

Hormonal and Histological Study on the Effect of Honey on Mice Male

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ABSTRACT

Honey is traditionally consumed as a nutrient, as well as for the enhancement of fertility. In this study, the effects of honey on reproductive hormones and histology of mice testis after 35 days of honey administrated orally. 18 adult male mice were divided into three groups: one control and two treated groups containing 6 mice in each group. Treated groups received honey in two different doses i.e 1.2 and 1.8 g/kg body weight, the results showed significant ($P<0.05$) increased in Testosterone, FSH and LH hormones between the three groups. Also there is significant increase in diameters of seminiferous tubules between control and the treated groups, while the results showed significant increased in primary spermatocytes and spermatids between 1.2 g/kg body weight treated group with other groups.

Keywords: Honey, Testosterone, FSH, LH, Histology Of Testis.

دراسة هرمونية ونسجية لتأثير العسل على ذكور الفئران

الخلاصة

العسل من المواد الغذائية الاستهلاكية التقليدية, وكذلك يستعمل لزيادة الخصوبة. في هذه الدراسة, تأثير العسل على هرمونات الجهاز التناسلي الذكري وانسجة الخصى بعد 35 يوما من التجريع عن طريق الفم. تمت الدراسة على 18 ذكر من الفئران البيض المختبرية والتي قسمت الى ثلاث مجاميع : واحد هي مجموعة سيطرة لم يتم تجريعها بالعسل ومجموعتين تتكون كل واحد منهما من 6 فئران تم تجريعهم بالعسل. تم تجريع كل مجموعة بتركيز معين من العسل والتركيز هي 1.2 و 1.8 غم/كغم من وزن الجسم, وظهرت النتائج ان هناك فروقات معنوية لزيادة نسبة هرمونات Testosterone, FSH and LH بين المجاميع الثلاثة. وهناك ايضا زيادة في قطر النبيبات المنوية بين مجموعة السيطرة والمجاميع المعاملة, وان هناك زيادة معنوية في spermatocytes and spermatids بين المجموعة المعاملة بالتركيز 1.2 غم/كغم من وزن الجسم مع المجاميع الاخرى.

INTRODUCTION

Honey is a natural product of bees formed from nectar collected from flowering vegetation. It is nutrient rich content, e.g. sugars such as fructose and glucose; minerals such as magnesium, potassium, calcium, sodium chloride, sulphur, iron and phosphates; as well as vitamins B1, B2, C, B6, B5 and B3 [1]. In addition, it possesses some biological properties such as antioxidant [2], antimicrobial [1], anti-inflammatory [3] and immunomodulatory [4]. In Arab countries honey is considered to increase human male potency. It had been reported that honey increased spermatogenesis in rats [5]. Also found significantly increase testosterone level, body weight, relative weight of testis, relative weight of epididymis [6].

To date, there is a lack of data concerning the medicinal use of honey on reproductive performance and testicular dysfunction. The present study aimed to determine the effects of oral administration of original mountain honey on reproductive hormones and histology of testis of mice male.

MATERIALS AND METHODS

Administration Doses

The doses prepared from original mountain honey with concentrations of (1.2 g/kg body weight and 1.8 g/kg body weight) these concentrations, orally administrated daily for 35 days [7].

Treatment of males

Eighteen adult male mice (30-36 gm) were purchased from Biotechnology Research Centre-Al-Nahrain University and maintained on a 14:10-hour light dark cycle in the Animal house control and treated mice were provided with feed and water ad libitum, there were no differences in feed intake. Males were randomly divided into 3 groups, each composed of 6 mice. The first group was treated with 1.2 g/kg body weight; the second group was treated with 1.8 g/kg body weight orally administered daily for 35 days and the third group was given normal saline as a control group.

Hormonal analysis

Blood samples were withdrawn by cardiac puncture and immediately the serum was separated by centrifuge at 3000 rpm for 3 minutes.

Testosterone hormonal assay

Bio merieux Italia S.P. a vidia campligliano, 58 50015-point A EMA (F1) Italia miniVIDAS. Was used for the hormonal assay.

C. Bio merieux Sa.69230 marcy l'Etoile-France, testosterone for 30 sample (test), code No. 09345B.

In testosterone test the assay principle combines an enzyme immuno assay sandwich method with a final fluorescent detection (ELFA).

