Effect of Royal Jelly Diet on the Testicular Function of Hamsters

Michihiro Kohguchi, Shin-ichiro Inoue, Shimpei Ushio, Kanso Iwaki, Masao Ikeda and Masashi Kurimoto

Fujisaki Institute, Hayashibara Biochemical Laboratories, Inc., 675-1 Fujisaki, Okayama 702-8006, Japan

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To investigate the long-term effect of feeding royal jelly (RJ) on the testicular function, 32-week old male golden hamsters were fed diet containing RJ at doses of 0 μg/g diet (control), 50 μg/g diet or 500 μg/g diet for 12 weeks. At the end of the experiment, the hamsters were assessed for testicular function in terms of the amounts of intra-testicular free testosterone (TS) and histopathological changes. RJ diet groups showed higher TS levels and more intensive spermatogenesis than the control group in a dose-dependent manner. The intensity of spermatogenesis and TS levels in the 500 μg of RJ/g diet group showed significant differences of p < 0.01 and p < 0.05, respectively, when compared with those in the control group. These results indicate that the long-term feeding of RJ inhibits the age-associated decline in the testicular function of male hamsters.

Keywords: royal jelly, testis, testosterone

The oldest historical reference to the honeybee is considered to be that on a rock painting approximately 7,000 years old, located in Arana Cave in Spain (Rembold, 1965). Although only honey was used as a source of sugar up to the Middle Ages, in recent times, other beehive products have generated interest as therapeutic and nutritive agents, namely royal jelly (RJ), propolis and bee pollen. RJ that is produced by the hypopharyngeal and mandibular glands of the worker honeybees is well known to be a necessary food for the queen honeybee, and the physical properties of RJ are well known. It is a creamy, opalescent, and white liquid, and its major components are carboxylic acids including 10-hydroxy-2-decenoic acid, free amino acids, proteins, sugars, minerals, and vitamins (Rembold, 1965; Lercker et al., 1982). RJ has been used for many years as an anti-aging agent, a hormonal stimulant, an energy enhancer, for cholesterol control, as a wound healing agent, and for general healthcare use (Allen et al., 1958; Elkins, 1996). Various scientific works have shown that RJ has biological functions in mammals. We have also shown that RJ has collagen-inducing activity (Koya-Miyata et al., 2002), anti-allergic activity (Kataoka et al., 2002), anti-atopic activity (Taniguchi et al., 2003), anti-inflammatory activity (Kohno et al., 2004), and anti-aging functions (Inoue et al., 2003).

Regarding hormone-like effects, Townsend et al. (1940) reported that the fruit fly (Drosophila melanogaster) experienced a remarkable influence on the number of eggs and on the rate of reaching sexual maturity after being fed an ether-soluble fraction of RJ. Kato et al. (1988) also demonstrated that the weight of the testes, epididymides, seminal vesicles and prostate glands of male mice increased after the subcutaneous injection of RJ. Furthermore, Takahashi et al. (1962) reported that the intramuscular injection of the ether-soluble fraction of RJ increased spermatogenesis in the testes of mice and rats. Thus, various studies concerning the biological effects of RJ on the mammalian genital organs have been carried out parenterally. However, it remains to be resolved whether orally administered RJ promotes the testicular functions. In this study, we therefore fed RJ to old male hamsters and investigated the histopathological changes of the genital organs.

Materials and Methods

Experimental Animals This study was performed in accordance with the Guide for the Care and Use of Laboratory Animals, of the National Institutes of Health (1978). Male golden hamsters (31-week-old) maintained as a closed colony at our facility were used. The hamsters were acclimatized by feeding a commercial powdered diet NMF (Oriental Yeast Co., Osaka, Japan) ad libitum for 7 days. Two hamsters were housed per polycarbonate cage in an animal facility maintained at 23 ± 2°C with a 12 h light-dark cycle (light, 0700–1900 h). After acclimatization, 32-week-old hamsters weighing 150 g–190 g, were used.

Experimental diets Samples of native RJ were collected from the Paraibuna region of São Paulo, Brazil, and were kept frozen at −40°C until use. Pulverized RJ was prepared by adding nine volumes of the disaccharide trehalose (Hayashibara Biochemical Laboratories, Inc., Okayama, Japan), which prevents the degeneration of protein and fatty acid (Oku et al., 2002), to one volume of natural RJ. Experimental diets were prepared by adding the pulverized RJ to NMF diet at the amount of 50 μg or 500 μg of RJ per gram of diet. The compositions are summarized in Table 1.
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Table 1. Composition of experimental diets.

<table>
<thead>
<tr>
<th>Diet</th>
<th>Control</th>
<th>50 µg of RJ/g diet</th>
<th>500 µg of RJ/g diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>NMF-powdered diet</td>
<td>99.5%</td>
<td>99.5%</td>
<td>99.5%</td>
</tr>
<tr>
<td>Trehalose</td>
<td>0.500%</td>
<td>0.495%</td>
<td>0.450%</td>
</tr>
<tr>
<td>Royal jelly</td>
<td>0.000%</td>
<td>0.005%</td>
<td>0.050%</td>
</tr>
</tbody>
</table>

**Experimental design** After the adaptation period, hamsters were weighed and randomly assigned to each experimental group (n = 10). The hamsters were given free access to the respective diet and water for 12 weeks. Daily food intake and weekly body weight changes of individual animals were monitored throughout the experiment.

