

Effect of Honey on Testicular Functions in Rats Exposed to Octylphenol

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Abstract: Honey is traditionally consumed by the local Saudian population as a nutrient, as well as for the enhancement of fertility. The decline in male reproductive health and fertility in the last 30 years has been linked to environmental toxicants including octylphenol (OP). OP has been considered an endocrine disrupting substance causing reproductive dysfunction and increase in reactive oxygen species production in different organs including testis and honey has an antioxidant property. The aim of this study was to elucidate the protective effects of honey against reproductive toxicity induced by OP in male Sprague Dawley rats. Six experimental groups receiving a combination of OP (0.1 and 1.0 mg kg⁻¹ b.wt., corresponding to 1/100 and 1/10 LD₅₀) and/or honey (20 mg/ kg body weight/day) for 4 weeks were divided as follows: no treatment (control); low dose OP alone (Group A); high dose OP alone (Group B); low dose OP plus honey (Group C); high dose OP plus honey (Group D); and honey alone (Group E). OP caused a significantly decreased the fertility index and weight of testes. It induced testicular lesions characterized by moderate to severe degenerative changes of seminiferous tubules and incomplete arrest of spermatogenesis. Administration of Honey either alone or combined with OP ameliorated these toxic effects. Similarly, histopathological results revealed that OP caused alterations in the testes. In conclusion, Honey reduced the histopathological stress toxicity induced by OP in the reproductive system of male Sprague Dawley rats. A great attention should be taken during field application of octylphenol to avoid its deleterious effects in farm animals and occupationally exposed humans.

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1. Introduction

Honey is mentioned two times in the Holy Quran, the honey that we use is described in the Quran as a part of the bees' products. The Holy Quran describes the process of its formation by bees and the medical values of it and of the other bees' products. Surah 16 (Al-Nahl: 68-69) "And your Lord revealed the female Honeybee; build homes in mountains, and trees, and in the hives people built for you".

Honey is a natural product of bees formed from nectar collected from flowering vegetation. It has been reported that honey contains moisture, sugars such as glucose and fructose, enzymes such as catalase and glutathione reductase, trace essential elements such as iron, copper, zinc and calcium, vitamins such as vitamin A, C and E as well as some flavonoids and phenolic acids (Gheldof *et al.*, 2002; Al-Waili, 2003; Yao *et al.*, 2004; Michalkiewicz *et al.*, 2008). In addition, it possesses some biological properties such as antioxidant (Perez *et al.*, 2006), antimicrobial (Estevinho *et al.*, 2008), anti-inflammatory (Prakash *et al.*, 2008) and immunomodulatory effects (Timm *et al.*, 2008). Traditionally, honey is frequently consumed by the Muslim population as a nutrient, as well as for the enhancement of fertility and vitality. Recently, a

higher sperm count was observed in many studies following the oral administration of Malaysian honey for 28 days in rats (Mahaneem *et al.*, 2007). A significantly higher epididymal sperm count was also found in adult rats following the daily treatment of 5% Palestinian honey for 20 days (Abdul-Ghani *et al.*, 2008).

Several common pollutants have profound effects on reproduction and growth of animals because they mimic or suppress the actions of sex hormones. Of particular concern, alkylphenol-polyethoxylates (APEOs) are a large number of nonionic surfactants in commercial production of lubricating oil additives, resins, plasticizers, detergents, herbicides, paints and cosmetics (Hernandez-Rodrigues *et al.*, 2007). These compounds enter the aquatic environment mainly from sewage treatment (Trudeau *et al.*, 2002). Octylphenol (OP) and nonylphenol (NP), formed as hydrophobic metabolites of APEOs, act as estrogenic compounds (Blake *et al.*, 2004), and can accumulate within the internal organs of animals in concentrations enough to disrupt the reproductive and endocrine system (Ying *et al.*, 2002). octylphenol (para-octylphenol, 4-OP) metabolites, including linear and branched isoforms of octylphenol (n-OP

and t-OP, respectively), have been considered as endocrine disrupting substance causing reproductive dysfunction, and increasing production of reactive oxygen species in testis, liver, kidney, and brain in many animal (Zha *et al.*, 2007 Hsieh *et al.*, 2009). Male mice exposed to nonylphenol (200mg/kg) during sexual maturation period suffered from damaged reproductive development (El-Dakdoky and Helal, 2007).

