



Original Investigation

The Influence of Excessive and Prolonged Ingestion of Honey on Sex Hormones and Prostate Specific Antigen in Adult Male Wistar Rats

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Abstract

This investigation was designed to determine the effects of excessive and prolonged administration of honey on the reproductive hormones and prostate specific antigen in adult male Wistar rats. A total of twenty five wistar rats weighing between 210 – 220g were used for the study. The animals were divided into 2 experimental groups of ten rats each (n=10) and 1 control group of five rats, (n=5). Experimental groups 1 and 2 rats were administered with 5ml/kg body weight and 7.5ml/kg body weight of bee honey through orogastric tube (gavage) thrice a week for 10weeks. The serum male sex hormonal profile and prostate specific antigen (PSA) levels were determined at the end of administration and after 10weeks of treatment rest and results compared with control. Results at the end of treatment revealed that excessive consumption of honey has no significant effects on serum levels of follicle stimulating hormone; however, it depressed serum levels of luteinizing hormone, testosterone and increased the serum levels of progesterone and prolactin. Higher dose (7.5ml/kg body weight) had significant increase on serum levels of prostate specific antigen (PSA). After a period of 10weeks rest the serum hormonal levels became significantly higher except testosterone that was significantly depressed. Excessive consumption of honey appeared to have a deleterious effect on serum levels of testosterone but enhanced the production of follicle stimulating hormone, luteinizing hormone and prostate specific antigen (PSA).

Keywords: honey, excessive, prolong, hormone, reproduction, wistar rats.

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Introduction

Since ancient times, honey has been used for its medicinal properties in many cultures [1]. Currently, information on the use of honey for the treatment of many human diseases can be found in magazines, bee keeping journals and natural products leaflets, suggesting a wide variety of unfounded properties. In contrast, medical reports supported by tests are few and far between [2, 3]. Honey is also considered a part of traditional medicine [4]. It is effective in the healing of wounds and burns and the treatment of diabetic ulcers, [5-7]. Honey is produced from many floral sources and its content and activity vary with its origin and processing technique. Histological studies of honey applied to wounds have been reported to be safe [8] as it reduces inflammation in deep and superficial [9] burns as well as in wounds [10]. At a concentration of 1%, it stimulates growth of monocytes in cell cultures to release cytokines, tumour necrosis factor (TNF)-alpha, interleukin (IL)-1 and IL-6, which activate the immune response to infection [11]. The proliferation of peripheral blood B-lymphocytes and T-lymphocytes in cell culture has also been stimulated by honey concentration as low as 0.1% [12]. The bacterial destroying activity of macrophages may have been assisted by the carbohydrate content of honey principally, glucose and fructose [13] and by its pH [14] which is between 3 and 4. Honey is said to have an inhibitory effect to several species of bacteria including aerobes and anaerobes, gram-positives and gram-negatives [15] and an antifungal action on some yeasts and species of *Aspergillus* and *Penicillium* [16], as well as some dermatophytes [17]. Wounds infected with *Pseudomonas*, showing resistance to several antibiotics, have been rapidly cleared of infection with honey and allowing successful skin grafting. Application of honey to open wounds has been reported to be soothing [18] to relieve pain [19], and with no adverse effects [20].

In many contexts, the two main classes of sex steroids (hormones) are androgens and oestrogens, of which the most important human derivatives are testosterone and estradiol, respectively. Other contexts will include progestagen as a third class of sex steroids, distinct from androgens and oestrogens. Progesterone is the most important and only naturally occurring human progestagen. In general, androgens are considered "male sex hormones", since they have masculinizing effects, while oestrogens and progestagens are considered "female sex hormones [21] although all types are present in each gender,

albeit at different levels. Prostate Specific Antigen (PSA) derives its name from the observation that it is a normal antigen of the prostate but is not found in any other normal or malignant tissue. It is found in benign, malignant and metastatic prostate cancer. Since prostate cancer is the second most prevalent form of male malignancy, the detection of elevated PSA levels plays an important role in the early diagnosis. Serum PSA levels have been found more useful than prostatic acid phosphatase (PAP) in the diagnosis and management of patients due to increase sensitivity [22].

The purpose of this study therefore, was to establish whether excessive intake of honey alter serum levels of male reproductive hormones and prostate specific antigen. The study also intended to reveal whether the effect, if any, would reverse on cessation of honey administration.

Materials and Methods

Twenty five adult male wistar rats were used for the study. The rats were collected from the Animal House of the College of Medicine, Ambrose Alli University, Ekpoma, Edo State Nigeria. They weighed between 210-220g and were acclimatised for two weeks, had access to water *ad libitum*. Feeds (growers mash) were obtained from Bendel Flour Mills Plc, Ewu Edo State and honey was purchased locally from M.C Super Market Ekpoma. The animals were caged separately for the purpose of identification. The experimental protocol for animal research was approved by the ethics committee of the College of Health Sciences, Delta State University, Abraka

Experimental Design

Animals: Twenty rats were used as experimental and five as control. They were divided into three groups of two experimental and one control. The experimental rats were administered with two varying doses of bee honey. Group 1 experimental animals were administered with 5ml/kg body weight of bee honey through orogastric tube (gavage) once a day twice weekly for 10 weeks.

