

Original Article

Protective Role of Nigerian Honey on Sperm indices and Testis in Sucrose-Fed Rats

Oyelowo OT¹, Adekunbi DA², Dada KA¹

Abstract

Objective: This study aimed at investigating the effect of high sucrose diet on male reproductive function and if Nigerian honey could exert a protective role. **Methods:** Twenty-four (24) rats were randomly divided into four equal groups of six animals and given water (control); honey (H); high sucrose solution (30%w/v) (HSS); and both high sucrose solution (30%w/v) and honey (HSS+H). Each rat on honey received a daily dose of 10ml honey/kg/5ml of distilled water. Food intake, body weight, organ weight, fasting blood glucose, LH, FSH, testosterone and sperm functions were assessed. **Results:** This revealed that sperm motility ($p < 0.05$) and count increased in the HSS+ H and H- fed rats compared with HSS fed and control rats. Head and tail abnormalities sperm were also significantly reduced in the H fed rats ($p < 0.05$). MDA level in the liver and not in the testes was significantly increased in the HSS fed rats compared with control while SOD activity was significantly increased in HSS+H rats compared with the HSS fed rats. **Conclusion:** The results indicate that sucrose feeding impact negatively on sperm function while Nigerian honey supplementation confers protective function on male reproduction.

Key words: Nigerian honey, sucrose, sperm indices, antioxidants,

DOI: <http://dx.doi.org/10.3329/bjms.v13i2.18301>

Bangladesh Journal of Medical Science Vol.13(2) 2014 p.180-189

Introduction:

The available information on the effect of high sugar on male reproductive function is inadequate, though consumption of sweetened foods is not uncommon in many geographical areas of the World^{1, 2}.

As a reflection of global changes in dietary behavior, the prevalence of unhealthy diets characterized by high intake of food rich in fats and sugar has increased in women and men within the reproductive age range³. Indeed, lifestyle factors arising from unhealthy dietary habit exert a profound influence on reproductive function⁴. A number of studies have shown that various physiologic processes which include reproductive function can be affected by dietary manipulation. For instance, a high fat diet has been shown to impact negatively on both male and female reproductive function^{5, 6}.

Meanwhile, the possibility of a high sugar diet affecting reproductive function has not been verified experimentally to the best of our knowledge although consumption of a high-sugar diet has been associated with the development of obesity, insulin resistance, diabetes, dyslipidemia, fatty liver and high blood pressure⁷.

For a long time, honey has been the only available sweetener until its replacement by industrial sugar after 1800⁸. Honey, a natural mixture of fructose, glucose, and more complex sugars⁹ is not associated with the same metabolic effect as sucrose, thus its nutritional benefits^{10, 11}. The physiological importance of honey includes its antioxidant, anti-inflammatory, antibacterial and antitumor activity among others^{12, 13, 14, 15}. As far as reproduction is concerned, honey from different parts of the world has increased

1. Oluwakemi Tinuolaoluwa Oyelowo, Department of Physiology, College of Medicine of the University of Lagos, Lagos Nigeria;
2. Daniel Abiodun Adekunbi, Department of Physiology, Benjamin Carson (Senior) School of Medicine, Babcock University, Ilishan-Remo;
3. Kayode A Dada, Department of Physiology, College of Medicine of the University of Lagos, Lagos Nigeria.

Corresponds to: Oluwakemi Tinuolaoluwa Oyelowo (Mrs), Department of Physiology, College of Medicine of the University of Lagos, Lagos Nigeria; E-mail address: pinkkemi@yahoo.com

the sperm count in rats and monkeys and increased vaginal wall epithelium and muscle thickness, without showing any effect on circulating gonadotropins or testosterone¹⁶. Honey has also been reported to enhance spermatogenesis in rats if given at the appropriate dose¹⁷ and to reduce the toxic effects of cigarette smoke on spermatogenesis¹⁸.

In lay press, sucrose has been assumed to have negative implications on the male reproduction. With this background, this study attempts to investigate whether high sucrose intake will impact negatively on the male reproductive function and if Nigerian honey could exert a protective role.

Materials and Methods:

Honey Sample

Honey was obtained from a reliable supplier in Oyo State who freshly collected honey from sealed honeycombs, and the honey was certified pure¹⁴. Each rat on honey received a daily dose of 10ml honey/kg/5ml of distilled water¹⁹ by oral gavage.

Animals

Male Sprague-Dawley rats weighing 120-150g were obtained from the Laboratory Animal facility of the College of Medicine of the University of Lagos. Animals were housed in plastic cages with *ad libitum* access to commercial rodent pellet diet (Livestock Feed, Lagos, Nigeria). The light cycle was maintained at 12 h of dark, followed by 12 h of light. The room temperature was maintained at 24±2 °C and humidity of 50% - 64%. The rats were allowed to acclimatize for a period of two weeks before the commencement of the experiment. All animal handling and experimental protocols adopted in this study complied with the international principles for laboratory animals as obtained in the Helsinki's Declaration.

Study Design

Twenty-four (24) rats were randomly divided into four groups of six animals each. The groups are as follows:

Control: was administered water;

H- was administered honey once daily by oral gavage and treatment lasted for 4 weeks ;

HSS- had free access to high sucrose solution (HSS) (30 % w/v) as drinking water according to the method of ²⁰ with slight modification and treatment lasted for 4 weeks;

HSS+H- had free access to high sucrose solution (HSS) (30 % w/v) as drinking water as well as

administration of honey (H) once daily by oral gavage and treatment lasted simultaneously for 4 consecutive weeks.

Determination of food intake, body and organ weights

Food intake was measured at 0800hrs daily. Body weight was determined once a week using a digital weighing scale. On the last day of administration, the reproductive organs; testis, caudal epididymis, seminal vesicle and prostate gland were weighed.