- 1- Only remove the required reagents from the refrigerator and allow them to come to room temperature for at least 30 minutes.
- 2- Use one TES strip and one TES SPR from the kit for each sample, control to be tested.
- 3- Select TES to enter the code. The calibrator must be identified by "S1", and tested in duplicate. If the control needs to be tested, it should be identified by C1.
- 4- Mix the samples, the calibrator and/or the control using vortex type mixer.

- 5- Pipette 200 μ l of sample, control into the sample well.
- 6- Insert the VIDAS SPRs and strips into the positions indicated on the screen. Check to make sure the color labels with the three letter assay code on the SPRs and the reagent strip match.
- 7- Initiate the assay processing as directed in the operator's manual. All the assay steps are performed automatically by the instrument. The assay will be completed within approximately 60 minutes.
- 8- After the assay is completed, remove the SPRs and strips from the instrument.
- 9- After the assay is completed. Dipose of the used SPRs and strips into an appropriate recipient.

FSH & LH hormonal assay

Diagnostic automation, inc. for FSH & LH (Microwell ELISA Follicular – Stimulating Hormone (FSH) enzyme immunoassay test kit and Microwell ELISA Luteinizing Hormone (LH) enzyme immunoassay test kit for human was used. All reagent should be brought to room temperature, dilute 1 volume of wash buffer (50x) with 49 volumes of distilled water, dispense 50 μ l of standard, specimens, and controls into appropriate wells. Dispense 100 μ l of enzyme conjugate reagent in each well, thoroughly mix for 30 second. Incubate at room temperature (18-22 °C) for 60 minutes. Remove the incubation mixture, rinse 5 times with washing buffer. Dispense 100 μ l of TMB solution in each well and mix for 5 seconds, incubate at room temperature in the dark for 20 minutes, stop the reaction by adding 100 μ l of stop solution to each well. Gently mix for 30 seconds (it is important to make sure that all the blue color changes to yellow color completely), read optical density at 450 nm with a microtiter reader within 30 minutes [8].

Histological analysis

The perfuse-fixed testes placed in Bouin fluid overnight, and processed for routine paraffin embedding. The testes were cut into 5- μ m sections. Three serial sections per testes were mounted on slides, deparaffinized, rehydrated, and stained with hematoxyline - eosin stain. Sections of the testes were examined by light microscopy seminiferous tubules, interstitial spaces, primary spermatocytes and spermatids diameters were assessed in each testes using a previously calibrated Micrometers (Ocular micrometer, Stage micrometer).

The diameter of 25 seminiferous tubules was measured in 5 fields (5 seminiferous tubules per field). In similar manner diameter of primary spermatocytes, spermatids, leydig cells were measured in 5 fields and the mean value of each was calculated. The interstitial space observed between to consecutive seminiferous tubules by using the ocular micrometer [9].

Statistical analysis

Statistical analysis was performed to compare two different groups by using Chi-square and ANOVA-test. Statistical significance was determined at $P < 0.05$. [10].

RESULTS AND DISCUSSION

In this study we examined the effect of honey supplementation on reproductive hormones and histology of male reproductive system following 35 day of honey administration. The results show significant increased ($P < 0.05$) in

hormones level (Testosterone, FSH & LH) among the three groups (group I= control, group II= treated with honey 1.2 g/kg body weight daily, and group III= treated with honey 1.8 g/kg body weight daily) (Table: 1).

Table (1) Effect of honey on hormones Testosterone, FSH and LH after 35 days from treatment in mice.

Treatment groups	Testosterone ng/ml (mean±SE)	FSH IU/ml (mean±SE)	LH IU/ml (mean±SE)
Control	1.61±0.37	0.55±0.085	0.35±0.082
Treated with Honey (1.2 g/kg daily)	2.36±0.62	0.82±0.094	0.76±0.27
Treated with Honey (1.8 g/kg daily)	2.94±0.92	1.37±0.86	0.97±0.41

Significant Differences ($P < 0.05$) to compared rows by ANOVA test.

