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Beneficial Effects of Honey-Based Diet on Glycemic Control and Reproductive Potential in Diabetic Rats

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Abstract Reports of clinical studies on the global increase in male infertility prevalence are of public interest due to its social and economic burden. Honey is used in various foods, beverages and medicinal traditions to treat various ailments. This experimentally-controlled designed study aimed to determine the effect of honey-based diet on the sperm quality, testicular histology, reproductive hormones and glycemic tolerance in diabetic rats fed for 8 weeks. Thirty two adult male Wistar rats each weighing ≥ 200g at the beginning of the study were used and were randomly categorized into four experimental groups of 8 rats each: diabetic rats fed with honey-based diet - DHF, diabetic rats fed with standard feed (diabetic control - DNF), Non-diabetic rats fed with honey-based diet - NHF and non-diabetic rats fed with standard feed (normal control – NNF). Diabetes was inducted in DHF and NHF grouped rats using freshly prepared alloxan monohydrate solution (150mg/dL, intraperitoneally) after 15 hours overnight fast and was confirmed 4-7 days later using glucometer. All rats were fed according to the experimental design for eight week period while weekly weight and total food intake per group recorded. Fasting blood glucose (FBG) concentrations were measured at the entry point and 8th week of study. Glycemic tolerance test using D-glucose (2g/kg wt), hormonal (testosterone, FSH, LH) assays and sperm analysis were conducted after 8 weeks while sections of extracted testes were examined histologically. Data obtained were expressed as mean of eight replicates ± SEM. A significant ($P < 0.05$) reduction in total weight gain (DHF - 6.2%; NHF - 7.44%) with insignificant increase in food intake was observed in diabetic and non-diabetic rats fed with honey-based diet compared with their respective controls. Sperm analysis and hormonal assay revealed significant ($P < 0.05$) increase in sperm count, testosterone level and improved sperm morphology in diabetic and non-diabetic treated rats. Testicular histoarchitecture of honey-based diet-fed rats displayed a densely packed spermatogenic cells in the seminiferous tubular lumen while that of the control rats showed sparse distribution. An improved glycemic tolerance with significant ($P = 0.02$) reduction in FBG concentration was observed in DHF (5.1%) and NHF (10.73%) rats at the end of study. In conclusion, honey-based diet improves reproductive potential in diabetic rats with beneficial impact on the glycemic tolerance and control.

Keywords: *diabetic rats, glycemic tolerance, honey-based diet, reproductive hormones, sperm analysis, testicular histology*

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1. Introduction

The prevalence of male infertility is on the increase globally and is of public interest due to its social and economic burden [1,2]. Poor reproductive functions in males have been attributed to so many factors including environmental, genetic, nutritional, physiological, pharmacological and psychogenic [3,4]. The use of honey as a nutraceutical has become of increasing interest in the recent years largely due to an increase in the availability of evidence-based findings demonstrating the health beneficial effects of honey in treating diverse disease conditions including diabetes mellitus. The beneficial effects of honey in male reproductive performances were mostly investigated in healthy subjects [5,6], without much consideration of its impact on blood sugar level if it were to be recommended in diabetic subjects for similar indication. Thus, investigating the suitability of honey recommendation in diabetics for whatever use considering the fact that honey is a sugary substance is very essential. Therefore, this experimentally-controlled designed study aimed to determine the effects of honey-based diet on sperm quality, reproductive hormones, testicular parameters/histology and glycemic tolerance/control in adult male diabetic Wistar rats with the rationale to ascertain the suitability of honey in the treatment of reproductive disturbances in diabetics without adverse impact on blood sugar level, glycemic tolerance and control.

2. Materials and Methods

2.1. Experimental Animals and Design

Thirty two adult male Wistar rats (*Rattus norvegicus*) weighing ≥ 200 g were purchased from the animal house of the Department of Biochemistry, Bowen University Iwo. They were fed initially with standard rat chow and water *ad libitum* for the 2 weeks acclimatization in raised stainless steel cages with 6mm^2 mesh floor (to maintain same physical activity) kept in a well ventilated animal house (at 23°C and a 12 h light and dark cycle). Replaceable numbered blotters papers were placed under each cage to catch the spilled diet that was measured to make up for the daily serving ration. After acclimatization, the experimental rats were randomly divided into four groups of 8 rats each (as shown below) such that each group had the same approximate mean body weight (Table 2) and coefficient of variation. All animal weights were measured weekly and recorded.