Group 2 experimental rats were administered with higher dose of 7.5ml/kg body weight, once a day twice a week for the same duration. Group 3 serves as control.

At the duration of 10 weeks, five from each group of experimental and five controls were sacrificed by guillotine decapitation for hormonal and prostate specific antigen assays.

Hormonal and PSA Assays:

Blood samples were collected from the hearts of the sacrificed animals, centrifuged, then serum separated for the assays. The serum hormones consisting of testosterone, prolactin, follicle stimulating hormone, leutinizing hormone and progesterone were determined using reagent kits supplied by International Immuno Diagnostics (Foster City). Computerised radioimmuno assay machine reader stat fax 2100 model was employed in the assay. Also evaluated in the assay was prostate specific antigen (PSA). Serum levels for each of the hormones and PSA were obtained for experimental and control and compared.

Treatment Rest

The unsacrificed ten experimental rats, five from each group, were maintained with normal feed for 10 weeks then sacrificed and were processed for serum hormones and prostate specific antigen for possible reversal of influence.

Statistical Analysis All parameters studied of experimental and control groups were compared, using two-way analysis of variance (ANOVA) to test the observations.

Results

It was observed that there was significant increase ($p < 0.05$) in serum levels of prolactin, progesterone in both experimental groups and follicle stimulating hormone and testosterone in group 1 rats treated with lower dose of 5ml/kg body weight. No significant effect ($p > 0.05$) on serum testosterone levels in group 2 experimental rats treated with higher dose of 7.5ml/kg body weight at the end of treatment as compared with controls. On the other hand, there was significant decrease ($p < 0.05$) in the serum levels of luteinizing hormone, group 2 testosterone and follicle stimulating hormone as compared with control.

Comparison between the two experimental groups showed that serum levels of prolactin and luteinizing hormone were significantly higher in group 2 animals treated with (7.5ml/kg body weight) than group 1 treated with (5ml/kg body weight) while serum levels of progesterone and testosterone were higher in group 1 at end of administration.

The prostate specific antigen (PSA) level was significantly higher ($p < 0.05$) in group 2 experimental animals administered with higher dose (7.5ml/kg body weight) as compared

with control. There was also significant difference ($p > 0.05$) between experimental groups 1 administered with low dose (5ml/kg body weight) and control.

Table 1. ANOVA results of end of treatment on hormonal and prostate specific antigen (pas) assays

Hormonal Assay	Group 1	Group 2	Group 3	F- ratio	Exact Sig
FSH(miu/ml)	0.73± 0.12	0.16± 0.04	0.52± 0.19	23.62	0.001
LH (miu/ml)	0.06± 0.09*	0.24± 0.05*	3.94± 0.43	362.71	0.001
Testosterone (ng/ml)	0.98± 0.38	0.42± 0.05*	0.46± 0.07*	9.88	0.001
Progesterone (ng/ml)	12.84± 0.33	6.27± 0.66	2.59± 0.29	643.43	0.001
Prolactine (ng/ml)	6.29± 0.43	7.75± 0.49	2.40± 0.43	185.49	0.001
PSA (ng/ml)	0.41± 0.06	3.33± 0.05	0.07± 0.08	179.46	0.001

*The mean difference is not significant at 0.05 level, using Scheffe Post Hoc test.

FSH- follicle stimulating hormone

LH- luteinizing hormone

PSA-prostate specific antigen

FSH - Significant decrease in group 2 but significant increase in serum levels between experimental groups and control.

LH - Serum level of this hormone was significantly lower in the experimental groups as compared with control but no significant difference between experimental groups.

Testosterone - No significant difference in group 2 but significant increase in group 1 as compared with control.

Progesterone – There was significant increase in the plasma levels in the experimental groups. Group 1 level was significantly higher than 2.

Prolactin - Significant increase in plasma levels in the experimental groups as compared with controls.

PSA- Significant difference in plasma levels between experimental groups and control.

Post-Treatment Effect (Withdrawal)

The purpose of this second aspect of the study was to establish whether the effect of honey on the parameters studied would reverse, remain the same or become worse after a period of rest.

The effect on sex hormones did not reverse after cessation of treatment for ten weeks. It rather grew worse (progressive). Results of the reversal study showed astronomical increase in the levels of all the sex hormones as compared with the results of end of administration and control except testosterone level which was significantly less than the control as presented in the table below.

The serum level of prostate specific antigen (PSA) was significantly higher in group 2 than group 1 as compared with control.

Table 2: Showing hormonal and PSA assays after 10weeks of treatment rest.

