EFFECTS OF HONEY ON PROSTATE GLAND FUNCTION AND SERUM TESTOSTERONE LEVEL IN ADULT MALE WISTAR RATS

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Abstract
Assessment of effects of honey on the prostate functions and testosterone level in adult male wistar rats at statistical significant of P<0.05. Twenty four adult male wistar rats were procured from the Department of Human Physiology, College of Medicine, Abia state University, Uturu, Abia state, Nigeria. They were ethically and scientifically handled. Groups A, B and C received 0.15ml/Kg Body Weight(KBW), 0.30ml/KBW, 0.60ml/KBW of honey respectively, once daily. Group D was control. Three rats were selected at random after 4 weeks. The blood was collected for serum testosterone, Prostatic Acid Phosphatase (PAP) and Prostate Specific Antigen (PSA) levels. The prostate gland was preserved for histomorphology. The result showed significant correlation between honey and testosterone, PSA and PAP serum levels after four weeks. There was significant increase in serum testosterone and PSA after 4 weeks in groups B and C. There was significant increase in serum PAP after 4 weeks in group C. There was significant difference comparing 4 weeks and 8 weeks serum levels of testosterone in groups B and C. After 4 weeks, there was statistically significant increase in the relative prostate weight. Honey intake could be regarded as risk factor for Benign Prostate Hyperplasia and Cancer of Prostate.

Key Words: *Honey, Prostate Gland, Serum Testosterone.*

Introduction

The prostate is a combine tubuloalveolar organ. Characteristically the gland is lined by two strata of cells; a basal strata of low cuboidal epithelium enclosed by a strata of columnar secretory cells. In adult man, prostatic parenchyma can be separated into four naturally and anatomically discrete zones or regions; the marginal, central and transitional zones and the region of the anterior fibromuscularstroma(1). Three pathologic developments that frequently involve the prostate gland include; inflammation or prostatitis, (Acute and chronic bacterial and chronic abacterial and granulomatous) (2, 3), benign nodular enlargement or Bening Prostate Hyperplsia (BPH) and carcinomas. Benign nodular enlargement or nodular hyperplasia also referred to as Benign Prostatic Hyperplasia (BPH) is an enormously frequent ailment in men above 50 years of age (4, 5). Incidence is about 20% among men of 40 years, which increases to 70% by age of 60 years and 90% by age of 70 years. It is the commonest urologic disease suffered by elderly and one of the commonest chronic diseases of males (6, 7). In Nigeria, it was testified that 25% of men greater than 40 years presents with symptoms indicative of BPH (8).

Testosterone is metabolized to Dihydrotestosterone (DHT) which is the definitive moderator of prostate development. Dihydrotestosterone is produced in the prostate from peripheral blood testosterone by the action of the enzyme 5α-reductase type 2, mainly restricted in the stromal cells or in paracrine fashion by spreading into surrounding epithelial cells. DHT is ten times more active than testosterone which can also stimulate growth action because DHT separates from the androgen receptors more slowly. It's worthy of note, that nodular hyperplasia are not regarded to be a premalignant lesion. Risk factors include; age, race, family history, hormone levels and environmental effects are assumed to contribute (9, 10). Suggestions are for screening for prostate cancer should start at the age of 40 years and again at 45 years for both those men at higher risk and those not at risk. Proposed primary screening test for prostate cancer is the analysis of serum prostate specific antigen (PSA) levels. Other methods of screening such as Digital Rectal Examination or ultrasonography are secondary (11).

Honey is a biological produce known for its various biological or pharmacological activities spanning from; antioxidant, anti-inflammatory, antibacterial, anti-hypertensive to hypoglycemic effects (12). It is also revealed that honey has anti-emetic, anti-proliferative and anti-cancer as well as anti-infertility effects by enhancing testosterone secretion in males (13). Honey is a natural produce of bees made from nectar obtained from flowering plant. It's abundant nutrient content, includes; sugars such as fructose and glucose, minerals such as magnesium, potassium, calcium, sodium chloride, sulphur, iron and phosphates, as well as vitamins B1, B2, B6, B5, B3 and C (14). Honey can also be seen as a natural produce of bees and it comprise various compounds such as carbohydrates, normal minerals, proteins, vitamins, organic acids, enzymes and antioxidants such as catalase, peroxidases, alkaloids, polyphenols and flavonoids (15).

Its beneficial effects in male reproductive performances have been recorded by some researchers, where it enhanced spermatogenesis leading to increase in sperms. However, elevated sperm cell counts have been observed in rats administered with tualang honey over a period of time. The testosterone levels were observed to increase too and these results were probably due to the antioxidant protective influence of tualang honey (16).