The decline in male reproductive health and fertility for the past 30 years has been linked to environmental toxicants and xenobiotics (Sikka and Wang, 2008). One of the toxicants that have detrimental effects on male reproductive function is octylphenol (OP). To date, whether honey has any protective effect against the toxic effects of OP on testicular functions is rare to be reported. Therefore, this study was undertaken to investigate the effect of honey on testicular functions in adult rats exposed to OP.

2. Material and methods

Chemicals

Octylphenol (OP) is a family of closely related organic compounds. The term octylphenol represents a large number of isomeric compounds. The octyl group may be branched in a variety of ways or be a straight chain and may be located at either the 2-, 3- or 4-position of the benzene ring. Of these potential isomers, 4-tert-octylphenol is the most commercially important. OP was purchased from Sigma-Aldrich (Sigma-Aldrich chemical Co. St. Louis, MO, USA).

Honey sample

Honey used in this study was purchased from the local honey market in Bab-Makkah, Jeddah, Saudi Arabia. It is a wild multifloral honey collected from beehives built on Taief and Mekkah Al-Mokaramah Cave Mountains. This honey is also locally known as Seder honey. It was filtered to remove solid particles and concentrated (20% w/v water) by oven drying at 40°C by the supplier.

Animals

A total of 60 adult male Sprague Dawley rats, aged 10 weeks (270-320g) were obtained were selected from inbred colony maintained in the Animal House of King Fahd center of medical researches. Rats were individually housed in a polycarbonate cage and maintained on a 12- h light/dark cycle at 20-24°C. They were provided a standard pellet diet and water *ad libitum* and acclimatized to the environment for 2 weeks prior to experimental use.

Acute toxicity experiment:

For estimating the LD₅₀ of Nonylphenol, 30 male Sprague Dawley rats were distributed into five groups each containing 6 animals. Rats were given orally, by stomach tube, the tested organic compound

in graded doses. Toxic symptoms and the number of rats that died in each group after 48 h observation were recorded. The LD₅₀ of octylphenol was then calculated according to the toxicity method described in Gad and Weil (1982).

Experimental study

Effects of octylphenol at two dose levels (1/10 and 1/100 of the LD₅₀) and honey at acceptable daily intake (20 mg kg⁻¹ b.wt.) were estimated. For estimating the effect of octylphenol, honey and their combination on male fertility, 60 mature male rats were allocated into six equal groups each of 10 individuals. The 1st group (Control) was given orally 1.0 ml distilled water/day (vehicle) and kept as normal control. The 2nd and 3rd groups (A and B Groups) were given orally 1/100 (0.1 mg kg⁻¹ b.wt.) and 1/10 (1.0 mg kg⁻¹ b.wt.) of the LD₅₀ of octylphenol, respectively. The 4th group (Group C) of male rats was orally given 20.0 mg kg⁻¹ b.wt. of honey calculated from acceptable daily intake combined with 1/100 of the LD₅₀ of octylphenol. The dose of honey was based on the body surface area normalization method (Reagan-Shaw *et al.*, 2007). The 5th (group D) was given 20.0 mg kg⁻¹ b.wt. of honey and 1/10 of the LD₅₀ of octylphenol. The rats were given octylphenol and after 2-4 h rats were given honey. The 6th one (group E), rats was only orally given 20.0 mg kg⁻¹ b.wt. of honey. Oral administration of the tested compounds continued for 28 consecutive days to cover the spermatogenic states according to Amann (1982).

At the end of experiment, the rats were sacrificed and the left testis was removed and weighed. The testes were preserved in 10% neutral formalin solution till processed for histopathological examination (Bearden and Fluquary, 1980; Wilke and Utley, 1987).

Histopathological examination

Testes of the treated rats were taken and fixed in 10% neutral formalin solution. The fixed specimens were then trimmed, washed and dehydrated in ascending grades of alcohol. These specimens were cleared in xylene, embedded in paraffin, sectioned at 4-6 μm thickness and stained with Hematoxylin and Eosin (H&E) then microscopically examined.

Statistical analysis

Statistical analysis was performed using one-way analysis of variance (ANOVA) to assess significant differences among treatment groups. The criterion for statistical significance was set at *p* 0.05. All statistical analyses were performed using SPSS statistical version 16 software package (SPSS Inc., USA).

