proteins, free amino acids, lipids, carbohydrates, vitamins and minerals. RJ for years has been widely used in Europe and Asia as traditional medical product, health food or cosmetic to promote longevity. It has been demonstrated to possess several pharmacological activities such as: disinfectant action, antimicrobial, anti-oxidant, anti-hypercholesterolemic, anti-hyperglycemic, anti-inflammatory and anti-tumor activity that may be responsible for supporting the health welfare during aging.

Royal Jelly divergent pharmacological activities were described in pre-clinical research. Lot of works indicate that RJ which contains many active compounds may influence the nervous system cells performance estimated in in vitro examination. A unique to RJ component—an unsaturated fatty acid, 10-hydroxy-trans-2-decenoic acid (HDEA) implies on brain-derived neurotrophic factor (BDNF) production (Ito et al., 2003) and on neurogenesis, in neural stem/progenitor cells (Hattori et al., 2007a). Adenosine monophosphate (AMP) N(1)-oxide, also found only in RJ, may exert neurothropic activity, potentiate the development of astrocytes as well as induce neuronal differentiation (Hattori et al., 2006, 2007b, 2010).

Royal Jelly influences intracellular signaling pathways and this in turn results in hippocampal long-term potentiation changing the ability for learning and memory. RJ may facilitate restoration of the cognitive skills in mice (Hattori et al., 2011). It is also known that 10-hydroxy-trans-2-decenoic acid presents antidepressant activity in stress-inducible depression-like mouse model (Ito et al., 2012). Royal Jelly given orally alleviated the adverse effects of intracerebroventricular injection of streptozocin on spatial learning and

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memory in the rat model of sporadic Alzheimer's disease (Zamani et al., 2012). It improved also memory in the water maze of adult Sprague Dawley rats in D-galactose sub-acutely aging model (Peng et al., 2011).

Chronic administration of RJ may also affect carbohydrate and lipid metabolism. (Nomura et al., 2007; Zamami et al., 2008) as well as modify vascular responsiveness. Then RJ may have some effect in preventing insulin resistance combined with the development of hypertension. Due to RJ extract features it may be found efficacious in the view of prevention of age—related changes, especially within brain and vessels.

In the framework of our studies on bee-keeping products (Melliou and Chinou 2005; Vucevic et al., 2007; Gašić et al., 2007; Moutsatsou et al., 2010; Dzopalic et al., 2011) the research project addresses chemical characterisation of Greek RJ by recent analytical methods as well as the effect of its chronic pre-treatment on old rat performance in the Morris water maze task and on brain neurotransmission.

The behavioral and biochemical effects of long-term RJ administration in naturally aged Wistar male rats are not known so far. In the view of the process of ageing of human population there is a great need for establishing the natural food products of small toxicity and big potency in prophylaxis against the development of civilization diseases including cognitive dementia.

In this study we are examining the influence of the 60-day oral RJ pre-treatment on spatial cognitive abilities in Morris water maze in 18-month old male rats and on neurotransmitter content in prefrontal cortex, hippocampus, striatum and hypothalamus.

2. Materials and methods

2.1. Animals

In the present study, we investigated the effect of chronic Greek Royal Jelly pre-treatment on rat performance in the Morris water maze task. Chronic treatment was administered for 60 days in 18-month old male Wistar Albino Glaxo (WAG) rats. All animal testing was carried out according to the Directive 2010/63EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes, after approval of the Ethical Committee for Animal Experiments at the Medical University of Warsaw.

2.2. Chemicals

In the experiment 1 kg of Royal Jelly sample (ATTIKI Bee Culturing Co., Greece) was lyophilized and used to feed the rats. Royal Jelly powder was added to 0.9% NaCl solution (1 ml) to form the suspension that was given to the rats by the gastric gavage. The animals were divided into three groups—RJ50 (rats were treated with 50 mg of powder/kg b.w./day, $n=9$), RJ100 (100 mg of powder/kg b.w./day, $n=9$) and control rats (Con, given also by the gavage the 1 ml of 0.9% NaCl solution/kg b.w./day, $n=8$). The 18-month old rats were treated for 60 days and later for 6 days over the period of behavioral testing.