**Sampling and analytical procedures** After 12 weeks of the experimental period, at the age of 44 weeks, blood was collected from the heart under anesthesia induced by diethyl ether inhalation, and transferred to a blood collection tube (Venoject II, Terumo Co. Ltd., Tokyo, Japan). The serum was separated by centrifugation at 2,500 rpm at room temperature for 20 min, and stored at -40°C until analysis. After blood collection, both testes were removed and weighed. The right testis was fixed in 15% buffered formalin, and embedded in paraffin for histology to examine any morphological changes. Paraffin blocks were cut into 2 µm thick sections and stained with hematoxylin-eosin for microscopic examination. The other testis was stored at -40°C until measurement of free testosterone (TS) levels.

Serum lipid hydroperoxide (LPO) levels, as a marker of oxidative stress that increases during senescence (Yanagawa et al., 1999; Yasui et al., 2003), were measured with commercial kits (Lipid Hydroperoxide Assay Kit, Funakoshi Co. Ltd., Tokyo).

To determine male genital function, the testis was homogenized by using an ultrasonic homogenizer (Sonifier, Branson Ultrasoundics Corp., Danbury, CT, USA) for approximately 30 sec, and the supernatant was separated by centrifugation (15,000 rpm × 1 min). TS levels in the supernatants were measured using commercially available RIA kits (DPC Corp., Los Angeles, CA, USA).

Histopathological analysis of the testicular tissues was performed under the light microscope according to the method of Dostal et al. (1988). In brief, from 5 randomly chosen fields (magnification 40 x) of the testicular tissues, both normal and abnormal seminiferous tubules showing atrophy, degeneration and loss of germ cells were separately counted (magnification 100 x). After calculating the sum of the seminiferous tubules in the 5 chosen fields, the intensity of spermatogenesis was presented as the proportion of normal seminiferous tubules.

Table 2. Comparison of daily food consumption.

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of hamsters</th>
<th>Daily food intake (g)</th>
<th>Daily RJ intake (mg/kg body weight)</th>
<th>Body weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10</td>
<td>7.9 ± 0.38</td>
<td>0</td>
<td>164.0 ± 10.8</td>
</tr>
<tr>
<td>50 µg of RJ/g diet</td>
<td>10</td>
<td>8.1 ± 0.30</td>
<td>2.3 ± 0.2</td>
<td>163.4 ± 13.6</td>
</tr>
<tr>
<td>500 µg of RJ/g diet</td>
<td>10</td>
<td>8.3 ± 0.62</td>
<td>24.2 ± 2.6</td>
<td>163.9 ± 12.3</td>
</tr>
</tbody>
</table>

Each value represents the mean ± S.D. for each group.
severe degenerative changes were not observed in RJ-fed hamsters (Fig. 2B and C). The proportion of the normal seminiferous tubules, which was calculated by counting the number of normal and abnormal seminiferous tubules in the 5 randomly chosen fields, is shown in Fig. 3. The percentage of testicular tubules showing vivid spermatogenesis was significantly \( p < 0.01 \) higher in 500 \( \mu \)g of RJ/g diet-fed hamsters than that in hamsters of the control group.

**Discussion**

RJ has been traditionally used for years as an anti-aging agent, hormonal stimulant, energy enhancer, natural antidepressant, cholesterol control, and for many other physiological functions. However, the biological effects of RJ have not been fully elucidated. Although early studies reported on the gonadotropic or estrogenic effects in experimental animals by parenteral RJ administration (Townsend et al., 1940; Kato et al., 1988; Takahasi et al., 1962; Heyl, 1939), no experiments to examine the effect of orally administered RJ on the testicular function have been reported. In this study, we confirmed that the feeding of RJ delays the decline in the testicular function of male hamsters. Histological degenerative changes, such as atrophy, diminishment and detachment of germ cells, were not observed in the RJ-fed hamsters. Furthermore, long-term feeding of RJ inhibited the age-associated decline in the intra-testicular TS levels. Considering that TS plays crucial roles in spermatogenesis, these results suggest that orally administered RJ inhibited the loss of TS secreting cells such as Leydig cells in the testis. In this regard, Castro et al. (2002) have shown that the number of Leydig cells per gram of testis correlates with both plasma and testicular levels of TS.

The hormone-like effects of RJ extracts in experimental animals were reported in a very early study (Heyl,
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1939), and later by Vittek et al. (1982) who demonstrated that TS-like compounds are present in RJ as determined by a RIA method. These results further suggest that the compounds like a gonadotropic hormone contained in the RJ may inhibit the decline of testicular function in mammals via activating Leydig cells.

It is well known that the decline of testicular function is attributable to age-associated changes. The mechanism of aging at the biochemical level can be explained by the production of free radicals, which are induced by oxidative stress, resulting in the cellular membrane damage. Free radicals attack proteins, nucleic acids, carbohydrates and lipids of the cells (Günther et al., 1991; Leibovitz et al., 1980; Sohal et al., 2002). It is widely accepted that free radicals induce LPO and play an important role in age-related pathological phenomena including the clustering of degenerative diseases (Günther et al., 1991; Harman, 1981; Tokumaru et al., 1996). Sugawara et al. (1990) demonstrated that testicular LPO levels in 28 week-old mice increase significantly compared with the levels in 6 week-old mice. Therefore, we investigated serum LPO levels as a marker of oxidative stress. Our results showed that feeding 500 µg of RJ/g diet significantly suppressed LPO levels compared with those in the hamsters of control group. The finding that the long-term daily intake of RJ inhibited the generation of LPO suggests that RJ could protect organs from free radical-induced cellular damage. These results further prompt us to speculate that RJ may have the oxygen free radical scavenging function in addition to the gonadotropic hormone function, and thereby inhibited the decline of male testicular function. Further studies will help to fully elucidate the mechanisms involved.

In conclusion, our study demonstrated for the first time that the long-term feeding of RJ inhibits the decline of male hamster testicular function.

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References