Prostatic Acid Phosphatase (PAP) was mainstay of prostate cancer diagnosis before introduction of Prostate-Specific Antigen (PSA) that enhanced revealing of early stage of prostate cancer and majorly displaced PAP. Presently, there is rekindled interest in PAP because of success in the immunotherapy of prostate cancer (17). PSA is more sensitive compared to PAP in diagnosis. However, the use of PSA had also caused "over-diagnosis" or "pseudo-disease" and excessive treatment of prostate cancer (18, 19, 20). Renewed interest on PAP is also based on the fact that PAP has significantly higher correlation with prostate cancer

progression (21). PAP seems timely in re-evaluation during therapy (22).

Prevalence of prostatic cancer and BPH are high in Nigeria. Awareness of prostate cancer is poor among Nigerian men (23, 24). Majority present in the hospital at already advanced stages (25). Few researches on honey revealed that it increases secretion of testosterone which can leave a man at risk of BPH or Cancer of Prostate. Hence, this study was to determine, the influences of different doses and duration honey intake on the serum levels of testosterone, Prostate Specific Antigen, Proastatic Acid Phosphatase and Histomorphology of prostate gland in adult male wistar rats, which can be extrapolated to man.

Materials and Methods

Twenty four adult male wistar rats (age ≥ 3 months), weighed 88–120 gram were procured from the animal house of Department of Human Physiology, College of Medicine, Abia state University Uturu, Abia state, Nigeria. The rats were acclimatized for two weeks, fed *ad libitum* with guinea® feed rat pellets and water at animal house Anatomy Department University of Nigeria Nsukka. The animals were exposed to 12 hours day light and 12 hours darkness. Ethical approval was obtained from Faculty of Veterinary Medicine, University of Nigeria (FVM-UNN-IACUC-2019-0249). The rats were shared into 4 groups of 6 rats. Group A received half of normal dose of honey (0.15ml/Kg body weight) once daily. Group B received normal dose (0.30 ml/Kg body weight) once daily which is normal recommended dose.[13] Group C received double dose (0.60 ml/Kg body weight) once daily. Group D served as control.

Three rats were randomly selected and sacrificed after 4 weeks intervals of intervention from each group. The blood was collected, after over-night fast through cardiac puncture, then transferred into a plain tube. The blood was allowed to clot and retract then, serum collected for biochemical analyses.

The prostates were dissected out, weighed and preserved in 10% neutral buffered formal saline for histomorphology.

Biochemical analyses included serum Prostate Specific Antigen (PSA), Prostatic Acid Phosphatase (PAP) and testosterone, were done in a chemical pathology laboratory. The analyses were done in triplet and result reported as mean ± SEM.

The honey was procured from a trusted dealer at Uturu, Abia state Nigeria. The honey was sent to Botany Department for confirmation.

The PAP was carried out using RANDOX® kit in a spectrophotometer.

The PSA and Testosterone were estimated using MONOBOND® kit in Microplate using Enzyme Immunoassay technique and read with Colourimeter.

The prostate tissues were sent to a Histhopathology Laboratory for paraffin section tissue processing and stained following standard Haematoxylin and Eosin (H and E) staining technique. Slides were sent to cellular Pathology Scientist and Pathologist for microscopy and photomicrography.

The results were analysed using Pearson correlation coefficient (r) and Student's T-test at statistical significance level, $P < 0.05$ with Microsoft Excel.

Results

Table one revealed that after 4 weeks, there was highly significant higher testosterone level among double dose treated group compared to control and significantly higher testosterone level among normal dose treated compared to control group. There was no significant higher testosterone level comparing half dose and control group.

After 8 weeks, there was no significant higher testosterone level among all the treated compared to control group.

Comparing intra-group results of 4 and 8 weeks, there was highly significant increase in testosterone

level in the control and normal dose groups and significant increase in double dose group. There was no significant increase in half dose group.

Table 2 showed that after 4 weeks, there was significantly higher PSA level among normal dose and double dose compared to control group. After 8 weeks, there was no significantly higher PSA level among all the treated groups compared to control group.

Intra-group comparison after 4 and 8 weeks of treatment showed very highly significant increase in PSA level among all the groups.

Table 3 revealed that after 4 and 8 weeks treatment, there was no significantly higher PAP level comparing all the treated and control groups.

Intra-group comparison after 4 and 8 weeks treatment revealed no significant increase among all the groups.

Table 4 revealed that after 4 weeks, there was highly significant higher Relative Prostate Weight (RPW) in half and double dose compared to control group and significantly higher RPW normal dose compared to control.

