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Short Communication

Effect of Palestinian Honey on Spermatogenesis in Rats

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ABSTRACT Treatment of male albino rats with 5% honey for 20 days had no significant effect on total body weight or on the relative weight of other organs like the testis, seminal vesicles, spleen, kidneys, liver, heart, or brain. The only significant change was a 17% increase in the relative weight of the epididymis ($P \leq .01$). The relative weight of all the other organs was similar to those in control animals treated for the same period with drinking water. Treatment of rats for the same period with the same concentration of 5% sucrose produced no significant changes in absolute or relative weight of tested organs compared to control animals. The same treatment with Palestinian honey increased significantly the epididymal sperm count by 37% ($P \leq .05$). The activity of testicular marker enzymes for spermatogenesis such as sorbitol dehydrogenase (SDH) was increased by 31% ($P \leq .05$), and lactate dehydrogenase (LDH) was reduced by 48% ($P \leq .05$), which indicates that treatment with honey induces spermatogenesis. Similar treatment with sucrose had no significant effect on any of the key enzymes or epididymal sperm count. In conclusion, our results show that ingestion of honey induces spermatogenesis in rats by increasing epididymal sperm count, increasing selectively the relative weight of the epididymis, and increasing SDH activity and reducing LDH activity.

KEY WORDS: • ***honey*** • ***lactate dehydrogenase*** • ***sorbitol dehydrogenase*** • ***spermatogenesis*** • ***sperm count***

INTRODUCTION

HONEY IS CONSIDERED a part of traditional medicine. It is effective in the healing of wounds and burns and the treatment of diabetic ulcers.[1–5] It provides gastric protection against gastric lesions,[6] in addition to its antibacterial activity, which is related to hydrogen peroxide produced enzymatically in the honey[7,8] and also to other substances.[9,10]

Many biological complications are caused by free radicals, which cause oxidative damage to lipids and proteins, thus explaining the importance of antioxidants in the treatment of carcinogenesis and mutagenesis. Honey contains phenolics, which have an antioxidant activity,[11–14] in addition to other compounds with antioxidant activity such catalase.[15] Thus, honey contains both aqueous and lipophilic antioxidants.[15]

Preliminary evidence that honey has an effect on reproduction was reported at a 2006 conference.[16–19] As far as reproduction is concerned, honey has increased the sperm count in rats and monkeys and increased vaginal wall epithelium and muscle thickness, without showing any effect on circulating gonadotrophins or testosterone.[16] In Malaysia honey is used traditionally in postmenapousal women to treat vaginal atrophy and dryness.[17] Honey was reported to enhance spermatogenesis in rats if given at the appropriate dose[18] and to reduce the toxic effects of cigarette smoke on spermatogenesis.[19]

In Palestine honey is frequently consumed for enhancement of male fertility; therefore the objectives of this study is to determine spermatogenic activity of Palestinian honey, by measuring its effect on epididymal sperm count and the activities of key enzymes involved in the process. Besides the preliminary reports cited above, to our knowledge, this is the first study on honey and reproduction.

EXPERIMENTAL METHODS

Chemicals

All the chemicals used were of analytic grade and purchased from Sigma Aldrich Co. (St. Louis, MO).

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Honey samples

Honey was purchased at the local market in the Ramallah District of the West Bank area of Palestine. It was diluted with drinking water (5% solution) and supplied continuously to the treated group with the drinking water. According to the supplier, the honey was freshly collected from sealed honeycombs, kept in dark glass bottles, and preserved at 10°C.

Animals

White albino male rats 12–14 weeks old weighing 250–300 g each were used in the experiments in groups of five to 12 rats per group. The animals were divided into three groups: group A was treated with 5% solution of Palestinian honey, group B was treated with 5% sucrose, and group C was treated with drinking water for 20 days. The animals were kept in cages with free access to food at 22 ± 2°C.

All the experimental animals in groups A, B, and C were maintained under normal conditions of humidity, food, circadian cycle, and temperature. At the end of the experiment the rats were anesthetized with ether, and the testis, epididymis, seminal vesicles, penis, spleen, kidneys, liver, heart, and brain were removed for the measurement of absolute and relative weight.