Blood glucose measurement

The experimental rats were fasted overnight before blood samples were collected via tail tipping for blood glucose measurement at the 4th week of treatment. The glucose levels were measured using a blood glucose monitoring system (Accu-Chek Glucometer, Roche, Germany).

Sperm Analysis

The testes from each rat were carefully exposed and one of them was removed together with its epididymis. The epididymis was separated and the epididymis fluid was collected while the progressive sperm motility, sperm count and sperm viability was determined²¹.

Hormonal Assay

Testosterone, Luteinizing hormone (LH) and Follicle-Stimulating Hormone (FSH) in rat serum were measured by enzyme immunoassays using commercially available kits from Endocrine Technologies (USA), Uscnlife (China) and Biocodex (Belgium), respectively. Samples were run in the same assay to avoid inter-assay variations.

Lipid peroxidation level

As a marker of lipid peroxidation, the level of malondialdehyde (MDA) in the liver and testes was measured by the method of ²² as thiobarbituric acid reactive substances (TBARS). The development of a pink complex with absorption maximum at 535nm is taken as an index of lipid peroxidation.

Antioxidant activities

Oxidative analyses of the liver and testes were carried out using previously described standard methods. The reduced glutathione (GSH) was determined using the method described by Van Dooran²³. The GSH determination method is based on the reaction

of Ellman's reagent 5,5' dithiobis (2-nitrobenzoic acid) DNTB) with the thiol group of GSH at pH 8.0 to produce 5-thiol-2-nitrobenzoate which is yellow at 412nm. Activity of the superoxide dismutase (SOD) was also determined according to the method described by Sun and Zigman²⁴. The reaction was carried out in 0.05m sodium carbonate buffer pH 10.3 and was initiated by the addition of epinephrine in 0.005N HCl. Catalase (CAT) activity was determined by measuring the exponential disappearance of H₂O₂ at 240nm and expressed in units/mg of protein as described by Aebi²⁵. Absorbance was recorded using Shimadzu recording spectrophotometer (UV 160) in all measurements.

Statistical Analysis

Data are expressed as mean ± standard error of mean (SEM) and analysed using the ANOVA followed by SNK post-hoc test. P < 0.05 was accepted as significant. All the analyses were carried out using the GraphPad InStat Version 3.05 for Window Vista, GraphPad Software, San Diego California, USA.

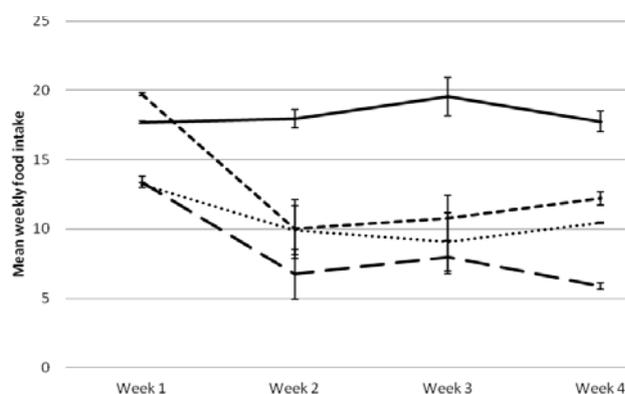


Figure I: Food intake of rats following administration of treatment for four weeks

Body weight

The body weight was measured and recorded weekly as shown in Table I. The H-fed rats had a significant increase (P<0.05) in body weight at week 1, 2 and 4 of treatment when compared with the control and HSS group as indicated.

Table I: Weekly body weight of the experimental groups

	CONTROL	HSS	HSS +H	H
WEEK 1	97.0 ± 5.3	107.6 ± 2.5	119.8 ± 9.6	127.6 ± 7.9 *
WEEK 2	117.4 ± 7.9	114.6 ± 4.2	126.0 ± 15.5	146.0 ± 9.4 * #
WEEK 3	119.8 ± 10.4	130.4 ± 6.5	154.7 ± 15.3	146.0 ± 12.2
WEEK 4	137.3 ± 12.3	133.2 ± 9.2	159.7 ± 17.9	164.0 ± 12.3 * #

Results:

Food Intake

The food intake of all rats measured and recorded from week 1 to 4 was shown in Figure 1. At the first week of study, HSS fed rats had reduced food intake (P<0.05) compared with control. Meanwhile, the H fed rats had a significant increase in food intake compared with the other experimental groups. The food intake in the HSS fed rats at week 2 was also significantly reduced compared with control, while H fed rats maintained an increase in food intake when compared with HSS and HSS+H fed rats. However, at week 3, both the HSS and the H fed rats had a significant reduction in food intake compared with control. At week 4, the HSS fed rat still had a significant decrease in food intake when compared with control, but this was however significantly lower (P<0.05) than the HSS+H fed rats. The food intake was reduced (P<0.05) in the H fed rats compared with control, nevertheless, the food intake was significantly higher than that of the HSS and HSS+H fed rats.

Values are Mean±SEM; n=5. *-compared with control; # -compared with sucrose P < 0.05, compared to control .HSS- high sucrose solution, HSS+H- high sucrose solution+ honey, H- honey

Fasting blood glucose

Table II shows the fasting blood glucose of the experimental rats taken at the end of the 4-week treatment. Sucrose and honey consumption did not result in any significant change in the blood glucose of the experimental rats for all comparison.

Table II: Fasting blood glucose (FBG) of the experimental rats

	CONTROL	HSS	HSS+H	H
Fasting Blood Glucose (mg/dl)	112.5 ± 2.5	104.7 ± 6.5	120 ± 15.0	109.7 ± 8.5

Values are Mean±SEM; n=5. P > 0.05, compared to control .HSS- high sucrose solution, HSS+H- high sucrose solution+ honey, H- honey

Reproductive Organ weights

Table III shows the weight of the reproductive organs recorded at the end of the experiment. The weight of the testes and seminal vesicle was significantly increased (P<0.05) in HSS+H and H- fed rats compared with HSS fed rats. The weight of the epi-

didymis was significantly increased in the H-fed rats compared with HSS fed rats.

