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Effect of royal jelly on sexual efficiency in adult male rats

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Abstract

The study was designed to investigate the efficacy of treating the adult male rats with royal jelly (1g/kg B. Wt. orally) for one month with or without hydrogen peroxide (0.5%) in drinking water on sexual efficiency, glutathione and malondialdehyde tissue testis levels. The current study demonstrated that male rats receiving hydrogen peroxide caused a significant decrease ($P<0.05$) in the sperm count, percentage of live sperm and glutathione level, accompanied with a significant increase ($P<0.05$) in the malondialdehyde level and percentage of abnormal sperm deformity compared with control group. No significant difference was found in the weight of testis, epididymus, prostate, seminal vesicles, testosterone hormone level and body weight compared with control group. The treatment of adult male rats with royal jelly concomitantly with hydrogen peroxide caused a significant increase ($P<0.05$) in testicular weight and the body of epididymus, sperm count, testosterone hormone and glutathione level, and decrease in sperm deformity percentage, while no significant differences in the prostate weight, seminal vesicles, the percentage of live sperm, malondialdehyde level and body weight compared with hydrogen peroxide group. The treatment of adult male rats with royal jelly alone produced a significant increase ($P<0.05$) in the weights of testis and body of epididymus, sperm count, testosterone hormone, the percentage of live sperm, and glutathione level and retuned to control value, accompanied with a significant decrease ($P<0.05$) in malondialdehyde level and the percentage of sperm abnormality. It could be concluded from this study that royal jelly is a beneficial treatment of male adult rats receiving hydrogen peroxide (to induced oxidative stress) specially on sperm count, testosterone hormone level, the percentage of live sperm, and improvement of glutathione and malondialdehyde tissue testis.

Keywords: Royal jelly, Sexual efficiency, H_2O_2, Testosterone, Glutathione, Malondialdehyde.
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تأثير الغذاء الملكي في الكفاءة التناسلية في ذكور الجرذان البالغة

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فرع الفسلجة، كلية الطب البيطري، جامعة الموصل، الموصل، العراق

الخلاصة

صممت الدراسة لمعرفة تأثير المعاملة الغذاء الملكي (١غم / كغم من وزن الجسم عن طريق الفم) لمدة شهر لذكور الجرذان البالغة المعاملة مع وبدون ببيرو كسيد الهيدروجين بتركيز (0.5%) في ماء الشرب على الكفاءة التناسلية ومستوى الكلوتاثايون والمالونداياليديهايد في نسيج الخصية. بينت الدراسة الحالية ان معاملة ذكور الجرذان ببيروكسيد الهيدروجين سبب انخفاض معنوي ($P<0.05$) في عدد النطف والنسبة المئوية للنطف الحية ومستوى الكلوتاثايون رافقها ارتفاع معنوي ($P<0.05$) في مستوى المالونداياليديهايد والنسبة المئوية للنطف المشوهة مقارنة مع مجموعة السيطرة. بينما لم يحدث اختلاف معنوي في وزن الخصية والبربخ والبروستات والحويصلات المنوية ومستوى هرمون التيستيستيرون ووزن الجسم مقارنة مع قيم مجموعة السيطرة. أدت معاملة الجرذان بالغذاء الملكي سوية مع بيروكسيد الهيدروجين الى حدوث ارتفاع معنوي ($P<0.05$) في وزن الخصية و جسم البربخ وعدد النطف وهرمون التيستيستيرون ومستوى الكلوتاثايون وانخفاض في نسبة المئوية للنطف المشوهة مع عدم حدوث اختلاف معنوي في وزن البروستات والحويصلات المنوية والنسبة المئوية للنطف الحية ومستوى المالونداياليديهايد ووزن الجسم مقارنة مع