DHF GROUP: Diabetic rats fed on honey-based diet.

DNF GROUP: Diabetic rats fed on normal diet - Diabetic Control.

NNF GROUP: Non-diabetic rats fed on normal diet - Normal Control.

NHF GROUP: Non-diabetic rats fed on honey-based diet.

This study using experimental animals was conducted in accordance with the internationally accepted principles for laboratory animal use and care [7] with the approval of the Animal Care and Use Review Committee at Bowen University, Nigeria.

2.2. Induction of Diabetes

After 15 hour overnight fast following acclimatization , rats in DHF and NHF groups (n=8, with each weight >200g) were injected by single intraperitoneal injection of 150 mg/kg body weight of freshly prepared 2% alloxan monohydrate (Sigma chemicals, USA) dissolved in sterile 0.9% normal saline in a standard volumetric flask strapped with foil to prevent alloxan instability. Diabetes was confirmed 4-7 days later by use of glucometer (On Call Plus Blood Glucose Monitoring System, ACON Laboratories, Inc. San Diego, USA.) and compatible strips. Rats with Fasting Blood Glucose (FBG) level > 150mg/dl were considered diabetic and used for this study since the level of serum glucose considered to be normal in *rattus norvegicus* ranges from 50 – 135mg/dL [8]. Diabetes was allowed to stabilize for 5 days before exposure to experimental diets. Blood glucose level of all rats in each experimental group was assessed on alternate days thereafter.

2.3. Test Diets and Feeding

The composition of the diets in this study was based upon the standard diet formulas used to assess weight gain in rodents during commercial feeding studies. The honey-based (test) diet was prepared by mashing the standard rat feed pellet (50% of carbohydrate, 15% fat, 12% protein, 20% fiber, 2% normal supplement and 1% vitamin and water) purchased from a commercial branch depot (Ladokun feed Plc. Nigeria) and then mixed it with honeycomb honey purchased from a local market in western part of Nigeria where it is locally called 'Afara Oyin'. The amount of honey used in the test feed for daily serving size was based on the total weight of the number of rats per group at a rate of 1g/kg body weight (equivalent to 100g of honey per kg of feed) such that the determined calories of the honey type used per serving was approximately 400 calorie to ensure no differential calories treatment in both control and test diets. The mixture was allowed to air-dry at room temperature (23°C) prior to feeding. The standard rat chow (50% of carbohydrate, 15% fat, 12% protein, 20% fiber, 2% normal supplement and 1% vitamin and water) without honey was used as control diet. The animals were fed according to the experimental design for 8 weeks with water *ad-libitum*. Body weight and total food intake of each group of rats were measured and recorded weekly while the food conversion ratio (food intake/weight gain) was calculated. The diet compositions are given in Table 1 below:

Table 1. Composition of Test and Control Diets (g/kg)

Ingredients	Control diet	Test diet
Carbohydrates	500	400
Protein	120	120
Lipid	150	150
Dietary fibre	200	200
Normal supplement	20	20
Vitamin + water	10	10
Honey	0	100

2.4. Blood Collection

The blood samples were collected from the tail veins (tail snipping) and the heart (cardiac puncture) for oral glucose tolerance test and hormonal assay respectively.

2.5. Oral Glucose Tolerance Test

Animals in all groups were fasted overnight with free access to water before the day of experiment (last day of eight-week) and were administered oral D - glucose load of 2g kg^{-1} (dissolved in distilled water) by means of cannula after taking the initial fasting blood glucose (FBG) concentration. Thereafter, blood samples were withdrawn from the cordal vein of each animal (tail snipping) to determine the fasting blood sugar concentration at intervals of 30, 60, 90, 120 and 150 minutes using glucose analyzer (On Call Plus Blood Glucose Monitoring System, ACON Laboratories, Inc. San Diego, USA.).

2.6. Hormonal assay

Levels of testosterone, luteinizing hormone and follicle-stimulating hormone in rats' sera obtained from cardiac blood samples were measured by enzyme immunoassays method using commercially available rodent Testosterone ELISA test kit, LH ELISA test kits and FSH ELISA test kit (Cosmo Bio Co. USA Inc.) respectively. Each hormone was carried out using same assay for all groups to avoid inter and intra variation which may affect the interpretation of result.