After 8 weeks, there was no significantly higher RPW among all the treated groups compared to control group.

Comparing intra-group results after 4 and 8 weeks, there was no significant difference in RPW in all the groups.

Table 5 revealed that after 4 weeks, there was significantly higher percentage body weight increase (PBWI) among double dose treated compared to control group, but no significantly higher PBWI in half dose and normal dose treated compared to control group.

After 8 weeks there was no significant difference in PBWI in all the treated groups compared to control.

Intra-group comparison after 4 and 8 weeks revealed very highly significant increase in half dose

treated and significant increase in control group. There was no significant increase in PBWI in normal and double dose groups.

Table 6 showed that after 4 weeks, there was significant correlation between honey intake and the following parameters; testosterone, PSA and PAP serum levels. There was no significant correlation between honey and RPW and PBWI. There was significant correlation between testosterone and PSA level. There was no significant correlation between testosterone level and the following; PAP, RPW and PBWI.

After 8 weeks, there was highly significant correlation between honey and RPW and significant correlation between honey and the following; testosterone and PAP level. There was no significant inverse correlation between honey intake and PSA and PBWI. There was significant inverse correlation between testosterone and PBWI. There was no significant correlation between testosterone level and PSA and PAP levels after 8 weeks.

Figure 1 was Heamotoxylin and Eosin stained sections. They revealed that after 4 weeks and 8 weeks, almost all of the prostate gland acini in control groups (slides A and E) contained eosinophilic secretions. Slide of the treated groups after 4 weeks (slides B, C and D) and after 8 weeks (slides F, G and H) showed mixture of few eosinophilic secretion retaining acini and majority of empty acini or generally empty acini of the prostate sections.

Discussion

The result of present study showed that after 4 weeks, honey significantly increased the secretion of testosterone comparing the control and the treated groups. There was also significant correlation between honey and testosterone secretion. This is in accordance with previous studies which revealed that honey enhances fertility and vitality among males in some populations (13). The result also agreed with previous reports that, 'consumption of honey helped to maintain testosterone while lowering estrogen level', though it's for short term. This was achieved because of

flavonoids, specifically chrysin. Chrysin inhibits aromatase enzyme activity, which catalyze the conversions of androstenedione and testosterone into estrone and estriol respectively, hence increases testosterone level (26, 27).

After 8 weeks, there was no significant correlation between honey and testosterone secretion among the control and treated groups. There was no significant decrease in group-A compared to control group. This might be in accordance with the previous study, where authors' treatment was for 10 weeks using 5ml/Kg BW and 7.5ml/Kg BW. They concluded that excessive and prolonged consumption of honey depressed serum level of testosterone and luteinizing hormone but increased serum level of PSA at higher dose (28). This could be due to saturation point or equllibrium or negative feed-back mechanism effects of honey and testosterone.

The result of our research after 4 weeks revealed that PSA significantly increased, respectively with increase in concentration of honey among treated groups compared to control. There was also significant correlation coefficient between honey and PSA. The result also showed significant correlation between testosterone level and PSA level. This agreed with teach=also agreed with definitive treatment for prostate cancer, medical orchiectomy or surgical orchiectomy to prevent testosterone secretion (19). Present study helped to answer the suggestions by some researchers, who reported that no strong evidence had proved the role of testosterone in the occurrence and progression of prostate cancer, so they requested for further investigation on the association between testosterone and prostate cancer.

Result of this present study after 4 weeks revealed that Prostatic Acid Phosphatase (PAP) secretion also significantly increased relatively in honey treated rats with significant correlation. Testosterone increased the activity of the prostate gland in treated groups, as also shown on the histomorphology studies of prostate where the control rats contained more secretions unlike treated group that had released their secretions into the blood.

After 4 weeks of treatment, there was significant increase in RPW comparing the control to all the treated groups which signified that honey increased prostate enlargement and functions, unlike after 8 weeks, when there were no significant increase comparing treated groups to control. Though, prior study concluded that honey didn't significantly changed the body weight and male reproductive organ weights (16).

After 4 weeks of treatment, the significant increase in the RPW coincided with the significant increases in PBWI in group C and significant correlation coefficient between honey and increased PBWI. This was in accordance with reports where the authors, in their study concluded that prohormones of testosterone; androstenedione, dehydroepiandrosterone and androstenediol were promoted to exert the same androgenic influences on building muscle mass and strength as does anabolic-androgenic steroids (29). However, some authors concluded that the use of testosterone as anabolic agent to skeletal muscle were made based on assumption (not necessarily evidence-based process) that growth hormone and testosterone enhance anabolic mechanisms that lead to skeletal muscle protein accumulation and hypertrophy. They concluded that local systems that are basic to the skeletal muscle tissue performing resistive contraction (that is weight lifting) are predominant in stimulating anabolism (30).