Measurements of sperm count

Sperms were collected from the epididymis of each rat by flushing with the same volume (10 mL) of suspension medium containing 140 mmol of NaCl, 0.3 mmol of KCl, 0.8 mmol of Na_2HPO_4, 0.2 mmol of KH_2PO_4, and 1.5 mmol of D-glucose (pH adjusted to 7.3 by adding 0.1 *N* NaOH). Collected samples were centrifuged at 100 *g* for 2 minutes, and the precipitate portion was resuspended in 10 mL of fresh suspension medium. A fraction of the suspension (100 μL) was mixed with an equal volume of 1% Trypan blue in the same medium, and numbers of sperms were counted in four chambers (used for counting of white blood cells) of the hemocytometer slide as described elsewere.[20] At this concentration of Trypan blue (0.5%), the dye was completely excluded by intact sperms, which appeared bright and colorless, but taken up by dead and damaged sperms, which showed blue heads. The sperm number was expressed per milliliter of suspension.

Enzymatic measurements

Testicular lactate dehydrogenase (LDH) and sorbitol dehydrogenase (SDH) activities were measured using kits purchased from Sigma Aldrich Co. Testicular tissue was homogenized in ice-cold 0.1 *M* Tris EDTA buffer (pH 8.0) at a tissue concentration of 10 mg/mL, and then the homogenized mixture was centrifuged at 10,000 *g* for 30 minutes at 4°C. One hundred microliters of the supernatant was used for the measurement of SDH activity, and 20 μL of the supernatant was used for the measurement of LDH activity; the procedure was carried out according to the instructions with the kit. Enzyme activity was expressed as micromoles of substrate converted per minute per milligram of protein.[21]

Measurement of protein concentration

Protein concentrations were determined by the method of Lowry *et al.*[22]

Statistical analysis

All the values are presented as mean ± SEM for the number of experiments indicated in parentheses, and the data were statistically analyzed using Student's *t* test.

RESULTS

Effect of honey on relative weight of different organs

Our results show that body weight gain in control animals during 20 days was 17.42%. A similar increase was achieved with animals treated for the same period with 5% sucrose solution or with 5% honey solution. No significant changes were observed in the relative weight (in comparison to body weight) of the following organs: spleen, 0.52 ± 0.04 ($n = 7$); kidneys, 2.14 ± 0.69 ($n = 7$); liver, 9.95 ± 0.35 ($n = 7$); heart, 0.97 ± 0.04 ($n = 7$); and brain, 1.67 ± 0.03 ($n = 7$). In reproductive organs similar trends were observed: testis, 1.21 ± 0.03 ($n = 14$); seminal vesicles, 0.58 ± 0.02 ($n = 14$); and penis, 0.32 ± 0.02 ($n = 7$).

The only significant increase (17%) occurred in relative weight of epididymis, from an initial value of 0.36 ± 0.01 g ($n = 10$) to a final value of 0.42 ± 0.01 g ($n = 14$) ($P \leq .01$). No significant changes in epididymal relative weight were observed in animals treated with 5% sucrose or with control animals.

Effect of honey ingestion on epididymal sperm count

Ingestion of 5% solution of Palestinian natural honey ($n = 12$) increased significantly the epididymal sperm count per gram of epididymis by 37%, from 2,236.80 ± 255.70 × 10^6 ($n = 10$) to 3,119.88 ± 203.48 × 10^6 ($n = 12$) ($P \leq .01$). In animals treated for the same period with 5% sucrose no significant effect on epididymal sperm count was observed (Table 1).