2.7. Sperm Analysis and Organ Extraction

After conducting the OGTT, all grouped rats were anaesthetized in a glass dome under ethyl ether for collection of sperm and testicular extraction. Sperm specimens were collected by aspiration from the caudal epididymis which involves making an incision in the right caudal ductus deferences of the testicle. Semen examination was carried out according to the method of Cheesebrough [9].Two drops of semen were placed on the microscope slide and two drops of warm 2.9% sodium citrate were added. This was then covered with the cover slip and examined under reduced light microscope using objective lens of × 400 magnifications. Sperm count, motility, viability and morphology were carried out using the new improved neubauer`s haemocytometer counting chamber. Sperm motility was assessed using world health organization (WHO) classification [10] while sperm morphology was assessed by percentage population of the rapidly motile, sluggish and immotile/dead cells. Sperm count was expressed in percentage population.

2.8. Testicular Extraction and Histology

The animals were eventually sacrificed and the testes extracted to measure the testicular parameters of weight, length and width for comparison. Transverse sections of the testicular tissues were cut and fixed in bouin's solution while fixed tissues were processed by appropriate histological technique and eventually stained with Haematoxylin and eosin stain prior to examination under light microscope.

2.9. Statistical Analysis

Data were analyzed using appropriate statistical methods and program of Microsoft Excel and SPSS version 20. Results (all mean values) are expressed as groups mean ± SEM (Standard Error of Mean). Comparisons between groups and the significant difference between the control and the treated groups were analyzed using one way analysis of variance (ANOVA) followed by Duncan's multiple range tests. A (9 x 3) repeated measures ANCOVA was performed on the weight gain data (using the total food intake as a covariable) to determine if there were any diet x time interactions. P values of < 0.05 were considered statistically significant.

3. Results

3.1. Effect of Test Diet on Overall Weight Gain and Food Intake

Table 2. Effects of Test Diet on Food Intake and Body Weight of Grouped Rats (n = 8/group)

	Experimental Animal Categories			
Parameters	**Diabetic**		**Non- diabetic**	
Body Weight (g)	**DHF**	**DNF**	**NHF**	**NNF**
Initial	200.15 ± 0.12	200.05 ± 1.00	200.25 ± 0.05	200.10 ± 0.01
Final	246.43 ± 2.06[a]	260.86 ± 1.59[b]	243.46 ± 1.83[a]	258.00 ± 1.66[b]
Overall % weight gain	23.18%	30.41%	21.61%	29.05%
Total food intake (g/8 weeks)	1672 ± 67	1602 ± 46	1700 ± 72	1634 ± 52

Values are expressed in mean±SEM. Values with different superscript letters on the same row are significant (P value = 0.021).

The main effect of honey-based diet on body weight and total food intake is presented in Table 2. Overall percentage weight gain after 8 weeks was significantly reduced ($P < 0.05$) in test diet-fed rats (DHF- 23.18%; NHF- 21.61%) compared with their respective controls (DNF- 30.41%; NNF- 29.05%) as suggested by standard ANOVA. Honey-based diets have similar effects on weight gain in diabetic and non-diabetic rats. Total food intake was insignificantly higher ($P < 0.05$) in DHF and NHF rats compared with DNF and NNF rats respectively. This might be possibly due to the increased palatability and taste conferred by honey. However, the repeated measures ANCOVA (using the total food intake for each animal as a covariable) revealed that whilst there was no interaction of diet x time over the 8-week period, there was a significant effect of diet. Mean weights were significantly lower ($p < 0.05$) in honey-fed rats compared with the control diet-fed rats. No difference observed in the food conversion ratio (food intake/weight gain) between groups.

3.2. Effects of Test Diet on Blood Glucose Level and Glycemic Tolerance

Honey-based diet caused a significant reduction in FBG of the DHF (5.12%) and NHF (10.73%) grouped rats at the end of 8th week (Table 3) with improved glycemic tolerance over that of the DNF and NNF rats (controls) as assessed by the incremental areas under the glycemic response curves shown in Figure 1 below.