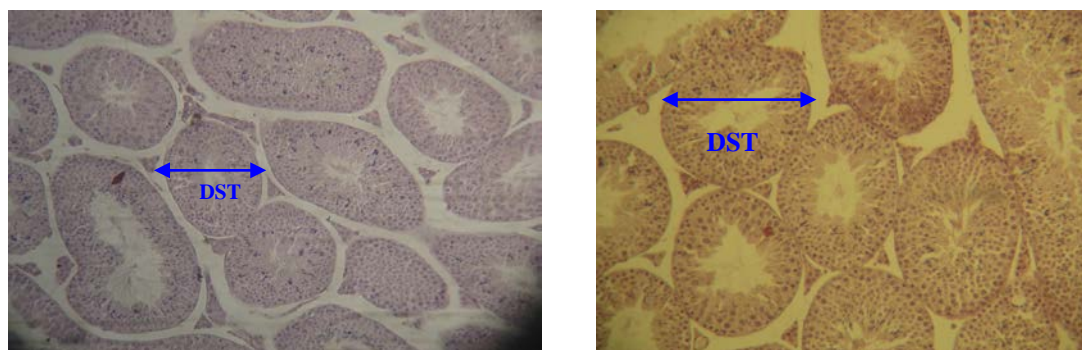
This result disagree with Mohamed *et al*, 2011 [11], there were no significant differences for the serum levels of luteinizing and follicle-stimulating hormones among the groups. The levels of these reproductive hormones were also similar between control and honey groups.

According to the histological study of mice male reproductive system, the results showed significant increased in diameters of seminiferous tubules when treated with honey compared with control group Table (2) Figure (1).

Table (2) Diameter of seminiferous tubules and interstitial space in both treated and control groups.

Groups	Diameter of seminiferous tubules (μm) (mean±SE)
Control	187.33±11.53
Treated with Honey (1.2 g/kg daily)	212.64±21.42
Treated with Honey (1.8 g/kg daily)	218.27±23.66

Significant Differences ($P < 0.05$) by ANOVA test.



A

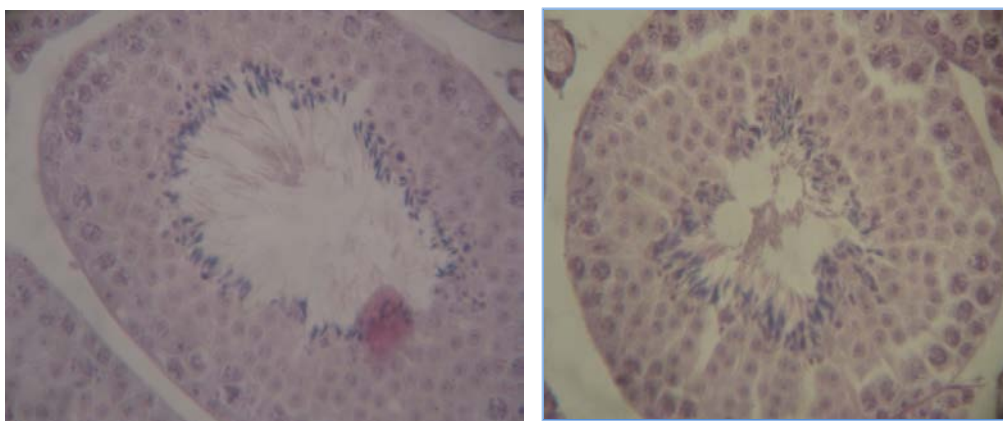
B

Figure (1)Photomicrograph of testes of mice. A: control group showing normal structure of seminiferous tubules. B: treated group showing increase in diameter of seminiferous tubules (DST- Diameter of Seminiferous tubules, IS- Interstitial space) (X 10).

Also in primary spermatocytes, spermatids there is significant differences between the group treated with Honey (1.2 g/kg daily) with other groups Table (3) Figure (2), Spermatogenic cell layers of control showed less dense packing of spermatogenic cells than the honey treated group. The lumen of control was less densely filled as compared to that of honey treated group which was filled with sperm tail this agreed with Syazana *et al.* 2011 [12]. The diameter of leydig cells Table (3) in the present study did not reveal any alteration after honey administration with respect to control. This could be due to either increasing age for heterogeneous population of leydig cells in the testes [13] which made non-significant results through changes in morphological form from round to oval in shape was observed.

Table (3) Diameter of primary spermatocytes, spermatids and No. of leydig cells in treated and control groups.

Groups	Primary spermatocytes(μm) (mean±SE)	Spermatids (μm) (mean±SE)	Leydig cells(μm) (mean±SE)
Control	6.54±0.83	4.32±0.61
Treated with Honey (1.2 g/kg daily)	7.54±0.96	6.58±1.31	5.41±0.82
Treated with Honey (1.8 g/kg daily)	7.62±0.91	6.82±0.94	6.03±0.97



A

B

Figure (2)Photomicrograph of testes of mice. A: control group showing normal structure of Spermatogenic cell layers. B: treated group showing increase in spermatogenic cells (X 40).

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