Table III: Reproductive organ weights (g) of rats following administration of treatments for four weeks

	CONTROL	HSS	HSS+H	H
Fasting Blood Glucose (mg/dl)	112.5 ± 2.5	104.7 ± 6.5	120 ± 15.0	109.7 ± 8.5

Values are Mean±SEM; n=5. # P < 0.05, compared to HSS .HSS- high sucrose solution, HSS+H- high sucrose solution+ honey, H- honey

Sperm indices

Epididymis sperm count and motility were significantly reduced (P<0.05) in the HSS fed rats compared with control, while, the sperm count and motility were significantly increased (P<0.05) in the H fed rats and HSS+H fed compared with the HSS rats (Table IV). The Epididymis volume was not significantly different across treatment groups.

The most common abnormalities encountered during the morphological examination of the sperms was the ‘curved tail’ and ‘curved mid-piece’ in the rats that received the HSS treatment. The H group however showed fewer occurrences of the morphological aberration as recorded in Table V.

Table IV: Sperm indices of rats following administration of treatments for four weeks

	CONTROL	HSS	HSS+H	H
Motility (%)	86.30 ± 5.54	68.00 ± 6.63 *	91.70 ± 1.67 #	90.00 ± 3.54 #
Live/Dead ratio	96.50 ± 0.87	92.20 ± 3.38	97.00 ± 1.00	96.50 ± 0.87
Epididymal Volume	5.18 ± 0.03	5.18 ± 0.02	5.17 ± 0.03	5.15 ± 0.03
Count	121.25 ± 5.38	96.5 ± 3.93 *	120.67 ± 1.76 #	131.00 ± 5.82 #

Values are Mean±SEM; n=5. *-compared with control; # -compared with sucrose P < 0.05. HSS- high sucrose solution, HSS+H- high sucrose solution+ honey, H- honey

Table V: Sperm abnormalities of rats following administration of treatments for four weeks

Sperm variables	CONTROL	HSS	HSS+H	H
Tailless Head	4.3 ± 0.75	4.4 ± 0.60	4.7 ± 0.76	4.8 ± 0.63
Headless Tail	4.5 ± 0.65	4.6 ± 0.68	4.3 ± 0.88	4.3 ± 0.63
Rudimentary Tail	1.8 ± 0.48	1.8 ± 0.58	2.0 ± 0.58	1.8 ± 0.48
Bent Tail	11.3 ± 0.86	10.8 ± 1.16	10.7 ± 0.67	9.3 ± 1.11
Curved Tail	11.8 ± 0.25	11.0 ± 0.89	8.7 ± 0.33	9.0 ± 0.71*#
Curved mid-piece	11.0 ± 0.41	11.2 ± 0.97	9.7 ± 0.33	8.3 ± 0.25*#
Bent mid-piece	10.3 ± 0.25	9.8 ± 0.49	8.3 ± 0.88	9.0 ± 0.82
Looped Tail	1.8 ± 0.48	1.8 ± 0.37	2.0 ± 0.58	1.8 ± 0.48
Total No of normal sperm	406.3 ± 3.15	405.0 ± 2.23	401.7 ± 1.67	405.0 ± 2.04
Total No of abnormal sperm	56.5 ± 2.67	55.4 ± 1.83	50.3 ± 1.20	48.5 ± 1.66*
% of abnormal cells	13.9 ± 0.57	13.6 ± 0.46	12.4 ± 0.44	11.9 ± 0.49*

Hormonal Analysis

The Luteinizing Hormone (LH) level was significantly reduced (P<0.05) in the H fed rats compared with control and HSS fed rats. There was no significant difference in LH level in the HSS rats compared with control. The HSS+H fed rats however, had a significant reduction in LH level compared with the HSS fed rats. The H fed rats had a significantly reduced (P<0.05) Follicle-Stimulating Hormone (FSH) level compared with control. However the testosterone level of the H fed rats was significantly higher than in the control as well as the HSS rats (Table VI).

Table VI: Hormonal levels of rats following administration of treatments for four weeks

	CONTROL	HSS	HSS+H	H
LH (iu/l)	0.87 ± 0.06	0.78 ± 0.12	0.34 ± 0.3 #	0.3 ± 0.14* #
FSH (iu/l)	2.21 ± 0.24	1.47 ± 0.58	0.86 ± 0.64	0.54 ± 0.34 *
TESTOSTERON	18.4 ± 3.6	21.9 ± 5.1	22.85 ± 1.15	27.55 ± 8.05 * #
E (nmol/l)				

Values are Mean±SEM; n=5. *-compared with control; # -compared with sucrose P < 0.05. HSS- high sucrose solution, HSS+H- high sucrose solution+ honey, H- honey

Malonydialdehyde (MDA) estimation and antioxidant activity in the testis

There was no significant difference observed in MDA level in the testes when compared with control. The Superoxide dismutase (SOD) activity as well as the GSH level was not significantly different in the testes of all the experimental rats. Catalase activity (CAT) was significantly reduced (P<0.05) in the testes of HSS fed rats compared with control while the H fed rats had a significantly increased (P<0.05) level when compared with HSS and HSS+H fed rats (Table VII).