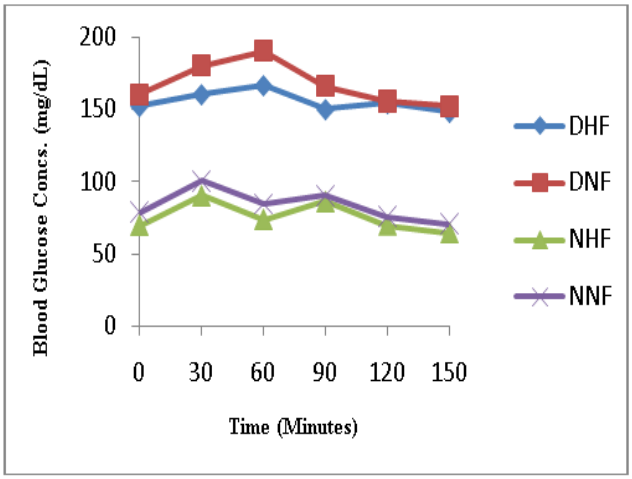

Figure 1. Glycemic tolerance curves of grouped rats (n = 8/group)

DHF = Diabetic rats on honey-based diet; DNF = Diabetic rats on normal diet (Diabetic control); NHF = Normal rats on honey-based diet; NNF = Normal rats on normal diet (Normal control).

Table 3. Fasting Blood Glucose (FBG) Concentrations of Grouped Rats (n=8/group)

	Experimental Groups			
	Diabetic		Non-diabetic	
FBG concentration (mg/dL)	**DHF**	**DNF**	**NHF**	**NNF**
Before 8 weeks (entry)	160.50 ± 5.45	158.66 ± 6.24	78.57 ± 5.59	80.01 ± 2.54
8 weeks after	152.26 ± 3.66	160.24 ± 3.86	70.04 ± 2.24	79.04 ± 1.68
Overall change (%)	5.12	0.01	10.73	0.01

Values are expressed in mean±SD.

3.3. Effect on Sperm Analysis and Testicular Morphometry

The impact of honey-based diet on sperm and testicular parameters are shown in Table 4 and Table 5. There were no significant differences in testicular parameters (weight, length and width) between groups (Table 4). In Table 5, Sperm count of DHF and NHF rats was significantly ($P < 0.05$) higher than their control grouped rats. However, higher values were observed in non-diabetic group (NHF: 108.22 ± 3.12 × 10^6/ mL versus DHF: 104.43 ± 15.25 × 10^6/ mL). Based on sperm morphology, DHF rats showed significantly higher percentage of normal sperm (96.83 ± 0.03%) as compared to DNF rats (93.35 ± 0.35%). Similar observation was made in the non-diabetic rats (NHF- 97.73 ± 0.01%; NNF- 94.24 ± 0.02%). Lower percentage ($P < 0.05$) of abnormal sperms was recorded in rats fed with test diet compared with those on control diet.

Table 4. Testicular Parameters of Grouped Rats (n = 8/group)

Experimental groups	Weights (g)	Length (mm)	Width (mm)
DHF	2.44 ± 0.06^a	18.42 ± 0.24^a	10.42 ± 0.07^a
DNF	2.12 ± 0.01^a	18.02 ± 0.36^a	10.36 ± 0.12^a
NNF	2.25 ± 0.02^a	18.12 ± 0.29^a	10.34 ± 0.03^a
NHF	2.16 ± 0.01^a	18.08 ± 0.32^a	10.28 ± 0.15^a

Values are expressed as mean±SEM.
Values with different superscript letters on the same row are significant at P value < 0.05.

Table 5. Sperm Parameters of Grouped Rats (n = 8/group)

	Experimental Groups			
Sperm Parameters	**DHF**	**DNF**	**NHF**	**NNF**
Total sperm count (x10^6/ml)	104.43 ± 15.25^b	94.22 ± 12.23^a	108.22 ± 16.12^c	96.78 ± 11.44^a
Motile sperm (%)	88.24 ± 5.21^a	89.33 ± 4.64^a	89.65 ± 6.55^a	90.32 ± 3.62^a
Normal sperm (%)	96.83 ± 0.03^b	93.35 ± 0.35^a	97.73 ± 0.01^b	94.24 ± 0.02^a
Abnormal sperm (%)	3.22 ± 0.02^a	5.66 ± 0.12^b	2.02 ± 0.01^a	4.44 ± 0.12^b
Non-motile sperm (%)	10.25 ± 1.46^a	9.91 ± 1.68^a	10.01 ± 1.12^a	8.65 ± 1.54^a

Values are expressed as mean±SEM.
Values with different superscript letters on the same row are significant at P value < 0.05.