مجموعة بيروكسيد الهيدروجين. أدت المعالة ذكور الجرذان بالغذاء الملكي لوحده ارتفاع معنوي (P<0.05) وزن الخصية وجسم البربخ وعدد النطف وهرمون التيستيستيرون والنسبة المئوية للنطف الحية ومستوى الكلوتاثايون والرجوع الى قيم مشابه للسيطرة، صاحبها انخفاض معنوي (P<0.05) في مستوى المالونداياليديهايد النسبة المئوية للنطف المشوهه. يستنتج من الدراسة ان المعاملة بالغذاء الملكي ذو فائدة قيمة في علاج ذكور الجرذان البالغة المعاملة ببيرو كسيد الهيدروجين (المحدث للكرب ألتأكسدي) وبالأخص عدد النطف ومستوى هرمون التيستيستيرون والنسبة المئوية للنطف الحية وقد حسن من مستوى الكلوتاثايون والمالونداياليديهايد نسيج الخصية.

Introduction

Royal jelly is a thick, extremely nutrition, milky white, creamy liquid secreted by the hypopharyngeal glands of worker bees (*Apis mellifera*) in relation to sexual determination of the bee (1). Considered as the major cause for difference between queen and bee workers, royal jelly is appreciated as a dietary complement because of its composition (1).

Royal jelly is an essential food for the queen bee larvae and the queen herself. All larvae fed royal jelly for three days, but the queen bee eats royal jelly exclusively which makes her fertile and able to live to seven years. Queen bees will produce 2000 eggs per day, with each day brood equal to 2.5 times her body weight (2). In contrast, worker bees are sterile and live just seven to eight weeks. Royal jelly contains considerable amounts of proteins, amino acids including 8 essential amino acids (3), hormone rich substance (testosterone has been identified in extremely small quantities in royal jelly about 0.012g/g fresh weight (4), lipid, and sugars, royal jelly also contains vitamin A, C, D, and E, mineral salts are in descending order: (K, Ca, Na, Zn, Fe, Cu, and Mn.), enzymes antibiotic components. It also has an abundance of nucleic acid-DNA and RNA (5).

Gelatin, one of the precursors of collagen, is also found in royal jell, collagen is a powerful anti-aging element that helps preserve the youth of the body (6). And is known to have several diverse physiological and pharmacological functions, these include vasodilative, hypotensive, anti-hypercholesterolemia, and anti-tumor activities (7). Royal jelly has been found to be of great help in boosting the body resistance to the harmful side effect of chemotherapy and radiotherapy (8). Also contains gamma globulin, which helps the immune system to fight infections. It also contains sterols, phosphorous compounds and acetylcholine, which is needed to transmit nerve messages from cell to cell (8). Al-Tai (9) demonstrated that the reactive oxygen species produced by administration of hydrogen peroxide are responsible for the pathophysiological changes of the male reproductive system and induced defect in the histophysiological aspect of this system in rats. Polyunsaturated fatty acids and phospholipids are key constituents in the sperm cell membrane and are highly susceptible to oxidative damage. Sperm produce controlled concentrations of reactive oxygen species, such as the superoxide anion, hydrogen peroxide, and nitric oxide, which are needed for fertilization; however, high concentrations of these free radicals can directly damage sperm cells (10).

The current study designed to investigate effect the administration of royal jelly orally for one month to the male adult rats induced oxidative stress by hydrogen peroxide on sexual efficacy and glutathione, malondialdehyde levels.

Materials and methods

Twenty adult male albino rats were obtained from the animal house of the Veterinary Medical College, University of Mosul, at aged 3-4 month, weighing 200- 300g. They were housed in polypropylene cages under controlled condition of temperature (24-26°C) and lighting (12hours light/12hours dark). The rats were supplied a standard diet and tap water *ad libitum.*