Hormonal assay	Group 1	Group 2	Group 3	F- ratio	Exact sig
FSH(miU/ml)	26.20±1.87	11.02±1.41	0.52±0.19	450.95	0.001
LH(miU/ml)	16.79*±1.20	16.36*±0.77	3.94±0.43	357.12	0.001
Testosterone(ng/ml)	0.32±0.07	0.01±0.01	0.46±0.07	91.53	0.001
Progesterone(ng/ml)	15.01±1.15	0.23±0.23	2.59±0.29	644.07	0.001
Prolactin(ng/ml)	7.59±0.35	22.66±1.85	2.40±0.43	446.28	0.001
PSA(ng/ml)	0.66±0.084	12.39±0.43	0.07±0.85	3529.0	0.001

*The mean difference is not significant at 0.05 level, using Scheffe Post Hoc test.

Follicle Stimulating Hormone- Plasma levels of experimental groups were significantly higher than control. There was also significant difference between groups 1 &2, higher in group 1 than 2.

Luteinizing Hormone- Significantly higher in experimental groups than control.

Testosterone – Significantly lower in both experimental groups than control.

Progesterone- Group 1 experimental was significantly higher than control, but group 2 was significantly lower than control.

Prolactin – Levels were significantly higher in the experimental groups than control, but significantly higher in group 2 as compared with group 1.

Prostate Specific Antigen (PSA) –Significant difference in plasma levels between experimental groups and control.

Discussion

This study focused on the effect of excessive and prolonged administration of honey on the male reproductive hormones and prostate specific antigen (PSA) in rats. The study showed that excessive and prolonged administration of honey, an exogenous substance inhibits the production of testosterone in male wistar rats but promoted the production of follicle stimulating hormone, leutinizing hormone, progesterone and prolactin. Though serum levels of follicle stimulating hormone of the two experimental groups were significantly low at the end of treatment as compared with control but became significantly high after ten weeks of rest. It may be that honey suppressed secretion of this hormone during treatment but withdrawal removed the suppressive effect, and excess quantity was released to compensate the initial loss.

The results at the end of treatment showed no significant effect on the plasma level of follicle stimulating hormone (FSH), but there was significant increase after 10 weeks of treatment rest (withdrawal). It may be that honey takes long time to have effect on plasma follicle stimulating hormone production. On the contrary, there was significant decrease in the serum level of luteinizing hormone (LH) on the two experimental groups at the end of 10 weeks of administration but there was significant increase after 10 weeks of treatment rest as compared with controls. The effect of honey on these hormones was not made manifest at the end of 10weeks treatment but became manifest after 10weeks of rest. It is possible that honey has a latent period that lasted for more than 10 weeks before exerting effects on these hormones.

There was appreciable significant increase in plasma levels of testosterone in animals treated with lower dose of 5ml/kg body weight at the end of administration but reduced

significantly after 10 weeks of treatment rest as compared with control. This post treatment effect was more pronounced in group 2 experimental rats than group 1.

Honey caused significant increase in the plasma levels of progesterone and prolactin at the end of treatment and after treatment rest as compared with control group. The increase was also significant between the two experimental groups at the end of 10 weeks of treatment and 10 weeks after treatment rest. In general, there was significant increase in the serum levels of the sex hormones after 10 weeks of rest except testosterone whose values were significantly reduced in both experimental groups.

It is possible that honey stimulates the pituitary gland by altering the feedback mechanism to produce elevated quantities of leutinizing hormone, progesterone and prolactin. Honey might have altered the pituitary gland control mechanism which may have accounted for the elevated plasma prolactin level.

The astronomical increase in the serum levels of the reproductive hormones except testosterone after ten weeks of treatment rest is an indication that the effect continued after cessation of treatment. Since the female sex hormones were the ones on the increase and testosterone on the decrease, it could be deduced that excessive ingestion of honey has feminization tendency.

The dose dependent elevated plasma levels of prostate specific antigen (PSA) in experimental animals at the end of treatment and after treatment rest could be due to disease of the prostate, benign progressive prostate hyperplasia. The hyperplastic diseased cells of the prostate caused excess release of the enzyme into the blood stream. Prostate specific antigen (PSA) is a protein derived from the prostate. It is a specific test for diagnosis and monitoring treatment of prostate disease [23]. An elevated plasma PSA occurs in prostate cancer and is widely used as a screening test for the disease, though PSA is also elevated in benign hyperplasia and prostaticitis [24].

Comparison of results between the experimental groups at the end of treatment and after treatment rest revealed significant differences in the levels of some of the reproductive hormones and prostate specific antigen. These values were higher in treatment rest than end of treatment, suggesting that the effect was long lasting. The possibility of reversal of effect on some of these parameters after a long abstinence is doubtful. The damage may be permanent in some, while reversal may be possible in others.

Conclusion

This study has demonstrated that excessive and prolonged use of honey inhibits testosterone secretion but boost secretion of other sex hormones of male rats. However, further investigation should be conducted to support or contradict these findings.

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