3. Results

Group A showed a significant decrease in testis weight ($p < 0.01$) when compared to the controls. The numbers of germinal ($p < 0.001$), Lydig ($p < 0.001$) and spermatocyte (primary & secondary) cells ($p < 0.01$) were also decrease when compared to the controls. These differences were highly significant between controls and group B, however, the numbers of spermatocytes in group B were significantly lower than in the control group (Table 1; $p < 0.01$). The numbers of germinal cells, Lydig cells and spermatocytes in group A were significantly lower than those in control [(9.11 vs. 9.84; $p < 0.001$); (10.21 vs. 10.97; $p < 0.001$); (44.78 vs. 46.11; $p < 0.01$)].

The diameters of seminiferous tubules were smaller in groups A and B, but a significant difference was seen only in group A compared to the control group ($p < 0.01$, Table 1). In C and D groups in which the rats were given octylphenol and after 2–4 hrs rats were given honey, all measurements were significantly lower than in the control group. Generally the numbers of germinal cells, Lydig cells, spermatocytes and seminiferous tubules were significantly lower in group D than those in group C ($p < 0.01$, Table 1). In the last group (group E), in which rats was only orally given 20.0 mg kg⁻¹ b.wt. of honey, there was no significant difference between this group and control one in all measurements (Table 1).

The structures of the seminiferous tubules in octylphenol –treatment groups were pathologically damaged (Figs. 1B, C) in comparison to the control group (Fig. 1A). Testicular structure of the control group showing normal and regular seminiferous tubules. Octylphenol –treated group B (0.1 mg/kg) showing the pathologic effects in which the structures of the seminiferous tubules were damaged while octylphenol –treated group (1.0 mg/kg), the tubules were severely damaged in which the thickness of the tubular walls was reduced. The number of germinal cells was greatly decreased with a disturbance in their diameter (Figs. 1 A, B and C).

The seminiferous tubules structure in octylphenol –treatment groups combined with honey was illustrated in (Fig. 2). Testicular structure of the low dose octylphenol and honey rat group showing sustainable normal but irregular seminiferous tubules with large intercellular space (Fig. 2A). High dose octylphenol and honey-treated group showing slightly decreased pathologic effects in which the structures of the seminiferous tubules were slightly damaged with bleeding in intercellular space (Fig. 2B). In honey –treated group, normal and regular seminiferous tubules were clearly appears. The germinal cells were greatly regulated with normal diameter and mild dilatation of the seminiferous

tubules with normal complete spermatogenic series (Fig. 2C).

4. Discussion

The present study shows that OP was able to induce testicular stress toxicity which is reflected by increased reproductive dysfunction. The toxic effect of OP on rat testes in group A was significantly higher than that in group B. These data suggest that the toxic effects of OP on rat testes are dose dependent. Although this reproductive dysfunction is typically characterized by disruptions in spermatogenesis and loss of fertility. Testicular damage was also proved by the decrease in testis weights, seminiferous tubule diameters, decreased germinal cell count and the present histological results. Moreover, the results showed that honey could provide advantages against OP intoxication which were consistent with a previous report (Perez *et al.*, 2006).

The reproductive dysfunction is typically characterized by disruptions in spermatogenesis and loss of fertility, the actual mechanisms involved in OP -induced infertility remain unclear. Chitra *et al.* (2002) explained that OP as a toxicant led to cell injury by mitotic toxicity, chromatin destruction and DNA disturbances. Some research has shown decreased testosterone production by Leydig cells in organophosphorus insecticide treated groups to be one of these mechanisms (Abdel-Aziz *et al.*, 1994; Maxwell and Dutta, 2005; Contreras *et al.*, 2006). The Leydig cells produce the testosterone needed in the seminiferous tubules to induce the differentiation of spermatogonia to spermatozoa (Contreras *et al.*, 2006). Because testicular Leydig cells play a critical role in male reproductive function, alterations in the Leydig cells could be due to many different pathological or experimental situations associated with spermatogenesis deficiency (Dufau, 1979; Walsh *et al.*, 2000).