Table VII: Malonydialdehyde (MDA) estimation and antioxidant activity in the testis following administration of treatments for four weeks

	CONTROL	HSS	HSS+H	H
MDA (µmol/l)	0.54 ± 0.17	0.38 ± 0.01	0.41 ± 0.10	0.23 ± 0.10
SOD (mmol/l)	2.02 ± 0.22	2.03 ± 0.19	3.77 ± 1.48	2.31 ± 0.55
CAT (mol/l)	0.96 ± 0.11	0.69 ± 0.01*	0.70 ± 0.05	0.93 ± 0.06 #π
GSH (µmol/l)	0.32 ± 0.03	0.40 ± 0.05	0.32 ± 0.04	0.43 ± 0.05

Values are Mean±SEM; n=5.*?-compared with control; # -compared with sucrose ?- compared with sucrose+ honey P < 0.05. HSS- high sucrose solution, HSS+H- high sucrose solution+ honey, H- honey

Malonydialdehyde (MDA) estimation and antioxidant activity in the liver

In the liver of HSS fed rats, there was a significant increase ($P < 0.05$) in MDA level compared with control. The activities of SOD was significantly higher ($P < 0.05$) in the liver of HSS+H fed rats compared with HSS fed rats. CAT and GSH activities in the liver were not significantly different among the experimental groups (Table VIII).

Table VIII: Malonhydiyaldehyde (MDA) estimation and antioxidant activity in the liver following administration of treatments for four weeks

	CONTROL	HSS	HSS+H	H
MDA ($\mu\text{mol/l}$)	0.56 ± 0.06	2.68 ± 0.40 *	2.07 ± 0.43	0.59 ± 0.16
SOD (mmol/l)	3.92 ± 0.92	2.07 ± 0.58	4.92 ± 0.77 #	3.77 ± 0.63
CAT (mol/l)	0.67 ± 0.06	0.70 ± 0.07	0.98 ± 0.20	0.69 ± 0.08
GSH ($\mu\text{mol/l}$)	0.44 ± 0.05	0.50 ± 0.06	0.55 ± 0.06	0.52 ± 0.05

Values are Mean \pm SEM; $n=5$. *-compared with control; # -compared with sucrose $P < 0.05$. HSS- high sucrose solution, HSS+H- high sucrose solution+ honey, H- honey

Discussion:

Different types of geographical locations have attributed different uses to honey, thus this study is in line with justifying the role of Nigerian honey on sperm indices in sucrose-fed rats. In Arab countries for instance, honey is considered to increase human male potency²⁶. In another study²⁷, it was observed in vitro, that diluted Egyptian bee honey and royal jelly had an enhancing effect on sperm motility, particularly in subnormal samples. A preliminary study involving a local Malaysian honey, referred to as Tualang honey, has been proposed to enhance spermatogenesis if given at appropriate doses and also to possibly reduce the toxic effect of cigarette smoke on rat spermatogenesis¹⁷. Nigerian honey has been shown to improve the growth rate and physiological welfare of broilers reared during hot-dry season²⁸. Some Nigerian honey samples have also shown varied antimicrobial activities²⁹. The known safe use of Nigerian honey without toxic effects suggests that it can be used to treat diseases due to bacterial pathogens³⁰.

The pattern of food intake was affected by HSS feeding throughout the period of the study. Most of the literatures have shown that adult and children are satiated by sucrose with satiety being prolonged when larger amount of sucrose is consumed³¹. Another study reported that sucrose feeding in rats increased serum leptin concentration³², thus the

observed reduction in food intake in the sucrose-fed rats may be due to the central effect of leptin initiating satiety. Previous studies have shown that honey supplementation decreases energy/food intake in rats¹⁰. However, the increase in food intake in the honey fed rats from the 1st to 2nd week of treatment followed by reduction in food intake from the 3rd to 4th week of treatment, suggests a prolonged initiation of satiety as against that of sucrose which was noticeable from the beginning of the study. Interestingly, by the 4th week of treatment the HSS+H fed rats reduced their food intake to a considerable level lower than that of the HSS-fed rats, this could possibly indicate a synergetic action of both honey and sucrose on food intake. Oligosaccharides such as palatinose (isomaltulose) present in honey have been reported to delay digestion and intestinal absorption of glucose resulting in reduced glycaemia^{33, 34}. Besides delaying absorption, report proposes that fructose consumption lowers food intake³⁵ and this reduced food intake is attributed to delay gastric emptying³⁶. This might be the reason for the reduced food intake in the H rats compared with the control.

There was a significant increase in the body weights of the animals in the honey (H) group. This is consistent with a study¹⁴. Some studies have also found that a composition of honey i.e. fructose consumption at high doses is associated with increased weight gain^{37, 38}.

Consumption of a high sucrose solution has been implicated in the development of insulin resistance and glucose intolerance in rats³⁹. In the present study, there was however no significant difference in fasting blood glucose among the experimental rats. Meanwhile, the duration of treatment may be an important factor to consider since most studies that have reported metabolic disorders due to high sucrose feeding were carried out over a longer duration of eight weeks and over⁴⁰. The short duration of high sucrose feeding (i.e. 4weeks) in this study may have hampered the metabolic changes that become pronounced over time. The fasting blood glucose level of the sucrose fed rats may have appeared to be reduced because serum glucose levels is known to be reduced in starved rats and animals were starved overnight⁴¹. Researchers have

studied non-diabetic subjects and reported that honey has a lower effect on increasing the levels of sugar in the blood, compared to sucrose; in addition, these authors have suggested that honey would cause fewer longterm health problems than fructose or sucrose, in part because it contains nutrients other than carbohydrates^{42,43}.

Honey supplementation has been shown to have great impact on the testis and other accessory sex organs according to a study by ⁴⁴. It is also important to note that the weight of the testes, epididymis, and seminal vesicle were significantly increased in the H and HSS+H fed rats when compared with HSS rats and increase in absolute weight of testes and epididymis have been attributed to increased androgen biosynthesis⁴⁵. The result from this present study is in agreement with the observation by Abdul-Ghani et al ⁴⁴ where ingestion of 5% honey for 20 days would induce spermatogenesis in rats by increasing relative weight of the epididymis.