After 8 weeks, there was significant inverse correlation between testosterone and PBWI. Increased testosterone level led to none significant reduction in weight of treated groups compared to control groups. This is supported by the report of prior studies, where they concluded that after 30 days intake of honey by over-weight and obese, there was mild reduction in body weight (1.3%) (31). An author concluded that prolonged testosterone intake in men suffering from testosterone deficiency produced remarkable and sustained weight loss, obvious reduction in waist circumference and body mass index and better in body composition (32).

Conclusions

Conclusions from present study were; that after 4 weeks, that was short term intake of honey, increased the secretion of testosterone and relative prostate weight. Honey also increased prostate function after 4 weeks showed by increased PSA and PAP serum levels and relative prostate weight. After 4 weeks honey caused increase in body weight.

After 8 weeks, that was long term intake of honey, had no effect on secretion of testosterone, PSA and PAP. After 8 weeks, honey caused reduction in PBWI.

Honey intake can be regarded as a risk factor for Cancer of Prostate and Benign Prostatic Hyperplasia.

We recommend that prostate patients or those at risk should take honey with caution or avoid the intake of honey. We can carry out the study on "honey intake of prostate patients". We recommend PSA and PAP check for males who take honey regularly. We recommend that the effect of honey on prostate gland be carried out on human participants. Also effects of honey should be carried out on prostate disease patients and compared with none patients. We also recommend that this study be done for a longer duration. Specific effects of different components of honey can be studied.

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TABLE 1: EFFECTS OF HONEY ON SERUM TESTOSTERONE LEVEL (ng/ml)

	4 WEEKS		8 WEEKS		4WKS V 8WKS
CONTROL	0.24±0.03		4.01±1.10		0.01**
0.30 ml/Kg BW	2.44±0.84	0.04*	5.28±0.17	0.15	0.01**

P< 0.05 = * (significant)
P< 0.01 = ** (highly significant)
P< 0.001 = *** (very highly significant)

TABLE 2: EFFECTS OF HONEY ON SERUM PROSTATE SPECIFIC ANTIGEN LEVEL (ng/ml)

	4 WEEKS		8 WEEKS		4 WKS V 8 WKS
CONTROL	0.21±0.01		4.10±0.13		0.001***
0.30 ml/Kg BW	0.77±0.18	0.02*	4.10±0.27	0.50	0.0002***

P< 0.05 = * (significant)
P< 0.01 = ** (highly significant)
P< 0.001 = *** (very highly significant)

TABLE 3: EFFECTS OF HONEY ON SERUM PROSTATIC ACID PHOSPHATASE LEVEL (IU/L)

	4 WEEKS		8 WEEKS		4 WKS V 8 WKS
CONTROL	0.52±0.02		0.92±0.45		0.33
0.30 ml/Kg BW	0.65±0.17	0.25	0.79±0.24	0.28	0.59

P< 0.05 = * (significant)
P< 0.01 = ** (highly significant)
P< 0.001 = *** (very highly significant)

TABLE 4: EFFECTS OF HONEY ON MEAN RELATIVE PROSTATE WEIGHT (%)

	4 WEEKS	8 WEEKS	4 WKS V 8 WKS
CONTROL	0.23±0.03	0.07±0.06	0.055
0.30 ml/KgBW	0.37±0.26 0.022*	0.40±0.62 0.349	0.124

P< 0.05 =*(significant)
P< 0.01 =** (highly significant)
P< 0.001=***(very highly significant)

TABLE 5: EFFECTS OF HONEY ON THE PERCENTAGE BODY WEIGHT INCREASE

	4 WEEKS		8 WEEKS		4 WKS V 8 WKS
CONTROL	21.2±8.1		98.2±18.8		0.03*
0.30 ml/Kg BW	36.4±12.1	0.119	88.8±22.8	0.352	0.084

P< 0.05 = * (significant)
P< 0.01 = ** (highly significant)
P< 0.001 = *** (very highly significant)