Reactive oxygen species (ROS) caused by organic toxicant treatment may be involved in the toxicity of various phenols (Walsh *et al.*, 2000). Increased ROS may decrease the effective concentration of antioxidant, increasing the harmful effects of ROS to reproductive tissue (Agarwal and Prabakaran, 2005). Nafstad *et al.* (1983) have shown that organophosphorus treatment caused an increase in lipid peroxidation (LPO) in erythrocytes. Because spermatozoa have large quantities of polyunsaturated fatty acids (PUFA) in their plasma membranes and their cytoplasm contains low concentrations of scavenging antioxidants (Agarwal and Prabakaran, 2005), a causal relationship is suspected. Thus, it is hypothesized that oxidative damage induced by OP may be one of these mechanisms which merit future study.

Table 1: Effect of octylphenol, honey and their combination on testicular measurements of male Sprague Dawley rats.

Parameters	Control	Group A	Group B	Group C	Group D	Group E
Testis weight (g)	1.62±0.21	1.33±0.16**	1.39±0.08	1.55±0.20*	1.44±0.23*	1.64±0.07
Germinal cell count	9.84 ± 0.64	7.34 ± 0.87*	9.56 ± 0.86	8.08 ± 0.62*	7.96 ± 0.44	9.97 ± 0.21
Lydig cell count	10.97 ± 1.25	8.52 ± 0.82*	10.31 ± 0.92	9.40 ± 0.66*	8.85 ± 0.34*	10.12 ± .25
Primary and secondary Spermatoocytes count	46.11 ± 2.58	41.39 ±2.90*	44.78 ± 2.74**	45.18 ± 2.55	44.11 ±74**	47.09 ± .32
Seminiferous tubule diameters (µm)	4.85 ± 0.36	3.98 ± 0.47**	4.56 ± 0.33	4.11 ± 0.48**	4.02 ± 0.41	4.33 ± 0.18

Results are presented as Mean ± SD * $p < 0.001$; ** $p < 0.01$; ** $p < 0.001$

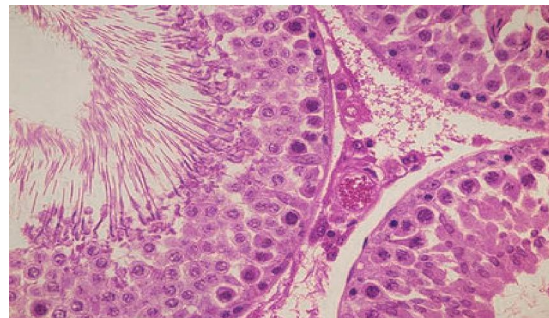
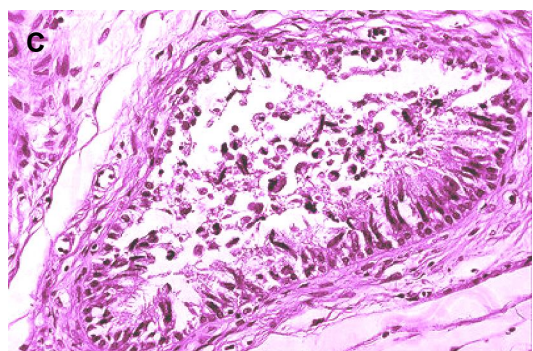
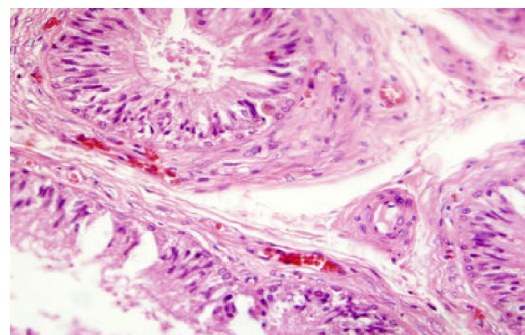
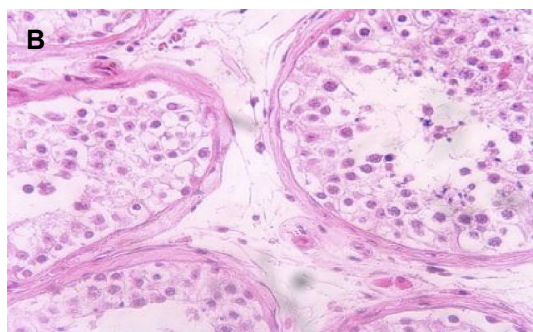
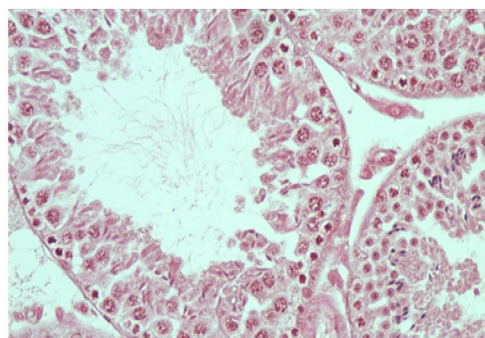
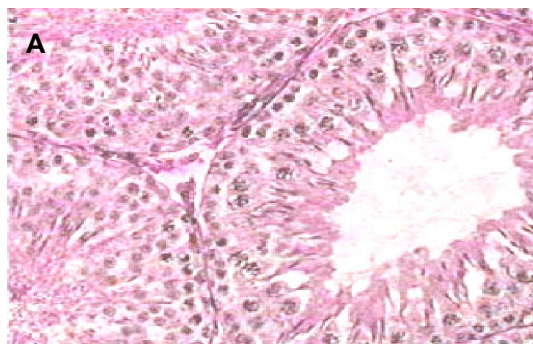