2.3. Chemical analyses of RJ sample

A small amount of lyophilized RJ (12 g) was extracted successively with dichloromethane and methanol and the extracts were analyzed. About 5 mg of each residue was mixed with 50 μl of dry pyridine and 75 μl of BSTFA and heated at 80 °C for 20 min.

The samples were first analyzed by GC-FID carried out on a Perkin-Elmer Clarus 500 gas chromatograph, fitted with a HP 5MS 30 m × 0.25 mm, 0.25 μm film thickness capillary column. The column temperature was programmed from 60 to 280 °C at a rate of 3 °C/min. The injector and detector temperatures were programmed at 230 °C and 300 °C, respectively. Helium was used as the carrier gas at a flow rate of 1 ml/min. The GC–MS analyses were carried out using a Hewlett–Packard 6890-5973 GC–MS system operating on EI mode (equipped with a HP 5MS 30 m × 0.25 mm, 0.25 μm film thickness capillary column). He (1 ml/min) was used as carrier gas. The initial temperature of the column was 60 °C and then it was heated to 280 °C at a rate of 3 °C/min. The identification of the compounds was based on comparison of their retention indices (RI), obtained using *n*-alkanes (C9–C25), and on comparison of their EI-mass spectra with reference compounds or on the basis of their general fragmentation using reference spectra.

2.4. Behavioral examination—Water maze test

The animals were tested in the modified version of the Morris water maze test (Widy-Tyszkiewicz et al., 1993; Vorhees and Williams, 2006). The experiment was performed during the light phase of the cycle (between 8.00 and 15.00 h). In the task animals were trained to find a hidden platform in a swimming pool. A circular white pool (140 cm in diameter and 50 cm deep) was filled to a height of 30 cm above the base with 23 °C water. The pool was located in the testing room which contained many objects that could be used by the rat for spatial navigation. The position of the cues were not changed throughout the period of testing. The pool was divided into four quadrants arbitrarily designed Northeast (NE), Northwest (NW), Southeast (SE) and Southwest (SW). A submerged plexiglass platform (10 cm × 10 cm) was hidden 1 cm below the water surface and placed in a constant location in the center of SE quadrant. Animals received 4 days of training with the hidden platform, each day included 4 training sessions with a 60 s intersession interval. Each trial was started by placing a rat with its face toward the wall of the pool at one of three start points. The start location was varied on each training trial and changed each day. The trial was terminated when the animal entered the platform. If the rat did not find the platform within 60 s it was placed on the platform by the experimenter for 15 s. During acquisition of the spatial navigation task all groups were given one session of four trials each day (day 1–4; trial 1–16). At the end of day's session the rat was removed from the pool, dried and returned to its cage. Spatial memory was evaluated in the probe trial, on fifth day (trial 17). The platform was removed and animals were allowed to swim for 60 s. The visible platform test was carried out on the 6th day. The animals had to find the well-signed platform placed 1 cm over the water surface in four trials from different starting points to the cued target.

Data from the water maze (latencies to locate the platform, distance travelled, swimming speed, number of crossings in the target area and time spent in the goal quadrant) were recorded by a VHS image collecting system and analyzed by Chromotrack software (Chromotrack, San Diego Instruments).

2.5. Biochemical assessment—Monoamines

The concentrations of monoamines and metabolites in selected brain regions were estimated. Biochemical measurements were carried out 24 h after the last behavioral trial. The rats were decapitated, their brains immediately removed and dissected out on an ice-cold plate according to the method of Glovinsky and Iversen (1966), into the following regions: prefrontal cortex, hippocampus, hypothalamus and striatum. Each tissue was placed in a dry ice-cooled polypropylene vial, weighed, and stored in a deep freezer at −80 °C until assayed. To precipitate proteins, tissues were homogenized in1ml of ice-cold 0.1 M perchloric acid ($HClO_4$), and centrifuged (13,000 × *g* for 15 min). The supernatant was filtered (0.2 μm pore size filter; Whatman) and

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