TABLE 6:   **VALUES OF CALCULATED CORRELATION COEFFICIENT**

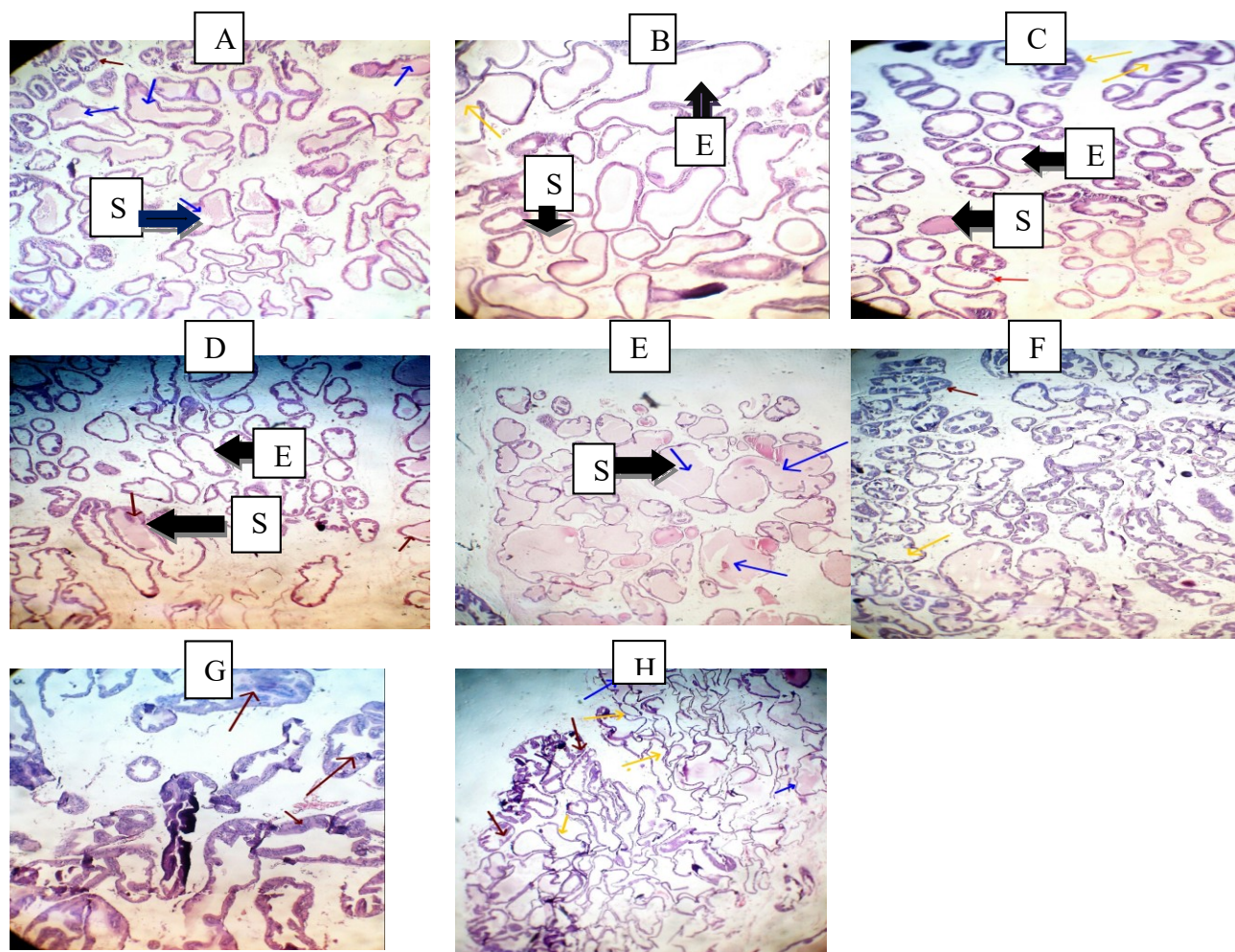|	H Vs T	H Vs PSA	T Vs PSA	H Vs PAP	T Vs PAP	H Vs RPW	T Vs RPW	H Vs BDY WT
AFTER 8 WKS	r =0.576 P > 0.05	r = -0.317 P > 0.05	r = 0.592 P > 0.05	r = 0.156 P > 0.05	r=0.215 P > 0.05	r = 0.994 P<0.005[**]	r = 0.663 P > 0.05	r= -0.378 P > 0.05

Figure 1: X400. Histomorphological photomicrograph of H and E staining of the prostate tissues from experimental male rats.

Slides A, B, C and D were from control, half dose, normal dose and double doses respectively after 4 weeks. Slides E, F, G and H were from control, half dose, normal dose and double doses respectively after 8 weeks.

Slides A and E showed that majority of acinus from control group after 4 and 8 weeks retained eosinophilic secretions and few or no empty acinus. Sldes B, C, D, F and G from treated groups showed mainly empty or mixture of empty acinus and few acinus retaining eosinophilic secretions.

Arrows labelled S were acinus containing eosinophilic secretions; arrows labelled E were emptied acinus.