Fig 1: Testicular structure of the (A) control Rats showing normal and regular seminiferous tubules, (B) Octylphenol -treated group B (0.1 mg/kg) showing the pathologic effects in which the structures of the seminiferous tubules were severely damaged and (C) Octylphenol -treated group (1.0 mg/kg) in which the thickness of the tubular walls was reduced. The number of germinal cells was greatly decreased with a disturbance in their diameter. X400 (H&E Stain).

Fig 2: Testicular structure of the (A) Low dose octylphenol and honey rat group showing sustainable normal but irregular seminiferous tubules with large intercellular space, (B) High dose octylphenol and honey -treated group showing decrease pathologic effects in which the structures of the seminiferous tubules were slightly damaged with bleeding in intercellular space and (C) honey -treated group (20 mg/kg) showing normal and regular seminiferous tubules with normal diameter and mild dilatation of the seminiferous tubules with normal complete spermatogenic series. X400 (H&E Stain).

Oxidative stress has been postulated as one of the mechanisms leading to testicular damage following exposure to phenols (El-Dakdoky and Helal, 2007). Honey has been reported to have some antioxidants such as vitamins A and E (Al-Waili, 2003), catalase (Gheldof *et al.*, 2002) and flavonoids (Yao *et al.*, 2004). Recently, it was reported that honey contained antioxidant such as phenols and possess anti-radical and antioxidant properties (Mohamed *et al.*, 2010). Therefore, it is plausible to suggest that the effect of honey in attenuating the OP-induced impaired testicular functions in this study could be partly mediated by its counteraction on oxidative stress within rat reproductive organs via its antioxidant properties.

Thus, further study is needed to determine the levels of oxidative stress markers in the rat testis to support this hypothesis. Apart from that, the impaired testicular functions following the exposure to OP could also be due to hypoxia, which in turn might decrease blood flow and inhibit vasomotion in the testis (Koskinen *et al.*, 2000). Phenolic compounds in plants, termed phytoestrogens that possess oestrogenic activity (Ososki and Kennelly, 2003), are also found in honey (Gheldof *et al.*, 2002, Mohamed *et al.*, 2010).

Moreover, there is a strong correlation between plasma oestrogen level and testicular blood flow in male mammals suggesting that oestrogen may play a role in testicular perfusion (Bollwein *et al.*, 2008). Indeed, the presence of oestrogen receptors and aromatase, an enzyme that transforms androgens into oestrogens, in germ cells of the testis might suggest that locally produced oestrogen may also be involved in spermatogenesis (Carreau *et al.*, 2007). Consequently, it is also possible that honey could ameliorate the toxic effect of OP on testicular function partly by improving testicular blood flow and spermatogenesis via the oestrogenic activity of its phenolic compounds and this requires further study.

In conclusion, administration of honey significantly attenuated the detrimental effect of OP on the spermatogenesis in rats. This study indicates that honey has a protective effect against OP-induced impaired testicular functions in rats, but further research to elucidate its exact mechanism of action is essential.

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