In the present result, several sperm indices were positively affected by honey. Significantly higher sperm count higher percentage of normal sperm and lower percentage of sperm head and tail abnormalities especially the 'curved tail' and curved mid-piece' were observed in the honey group in comparison to control and sucrose groups. The authors ⁴⁴ also reported that honey would induce spermatogenesis in rats by increasing epididymal sperm count and increasing sorbitol dehydrogenase activity. Sorbitol dehydrogenase is an enzyme in carbohydrate metabolism that converts sorbitol, the sugar alcohol form of glucose into fructose⁴⁶. A similar increase in sperm count was reported by¹⁶ in rats and monkeys, and enhanced spermatogenesis was reported by¹⁷. Although the present study was conducted using Nigerian honey, the results obtained were similar to that reported from the use of Malaysian honey²⁶ and Yousef ⁴⁶ who used propolis. Propolis could provide protection against infertility by improving sperm production, motility, sperm count and quality and increased the process of steroidogenesis and, hence, testosterone production. Royal jelly treatments significantly boosted testosterone level, increased ejaculated volume, increased seminal plasma fructose, improved sperm motility, increased sperm total output, reduced number of abnormal and dead sperm⁴⁸. Sperm motility and

concentration are important indicators of male fertility potential ⁴⁹. Epididymal histophysiology and acquisition of sperm motility are dependent on the presence of androgen⁵⁰. In the present study, the percentage of sperm with progressive motility was reduced in the HSS fed rats compared with both HSS+H and H fed rats. Alterations in motility parameters may lead to an inefficient sperm penetration of cervix mucus, impairing the ability of sperm to reach the oocyte. In addition, a high percentage of sperm with progressive motility is related to a high fertilization index⁵¹. Animals fed a high sucrose diet presented a reduced sperm concentration, but this was attenuated by honey supplementation in the HSS+H fed rats. Honey could possibly act as physiologic modulator of spermatogenic cells proliferation which influenced the spermatogenic cycle thus, increasing the sperm production⁵². These observations consequently demonstrate the protective role of honey on sperm motility and concentration as against the deleterious effect of sucrose on sperm function. Some authors have also reported that honey supplementation in normal rats improved spermatogenesis⁵³. A recent study also demonstrated the beneficial effects of honey on sperm motility and morphology in rats⁵².

The hypothalamic pituitary gonadal (HPG) axis was not altered by HSS feeding. Conversely, honey supplementation resulted in decreased level of LH and FSH. These gonadotropins exert a stimulatory effect on leydig cells of the testes for testosterone production. Although low LH and FSH is expected to result in reduced testosterone, there was however a significant increase in testosterone level of the H-fed rats. Contrarily, Mohamed et al ⁵² found no changes in LH and FSH level of rats supplemented with honey but reported increased testosterone level as well. In another study, no changes were reported on any of the sex hormones⁴⁴. Further studies are thus needed to characterize the effect of honey on the HPG axis, particularly its central effect.

The increased lipid peroxidation in the liver and not in the testes of the HSS fed rats makes it difficult to attribute the alteration of these sperm parameters to the oxidative status of the reproductive organ. However, it brings to fore, that metabolic insult was elicited by HSS feeding regardless on the fasting blood glucose; as the sucrose fed rats did not present

hyperglycemia. Meanwhile,, the increased CAT activity in the testes of the H fed rats demonstrates the antioxidant potential of honey^{54, 55, 56} on reproductive organs. Enzymatic scavengers like SOD, CAT and GSH protect the system from deleterious effects of ROS. The improved antioxidant status of the H fed rats in this study may have impacted on the sperm function according to the antioxidative nature of honey^{54, 57,58,59,60} since spermatozoa have little defence against oxidative damage and are highly sensitive to free radicals. Previous reports have shown that honeys with a high content of polyphenolic compounds have the capacity to decrease significantly the concentration of lipid hydro-peroxides produced during the lipid peroxidation process, in a process similar to that of other antioxidants like melatonin and vitamin E. The liver is susceptible to oxidative stress and damage; and the beneficial effect of antioxidants on hepatic oxidative stress has been documented^{61, 62}. In the liver of young and middle-aged rats, honey supplementation was reported to restore activities of CAT and GPx ⁶³, although in this study the activity of SOD was improved in the HSS+H rats compared to the HSS rats.

Conclusion:

Although several contributory factors have been implicated, the possible role of a sugar-driven dietary lifestyle as a causative factor has gained little or no attention. The result of the present study indeed indicates that a high sugar diet could affect sperm function as evidenced by decreased sperm motility and count in the sucrose fed rats. The observations of this study affirm the statement of hypothesis that a high sucrose diet will impact negatively on male reproductive function while honey consumption will confer a protective effect. The reproductive capacity of male rats is known to be higher than that of men ⁶; therefore, such a decrease in sperm quality as observed in the sucrose fed rats may be enough to alter fertility among human males, thus contributing to the alarming incidence of male infertility. Nigerian honey on the other hand appears to be a credible alternative sweetener and exhibits protective function against reproductive dysfunction via high sucrose feeding.

Conflict of no interest statement

We declare that we have no conflict of interest.