The adult male rats were randomly divided into four groups (5 rats /group). The first group received tap water serve as control. The second group received hydrogen peroxide (H_2O_2) (Laboratory reagent, India) (0.5%) in drinking water for one month (11). The third group received (H_2O_2) (0.5%) in drinking water for one month concomitant with royal jelly (Peking, China) at 1g/kg B. Wt. dissolved in distalled water and given at 1 ml/kg for one month orally by gavage needle (12). The fourth group received royal jell at a dose 1g/kg B. Wt. orally alone. The weight of rats recorded weekly. At the end of experiment blood samples were collected into clean dry centrifuge tubes allowed to clot, serum separated after centrifugation at 1500 rpm for 15 minute for testosterone hormone assay, using Enzyme Linked Immunosorbent Assay (ELISA) (BioCheck Company, USA). Rats were sacrificed by ether administration. The abdominal cavity was then opened; the weight of testis, epididymal, seminal vesicles and prostate were recorded. The testis placed in ice normal saline for glutathione estimation using Moron method as described by (13) and malondialdehyde (MDA) estimation using Gilbert method as described by (14). The epididymis was dissected out, sectioned and immediately the content of the tail of each epididymis was squeezed gently in clean watch glass,

diluted 10 times with isotonic solution of sodium citrate (2.9%) at (37° C), take one drop from isotonic solution on slide and added one drop of eosin - nigrosin stain and made smear, this technique was used for the percentage of live/dead and for morphological abnormal sperms to be counted (15). The content of the head of epididymis was squeezed immediately in clean watch glass contained 9.8 ml. buffer formalin with 0.1 ml. eosin 5%, this was used for counting the sperm concentration using hemocytometric technique (16).

Data were analyzed statistically using one way analysis of variance. Group differences were determined using Duncan multiple range test. Statistical significance was considered at ($P< 0.05$) (17).

Results

Table (1) showed that administration of hydrogen peroxide (0.5%) in drinking water for one month did not affect the weight of testis, epididymis (head, body, tail), prostate and seminal vesicles compared with control group value. Treatment of adult male rats with royal jelly (1g/kg orally) for one month with or without hydrogen peroxide produced a significant increase ($P<0.05$) in the weight of testis and body of epididymis whereas no significant changes in the weight of head and tail of epididymus, prostate and seminal vesicles compared with hydrogen peroxide group.

The current study revealed a significant decrease ($P<0.05$) in sperm count in hydrogen peroxide group compared with control group as shown in Table (2).

Treatment with royal jelly and hydrogen peroxide caused a significant increase ($P<0.05$**)** in sperm count compared with hydrogen peroxide group and retuned to the normal control value. The data of current study showed a significant increase ($P<0.05$**)** in the sperm count in royal jelly treated group compared with hydrogen peroxide group, and royal jelly concomitantly with hydrogen peroxide group and retuned to the control value as shown in (table 2).

The current study demonstrated that a significant decrease ($P<0.05$**)** in glutathione level in hydrogen peroxide group compared with control group, while administration of royal jelly with or without hydrogen peroxide caused a significant increase ($P<0.05$**)** in the glutathione level compared with hydrogen peroxide group and returned to the control group. The present study showed that a significant increase ($P<0.05$) in malondialdehyde level in hydrogen peroxide group compared with control group. Treatment the royal jelly concomitant with hydrogen peroxide did not affect significantly in malondialdehyde level, but treatment with royal jelly alone caused a significant increase ($P<0.05$**)** in malondialdehyde level as shown in (table2).

Table 1. Effect the treatment of royal jelly on the weight of the testis, epididymis (head, body, tail) prostate and seminal vesicle in rats receiving hydrogen peroxide for one month.