3.4. Effects on Testosterone, Follicle Stimulating Hormone (FSH) and Luteinizing Hormone (LH)

There were no significant differences observed in the serum levels of luteinizing and follicle-stimulating hormones between groups. However, rats fed with honey diet had significant ($P < 0.05$) higher testosterone level (DHF- 13.24±7.14ng/mL; NHF- 14.01±2.55 ng/mL) compared with those fed with control diet (DHF- 9.94±4.24ng/mL; NHF- 9.63±6.64 ng/mL) as shown in Table 6 below.

Table 6. Hormonal Profile of grouped rats (n = 8/group)

	Experimental Groups			
Hormones	**Diabetic**		**Non-diabetic**	
	DHF	DNF	NHF	NNF
Testosterone (ng/mL)	13.24 ± 7.14^b	9.94 ± 4.24^a	14.01 ± 2.55^b	9.63 ± 6.64^a
FSH (ng/mL)	4.22 ± 1.35^a	5.66 ± 0.12^a	5.22 ± 0.01^a	4.95 ± 0.12^a
LH (mIU/mL)	1.25 ± 1.42^a	0.97 ± 1.02^a	1.10 ± 1.12^a	1.00 ± 0.02^a

Values are expressed as mean±SEM.
Values with different superscript letters on the same row are significant at P value < 0.05.

3.5. Histological Analysis of the Testis

Photomicrographs (Figure 2 – Figure 4) of the testicular histology of rats fed with honey diet revealed a densely packed spermatogenic cells in the seminiferous tubular lumen with numerous sperm tails compared with those fed with control diet which showed sparsely packed spermatogenic cells with few sperm tails. However, density of spermatogenic cells and quantity of sperm tails were higher in non-diabetic rats.

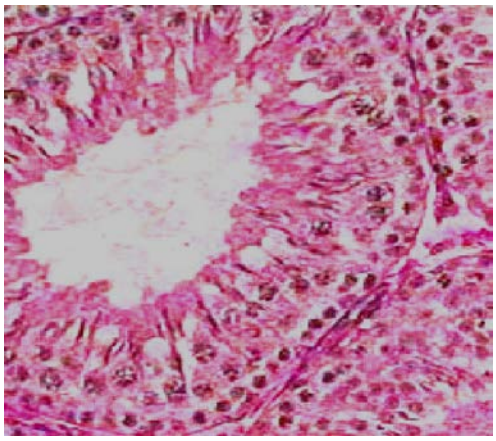

Figure 2. Photomicrograph of the testicular tissue of a normal control rat (NNF) showing normal and regular histoarchitecture of the seminiferous tubules with less dense spermatogenic cells and scanty sperm tails -filled wide tubular lumen (magnification x 400 H&E Stain)

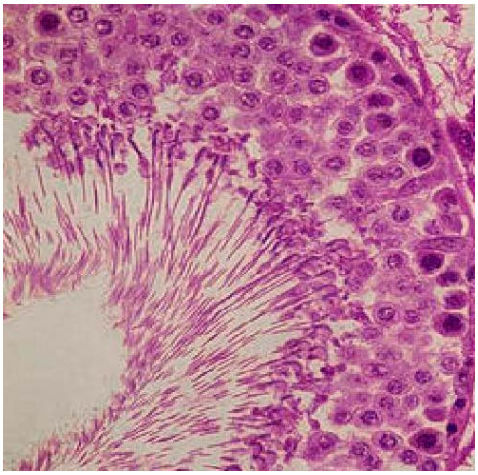

Figure 3. Photomicrograph of the testicular tissue of a normal rat fed with honey-based diet (NHF group) showing moderate densely packed seminiferous tubular lumen with spermatogenic cells and numerous sperm tails. Lumen reduced (magnification x 400 H&E Stain)