References:

1. Gray GA, Nielsen SJ, Popkin BM. Consumption of high-fructose corn syrup in beverages may play a role in the epidemic of obesity. *Am J Clin Nutr* 2004; **79**:537-543
2. Stanhope KL, Havel PJ. Fructose consumption: recent results and their potential implications. *Ann NY Academy Sci.* 2010; **1190**:15-24 <http://dx.doi.org/10.1111/j.1749-6632.2009.05266.x>
3. Vujkovic M, Ocke MC, van der Spek PJ, Yazdanpanah N, Steegers EA, Steegers, Theunissen RP. Maternal Western dietary patterns and the risk of developing a cleft lip with or without a cleft palate. *Obstet Gynecol.*2007; **110**:378-84 <http://dx.doi.org/10.1097/01.AOG.0000268799.37044.c3>
4. Homan GF, Davies M, Norman R. The impact of lifestyle factors on reproductive performance in the general population and those undergoing infertility treatments: a review. *Hum Reprod Update* 2007; **13**: 209-23 <http://dx.doi.org/10.1093/humupd/dml056>
5. Keen CL, Clegg MS, Hanna LA, Lanoue L, Rogers JM, Daston GP, Oteiza P, Uriu-Adams JY. The plausibility of micronutrient deficiencies being a significant contributing factor to the occurrence of pregnancy complications. *J Nutr.* 2003; **133**:597S-1605S
6. Fernandez CD, Bellentani FF, Fernandes GS, Perobelli JE, Favareto AP, Nascimento AF, Cicogna AC, Kempinas WD. Diet-induced obesity in rats leads to a decrease in sperm motility. *Repro Biol Endocrinol* 2011; **9**(32):1-10
7. Bizeau ME and Pagliassotti. Hepatic adaptations to sucrose and fructose. *Metabol.* 2005; **54**:1189-201 <http://dx.doi.org/10.1016/j.metabol.2005.04.004>
8. Crane E, History of honey. In Crane E (ed): "Honey, A Comprehensive Survey." London: William Heinemann, 1975; pp 439-488
9. Bogdanov S, Jurendic T, Sieber R, Gallmann P. Honey for nutrition and health: a review. *J Am Coll Nutr* 2008; **27**:677-689 <http://dx.doi.org/10.1080/07315724.2008.10719745>
10. Chepulis LM. The effect of honey compared to sucrose mixed sugars, and a sugar-free diet on weight gain in young rats. *J Food Sci* 2007; **72**:S224-S229 <http://dx.doi.org/10.1111/j.1750-3841.2007.00286.x>
11. Carmody EG, Li J, Potter H, Steffy-Thompson A, Rezende L, Nemoseck T, Stocker K, Kern M. Effects of honey versus sucrose on weight gain, adiposity and adipocentric markers of metabolism. *FASEB J* 2007; **21**:832 (A1060)
12. Bogdanov S. Nature and origin of the antibacterial substances in honey. *Lebensm.-Wiss-Technol* 1997; **30**:748-753 <http://dx.doi.org/10.1006/fstl.1997.0259>
13. Wang XH, Andrae L, Engeseth NJ. Antimutagenic effect of various honeys and sugars against Trp-p-1. *J Agric Food Chem* 2002;**50**:6923-6928 <http://dx.doi.org/10.1021/jf025641n>
14. Fasanmade AA, Alabi OT. Differential Effect of Honey on Selected Variables in Alloxan-Induced and Fructose-Induced Diabetic Rats. *African Journal of Biomedical Research* 2008;**11**:189-194
15. Beretta G, Granata P, Ferrero M, Orioli M, Facino RM. Standardization of antioxidant properties of honey by a combination of spectrophotometric/fluorimetric assays and chemometrics. *Anal Chim Acta* 2005; **533**:185-191 <http://dx.doi.org/10.1016/j.aca.2004.11.010>
16. Siti AS. Honey and reproductive hormones. *Malay J Med Sci* 2007; **14**(1):105-106
17. Mahaneem M, Siti AS, Yatiban MK, Hasnan J. Effect of Tualang honey on spermatogenesis in rats. *Malay J Med Sci* 2007; **14**(1):126
18. Mahaneem M, Siti AS, Islam MN, Zol Izhar MI, Yatiban MK, Nasir A: A pilot study to compare the effect of honey on spermatogenesis in rats exposed to cigarette smoke. *Malay J Med Sci* 2007;**14**(1):126
19. Busserolles J, Gueux E, Rock E, Mazur A, Rayssiuguier Y. Substituting Honey for Refined Carbohydrates Protects Rats from Hypertriglyceridemic and Prooxidative Effects of Fructose. The American Society for Nutritional Sciences. *J Nutr.* 2002; **132**: 3379 - 3382
20. Ribeiro RT, Lutt WW, Legare DJ, Macedo MP: Insulin resistance induced sucrose feeding in rats due to impairment of the hepatic parasympathetic nerves. *Diabetologia* 2005;**48**:976-983 <http://dx.doi.org/10.1007/s00125-005-1714-6>
21. World Health Organization WHO. Laboratory Manual for Examination of Human Semen and Semen-Cervical Mucus Interaction, 2nd ed. London: Cambridge University Press, 1987;pp. 1-10.
22. Uchiyama M, Mihara M: Determination of malonaldehyde precursor in tissues by thiobarbituric acid test. *Anal Biochem* 1978;**86**:271-278 [http://dx.doi.org/10.1016/0003-2697\(78\)90342-1](http://dx.doi.org/10.1016/0003-2697(78)90342-1)
23. van Dooran R, Liejdekker CM, Handerson PT. Synergistic effects of phorone on the hepatotoxicity of