Treated animals	Testis mg/100 g B. Wt.	Head of epididymus mg/100g B.Wt..	Body of epididymus mg/100g B.Wt	Tail of epididymus mg/100g B. Wt.	Prostate mg/100g B. Wt.	Seminal vesicle mg/100B. Wt.
Control	b 473.4±27.7	a 79.2±4.2	b 21.1±0.9	a 93.2±7.4	a 447.5±44.2	a 100.9±5.9
Hydrogen peroxide (0.5%)in drinking water for (1 month)	b 501.5±10.8	a 85.0±3.9	b 20.8±0.6	a 92.9±5	a 431±35.3	a 108.9±7.4
Hydrogen peroxide (0.5%)in drinking water for (1 month)+ royal jelly (1g/kg orally) for (1 month)	a 604.9±21.2	a 89.4±5.5	a 23.7±0.3	a 93.4±2.9	a 455.3±33.2	a 99.9±6
Royal jelly(1g/kg orally) for (1month)	a 636.1±21.7	a 76.5±4.1	a 24.1±0.3	a 90.4±1.5	a 427.1±26.7	a 105.7±6.4

Values were expressed as means ± SE from 5rats per treatment.
Values with different letters in the columns are significantly different at ($P<0.05$).

Table 2. Effect the treatment of royal jelly on sperm count, glutathione, and malodialdehyde levels in rats receiving hydrogen peroxide for one month.

Treated animals	Sperm concentration $\times 10^6$	Glutathione µmlg.	Malodialdehyde nm/g.
Control	ab 1.4320±0.02	a 1.04±0.02	b 264.82±12.48
Hydrogen peroxide (0.5%)in drinking water for (1 month)	c 0.8±0.02	b 0.59±0.02	a 311.0±17.6
Hydrogen peroxide (0.5%)in drinking water for(1 month)+ royal jelly (1g/kg orally) (1 month)	b 1.3440±0.13	a 1.23±0.14	ab 233.9±14.3
Royal jelly(1g/kg orally) for (1 month)	a 1.6260±0.02	a 1.26±0.02	c 219.7±5.7

Values were expressed as means ± SE from 5rats per treatment.
Values with different letters in the column are significantly different at (P<0.05).

Table 3 demonstrated that a significant decrease (P<0.05**)** in the percentage of the live sperms in hydrogen peroxide group compared with control group. Treatment the royal jelly concomitantly with hydrogen peroxide did not effect significantly in the percentage of the live sperms compared with hydrogen peroxide group, Whereas treatment with royal jelly alone caused a significant increase (P<0.05**)** in the percentage of the live sperms and retuned to normal control value.

The data of the current study revealed that a significant increase (P<0.05) in the percentage of sperms deformity in hydrogen peroxide group compared with control group. Treatment the royal jelly with or without hydrogen peroxide caused a significant decrease (P<0.05) in the percentage of sperm deformity compared with hydrogen peroxide group as shown in Table (3).

Same table shows no significant differences in the testosterone hormone level in hydrogen peroxide group compared with control group.

Administration of royal jelly concomitant with or without hydrogen peroxide caused a significant increase (P<0.05) in testosterone hormone compared with hydrogen peroxide group.

Table 4 demonstrated that no significant differences between groups in the body weight after (1, 2, 3, weeks) of treatment.

Table 3. Effect the treatment of royal jelly on the percentage number of live sperm, sperm deformity, and testosterone hormone concentration in rats receiving hydrogen peroxide for one month.

Treated animals	Live Sperm %	Sperm Deformity%	Testosterone Hormone ng/ml
Control	a 91.6±1.5	c 4.2±0.37	cb 2.37±0.16
Hydrogen peroxide (0.5%)in drinking water for (1 month)	b 84±1.51	a 11.2±1.06	c 1.72±0.30
Hydrogen peroxide (0.5%)in drinking water for (1 month)+ royal jelly (1g/kg orally)(1 month)	b 87±0.54	b 9.0±7.03	b 2.51±0.13
Royal jelly(1g/kg orally) for (1 month)	a 94.6±0.81	c 4.6±0.4	a 4.24±0.27

Values were expressed as means ± SE from 5rats per treatment.
Values with different letters in the column are significantly different at (P<0.05).

Table 4. Effect the treatment of royal jelly on body weights in rats receiving hydrogen peroxide for one month.