Measurement of enzyme activity

Table 2 shows that in animals treated with 5% honey, the activity of the testicular enzyme SDH was increased significantly by 31% from 0.542 ± 0.029 ($n = 7$) to 0.709 ± 0.035 ($n = 7$) μmol of substrate converted/minute/mg of protein, and the activity of LDH was reduced by 48% from 1,583.516 ± 157.500 ($n = 4$) to 823.209 ± 77.565 ($n = 5$) μmol of substrate converted/minute/mg of protein. No sig-

Table 1. Effect of Honey on Epididymal Sperm Count

Treatment	*Epididymal sperm count/g of epididymis*	*% change*	P
Control	$2{,}236.80 \pm 255.70 \times 10^6$ (10)		
Sucrose (5%)	$2{,}457.33 \pm 54.95 \times 10^6$ (9)	+8%	NS
Honey (5%)	$3{,}119.88 \pm 203.48 \times 10^6$ (12)	+37%	≤.05

Data are mean ± SEM values for the number of experiments indicated in parentheses. NS, not significant.

nificant changes were observed in the testes of rats treated with 5% sucrose.

DISCUSSION

From our results it is clear that ingestion of diluted Palestinian honey by male albino rats has no significant effect on body weight gain or on the relative weight of any of the body organs, except for a significant increase of 17% in epididymis and a 37% increase in epididymal sperm count. A similar increase in sperm count was reported by Siti[16] in rats and monkeys, and enhanced spermatogenesis was reported by Mahaneem *et al*.[18] Honey has a protective effect against the toxic effects of cigarette smoke on spermatogenesis.[19] No changes were reported on any of the sex hormones.

The effect of honey is not restricted to male fertility: in females, honey has increased the thickness of vaginal wall epithelium and muscle in ovariectomized rats,[23] and it was used for the treatment of vaginal atrophy and dryness in postmenopausal women.[17]

Honey can affect spermatogenesis by elevating SDH activity and by reducing the LDH activity. The SDH level has been used as an indicator for secondary maturation of sex organs.[23] Since spermatozoa contain a small amount of cytosol, the glycolytic pathway would not be a major source of energy, which emphasizes the role of SDH in converting sorbitol to fructose, which is faster than glucose in energy production. The resultant NADH, a by-product of SDH activity, can donate electrons to the electron transport system to synthesize ATP.[24–26]

The effect is not due to high concentrations of fructose, since animals treated for the same period with 5% sucrose solution did not show any spermatogenic activity.

Spermatozoa have little defense against oxidative damage and are highly sensitive to free radicals. Therefore treatment with honey, which contains several antioxidants as reported previously,[11–15] can reverse the oxidative damage as expressed by the increase in epididymal sperm count. Previous reports have shown that honeys with a high content of polyphenolic compounds have the capacity to decrease significantly the concentration of lipid hydroperoxides produced during the lipid peroxidation process, in a process similar to that of other antioxidants like melatonin and vitamin E.[12,14] Also, Malaysian honey has been used in treating male subfertility, by increasing sperm count without affecting circulatory gonadotrophins or testosterone in male rats.[16,18] The antioxidant activity of honey was found to protect male rats against the antispermatogenic activity of exposure to cigarette smoke.[19]

In conclusion, the present study shows that Palestinian honey increases the relative weight of the epididymis and the epididymal sperm count by affecting the activity of key enzymes in spermatogenesis and maturation. It increased SDH and reduced LDH activities. Further experiments are needed to elucidate the mechanism by which honey affects spermatogenesis. Also, experiments with other types of honey should be carried out.

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Table 2. Effect of Honey on SDH and LDH Activities

	Enzyme activity (μmol of substrate converted/minute/mg of protein)	
Treatment	*SDH*	*LDH*
Control	0.542 ± 0.029 (7)	1,583.516 ± 157.500 (4)
Sucrose (5%)	0.579 ± 0.044 (6)	1,543.465 ± 338.380 (6)
	+7%	−3%
	(NS)	(NS)
Honey (5%)	0.709 ± 0.035 (7)	823.209 ± 77.565 (5)
	+31%	−48%
	($P \leq .05$)	($P \leq .05$)

All the animals were treated with water (control), sucrose, or honey for 20 days. Data are mean ± SEM values for the number of experiments indicated in parentheses. NS, not significant.

AUTHOR DISCLOSURE STATEMENT

No competing financial interests exist.

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