- bromobenzene and paracetamol in mice. *Toxicol* 1978; **11**:225-233 [http://dx.doi.org/10.1016/S0300-483X\(78\)91389-6](http://dx.doi.org/10.1016/S0300-483X(78)91389-6)
24. Sun M, Zigman S: An improved spectrophotometric assay for superoxide dismutase based on ephinephrine autoxidation. *Anal Biochem* 1978;**90**:81-89 [http://dx.doi.org/10.1016/0003-2697\(78\)90010-6](http://dx.doi.org/10.1016/0003-2697(78)90010-6)
25. Aebi H. Catalase in vitro. *Methods Enzymol* 1984; **105**:121-126 [http://dx.doi.org/10.1016/S0076-6879\(84\)05016-3](http://dx.doi.org/10.1016/S0076-6879(84)05016-3)
26. SyazanaNS, Hashida NH, Majid AM, Durriyyah Sharifah HA, Kamaruddin MY. Effects of Gelam Honey on Sperm Quality and Testis of Rat. *Sains Malaysiana* 2011; **40**(11): 1243–1246
27. Abdelhafiz, A.T. & Muhamad, J.A. Midcycle pericoital intravaginal bee honey and royal jelly for male factor infertility. *International Journal of Gynecology and Obstetrics* 2008; **101**: 146-149 <http://dx.doi.org/10.1016/j.ijgo.2007.11.012>
28. Abioja MO, Ogundimu KB, Akibo TE, Odukoya KE, Ajiboye OO, Abiona JA, Williams TJ, Oke EO, Osinowo OA. Growth, Mineral Deposition, and Physiological Responses of Broiler Chickens Offered Honey in Drinking Water during Hot-Dry Season. Hindawi Publishing Corporation, International Journal of Zoology 2012; Article ID 403502, 6 pages doi : 1 0 . 1 1 5 5 / 2 0 1 2 / 4 0 3 5 0 2 <http://dx.doi.org/10.1155/2012/403502>
29. Omafuvbe BO, Akanbi OO. Microbiological and physico-chemical properties of some commercial Nigerian honey," *African Journal of Microbiology Research*, 2009;**3**(12), 891–896
30. Adetuyi FO, Ibrahim TA, Jude-Ojei, Ogundahunsi GA. Total phenol, tocopherol and antibacterial quality of honey *Apis mellifera* sold in owo community, Ondo State, Nigeria. *Electronic Journal of Environmental, Agricultural and Food Chemistry*, 2009;**8**, (8), 596–601.
31. Anderson H, Woodend G. Consumption of sugar and the regulation of short-term satiety and food intake. *Am J Clin Nutri* 2003;**73**: 843S-9S
32. Nemoseck TM, Carmody EG, Furchner-Evanson A, Gleason M, Li A, Potter H, Rezende LM, Lane KJ, Kern M. Honey promotes lower weight gain, adiposity, and triglycerides than sucrose in rats. *Nutri Res*.2011; **31**: 55-60 <http://dx.doi.org/10.1016/j.nutres.2010.11.002>
33. Kashimura J, Nagai Y. Inhibitory effect of palatinose on glucose absorption in everted rat gut. *J Nutr Sci Vitaminol (Tokyo)* 2007; **53**: 87-9 <http://dx.doi.org/10.3177/jnsv.53.87>
34. Lina BA, Jonker D, Kozianowski G. Isomaltulose (Palatinose): a review of biological and toxicological studies. *Food Chem Toxicol* 2002; **40**: 1375-81 [http://dx.doi.org/10.1016/S0278-6915\(02\)00105-9](http://dx.doi.org/10.1016/S0278-6915(02)00105-9)
35. Thibault L. Dietary carbohydrates: effects on self-selection, plasma glucose and insulin, and brain indoleaminergic systems in rat. *Appetite* 1994; **23**: 275-86 <http://dx.doi.org/10.1006/appe.1994.1059>
36. Gregory PC, McFadyen M, Rayner DV. Relation between gastric emptying and short-term regulation of food intake in the pig. *Physiol Behav* 1989; **45**: 677-83 [http://dx.doi.org/10.1016/0031-9384\(89\)90278-3](http://dx.doi.org/10.1016/0031-9384(89)90278-3)
37. Meirelles CJ, Oliveira LA, Jordao AA, et al. Metabolic effects of the ingestion of different fructose sources in rats. *Exp Clin Endocrinol Diabetes* 2011; **119**: 218-20 <http://dx.doi.org/10.1055/s-0031-1275276>
38. Bocarsly ME, Powell ES, Avena NM. High-fructose corn syrup causes characteristics of obesity in rats: increased body weight, body fat and triglyceride levels. *Pharmacol Biochem Behav* 2010; **97**: 101-6 <http://dx.doi.org/10.1016/j.pbb.2010.02.012>
39. Kawasaki T, Kashiwabara A, Sakai T, Igarashi K, Ogata N, Watanabe H, Ichiyanagi K, Yamanouchi T. Long-term sucrose drinking causes increased body weight and glucose intolerance in normal male rats. *Br J Nutr* 2005; **93**:613–618 <http://dx.doi.org/10.1079/BJN20051407>
40. Horton TJ, Gayles EC, Prach PA, Koppenhafer TA, Pagliassotti MJ. Female rats do not develop sucrose-induced insulin resistance. *Am J Physiol Regul Integr Comp Physiol* 1997; **272**:1571–1576 Seymour L, Arthur S. Feeding sugar overnight maintains metabolic homeostasis in rats and is preferable to overnight starvation. *Laboratory Animals Ltd. Laboratory Animals* 2000; **34**: 301-306
42. Shambaugh, P., V. Worthington, and J.H. Herbert. Differential effects of honey, sucrose, and fructose on blood sugar levels. *Journal of Manipulative and Physiological Therapeutics* 1990;**13**: 322-325
43. Shambaugh, P., V. Worthington, and J.H. Herbert. Differential effects of honey, sucrose, and fructose on blood sugar levels. *Journal of Manipulative and Physiological Therapeutics* 1991; **14**: 91-92
44. Abdul-Ghani, A.S., Dabdoub, N., Muhammad, R., Abdul-Ghani, R. & Qazzaz, M Effect of Palestinian honey on spermatogenesis in rats. *Journal of Medicinal Food* 2008; **11**(4): 799-802 <http://dx.doi.org/10.1089/jmf.2008.0085>