Treated animals	Weight (zero time)	Weight after one weeks	Weight after two weeks	Weight after three weeks.
Control	a 270.5±10.64	a 246.2±11.91	a 273.5±24.4	a 299.5±10.1
Hydrogen peroxide (0.5%)in drinking water for (1 month)	a 228.5±20.34	a 228.2±19.7	a 238.5±24.6	a 287.2±21.6
Hydrogen peroxide (0.5%)in drinking water (1 month)+ royal jelly (1g/kg orally) for (1 month)	a 263±8.09	223.5±20.01	a 235.2±21.9	a 286.2±14.3
Royal jelly(1g/kg orally) for (1 month)	a 265±23.47	a 250.2±17.06	a 247±26.5	a 305±34.07

Values were expressed as means ± SE from 5rats per treatment.

Discussion

The result of the present study demonstrated that administration of hydrogen peroxide resulted in a significant decrease in the sperm count, percentage of live sperm and glutathione level, accompanied with a significant increase in the malondialdehyde level and percentage of abnormal deformity sperm compared with control value. Similar results were obtained by other investigators (9,18-20).

Hydrogen peroxide caused an increase in oxidative damage to sperm membranes, proteins, and DNA is associated with alterations in signal transduction mechanisms that affect fertility (21). Numerous studies by Ollero *et al.,* (22) and Gill-Guzman *et al.,* (23) have shown that levels of (ROS) production in semen were negatively correlated with the percentage of normal sperm forms as determined by World Health Organization (24). These support the results of the present study which indicate that there was a relationship between oxidative stress induced by hydrogen peroxide and decrease in sperms count, percentage of live sperm and increased in the percentage of morphological abnormal sperms. Spermatozoa are particularly susceptible to oxidative stress induced damage because their plasma membranes contain large quantities of polyunsaturated fatty acids (PUFAs) (25).

The current study demonstrated that treatment with royal jelly produced a significant increase in the sperm count, live sperm percentage, testosterone hormone and glutathione levels and decreased in the malodialdehyde. Royal jelly is known as sexual tonic and used for treatment of impotence infertility, and significantly increase leutinizing hormone (LH) levels; this effect could be attributed to central effect of royal jelly. Royal jelly contains acetylcholine (1mg/g dry weight) (26).

Acetylcholine is one of peripheral and central neurotransmitters; Kobayashi *et al* (27) previously demonstrated a cyclic fluctuation of the biosynthetic enzyme choline acetyltransferase in the rats anterior hypothalamus with the activity of gonad (27). However some studies confirmed that acetylcholine helps to stimulate gonadotropine secretion of the hypothalamic level (28). Therefore, royal jelly could increase LH level by its effect at level of hypothalamus via its content of acetylcholine. This elevation of LH level, which is responsible for stimulation of testosterone secretion from interstitial cell (29).

Furthermore, testosterone could be elevated as a result of exogenous supplied by royal jelly, so it contains testosterone in amount 0.012g/g fresh weight (30). On the other hand elevation of testosterone level could be attributed to zinc found in royal jelly. So zinc deficiency causes low testosterone level, while zinc supplementation can raise testosterone level and increase fertility (31,32). Zinc sulphate also elevated LH and testosterone hormone (32).

Testosterone is essential for spermatogenesis from spermatogonium to spermatide (33). Royal jelly also contains L-argginine and carnitine amino acid, which essential for spermatogenesis (34). This study also showed that royal jelly increased in glutathione accompanied with decreased in malodialdehyde levels. This effect could be attributed to the royal jelly contain vitamin C, vitamin E and arginine (35). Vitamin E and C is a well-documented antioxidant and has been shown to inhibit free-radical-induced damage to sensitive cell membranes of the testis and reduced lipid peroxidation in tissue estimation by malodialdehyde, so vitamin E and C significantly decreased MDA, and increased in glutathione level (10).

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