45. Morakinyo AO, Adeniyi OS, Arikawe AP. Effects of Zingiber Officinale on Reproductive Functions in the Male Rat. *Afr J Biomed Res* 2008; **11**: 329 – 334
46. El-Kabbani O, Darmanin C, Chung RP. Sorbitol Dehydrogenase: Structure, Function and Ligand Design. *Current Medicinal Chemistry* 2004; **11**(4): 465-476 <http://dx.doi.org/10.2174/0929867043455927>
47. Yousef, M.I. & Salama, A.F. Propolis protection from reproductive toxicity caused by aluminium chloride in male rats. *Food and Chemical Toxicology* 2009; **47**: 1168-1175 <http://dx.doi.org/10.1016/j.fct.2009.02.006>
48. Elnagar, S.A. Royal jelly counteracts bucks' "summer infertility". *Animal Reproduction Science* 2010; **121**: 174-180 <http://dx.doi.org/10.1016/j.anireprosci.2010.05.008>
49. Ashby J, Tinwell H, Lefevre PA, Joiner R, Haseman J. The effect on sperm production in adult Sprague-Dawley rats exposed by gavage to bisfenol A between postnatal days 91-97. *Toxicol Sci* 2003; **74**(1):129-138 <http://dx.doi.org/10.1093/toxsci/kfg093>
50. Henderson NA, Robaire B. Effects of PNU157706, a dual 5 α -reductase inhibitor, on rat epididymal sperm maturation and fertility. *Biol Reprod* 2005; **72**(2):436-443 <http://dx.doi.org/10.1095/biolreprod.104.033548>
51. Donnell ET, Lewis SEM, McNally JA, Thompson W. In vitro fertilization and pregnancy rates: the influence of sperm motility and morphology on IVF outcome. *Fertil Steril* 1998; **70**(2):305-314 [http://dx.doi.org/10.1016/S0015-0282\(98\)00146-0](http://dx.doi.org/10.1016/S0015-0282(98)00146-0)
52. Asiyah HA, Syazana, NS Hashida NH, Durriyyah HA and Kamaruddin MY. Effects of nicotine and Gelam honey on testis parameters and sperm qualities of juvenile rats. *Sci. Res. Essays* 2011; **6**(26) 5471-5474 <http://dx.doi.org/10.5897/SRE10.1016>
53. Mohamed, M.; Sulaiman, S.A.; Jaafar, H.; Sirajudeen, K.N. Effect of different doses of Malaysian honey on reproductive parameters in adult male rats. *Andrologia* 2011; doi:10.1111/j.1439-0272.2010.01159.x <http://dx.doi.org/10.1111/j.1439-0272.2010.01159.x>
54. Gheldof N, Wang XH, Engeseth NJ. Identification and quantification of antioxidant components of honeys from various floral sources. *J Agric Food Chem.* 2002; **50**: 5870-7 <http://dx.doi.org/10.1021/jf0256135>
55. Al-Waili, NS. Effects of daily consumption of honey solution on hematological indices and blood levels of minerals and enzymes in normal individuals. *J Med Food.* 2003; **6**: 135-40 <http://dx.doi.org/10.1089/109662003322233549>
56. Yao L, Jiang Y, D'arcy B, Singanusong R, Datta N, Caffin N, Raymont K. Quantitative high-performance liquid chromatography analyses of flavonoids in Australian Eucalyptus honeys. *J Agric Food Chem.* 2004; **52**:210-4 <http://dx.doi.org/10.1021/jf034990u>
57. Al-Mamary M, Al-Meerri A, Al-Habori M: Antioxidant activities and total phenolics of different types of honey. *Nutr Res* 2002; **22**: 1041–1047 [http://dx.doi.org/10.1016/S0271-5317\(02\)00406-2](http://dx.doi.org/10.1016/S0271-5317(02)00406-2)
58. Gheldof N, Engeseth NJ: Antioxidant capacity of honey from various floral sources based on the determination of oxygen radicals absorbance and inhibition of in vitro lipoprotein oxidation in human serum samples. *J Agric Food Chem* 2002; **50**:3050–3055 <http://dx.doi.org/10.1021/jf0114637>
59. Schramm DD, Karim M, Schrader HR, Holt RR, Cardetti M, Keen CL: Honey with high levels of antioxidants can provide protection to healthy human subjects. *J Agric Food Chem* 2003; **51**:1500–1505 <http://dx.doi.org/10.1021/jf025928k>
60. Perez E, Rodriguez-Malaver AJ, Vit P: Antioxidant capacity of Venezuelan honey in Wistar rat homogenates. *J Med Food* 2006; **9**:510–516. <http://dx.doi.org/10.1089/jmf.2006.9.510>
61. Dias, AS, Porawski, M, Alonso M, Marroni N, Collado PS, Gonzalez-Gallego J. Quercetin decreases oxidative stress, NF-kappaB activation and iNOS over-expression in liver of streptozotocin-induced diabetic rats. *J. Nutr.* 2005;**135**: 2299–2304
62. Gumieniczek, A. Oxidative stress in kidney and liver of alloxan-induced diabetic rabbits: Effect of repaglinide. *Acta Diabetol.* 2005; **42**: 75–81 <http://dx.doi.org/10.1007/s00592-005-0182-2>
63. Yao LK, Razak SLA, Ismail N, Fai NC, Asgar MHAM, Sharif NM, Aan GJ, Jubri Z. Malaysian gelam honey reduces oxidative damage and modulates antioxidant enzyme activities in young and middle aged rats. *J. Med. Plants Res.* 2011; **5**:5618–5625