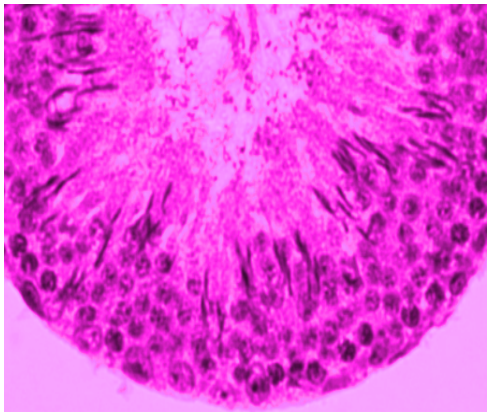

Figure 4. Photomicrograph of the testicular tissue of a diabetic rat fed with honey-based diet (DHF group) showing mild to moderate densely packed seminiferous tubular lumen with spermatogenic cells and few sperm tails. Lumen reduced (magnification x400 H&E Stain)

4. Discussion

This eight-week nutritional study determined and compared the effects of honey-based diet on the sperm quality, reproductive hormones, testicular parameters/ histology and glycemic control/tolerance between adult male diabetic and non-diabetic (normal) wistar rats. The findings obtained from the study revealed that honey-based diet improved reproductive functions in both diabetic and non-diabetic rats with beneficial impact on the glycemic tolerance and control. This improved reproductive function was evidenced by increased sperm count, elevated testosterone level and enhanced spermatogenesis. Comparison of the values obtained showed significant difference between diabetic and non-diabetic (normal) rats which may be explained by the consequence of diabetes mellitus on metabolic and reproductive functions.

In this study, consumption of honey-based diet caused significant reduction in weight gain in healthy and diabetic rats inspite of equal daily calories per serving and insignificant numerical increase in food intake observed in honey fed rats. Increase in food intake may result from the sweetening effect of honey on the diet, however, the repeated measures ANCOVA (using the total food intake for each animal as a covariable) revealed that whilst there was no interaction of diet x time over the 8-week period, there was a significant effect of diet. Mean weights were significantly lower in honey-fed rats compared with the control diet-fed rats. Some reasons have been given to explain the cause of weight reductions by honey consumption. The fructose in honey may enhance glucose uptake in diabetic rats. Studies have shown that fructose is an activator of glucokinase which increase glucose uptake [11]. The rich fructose content of the honey used in this study may have resulted in glucose uptake which otherwise could have contributed to weight gain. Weight gain reduction observed in non-diabetic rats was insignificantly higher than their diabetic counterpart and this might result from better handling capacity of glucose metabolism. A recent study [12] revealed that the antioxidant content of honey may also contribute to reduced weight gain. Certainly, further studies need to be undertaken to assess whether this effect of honey-based diet might be more obvious with larger sample numbers. Recently, research has demonstrated that even small amounts of glucose (e.g. 10% of a 75% glucose:fructose solution) may prevent fructose malabsorption [13]. Thus, it is likely that fructose malabsorption is not responsible for the decreased in weight gain seen in honey diet-fed rats. Instead, it is likely that other factors may be involved in the decreased weight gain observed in rats fed with honey diet. Conducting oral glucose challenge test further provided basic insight to the possible cause of weight gain reduction as it was done in this study. Whichever mechanism by which honey diet achieved this weight gain reduction, it is obvious that it could serve beneficial role in weight reduction in obese and overweight individuals.

Honey-based diet demonstrated beneficial blood glucose lowering effect in this study as it resulted in significant reduction in fasting blood glucose level with improved glycemic tolerance at the end of eight week feeding – period. However, the hypoglycemic effect was more marked in the diabetic than non-diabetic rats may be because of the obvious high blood glucose level in diabetics. This beneficial hypoglycemic effect observed in this study contrast with the finding of the study of Bahrami et al., [14] that reported the potential detrimental effect of honey administration on glycemic control based

on increased level of HbA1c observed in diabetic patients. Though, effect of honey diet on HbA1c was not carried out in this study which might be a limitation, the observed elevated levels of HbA1c following honey supplementation might be due to a number of factors including high doses of honey and unusually low fructose:glucose ratio of the honey used in their study which can enhance glycation [15]. From the above observations, it is obvious that the type of honey that should be used in diabetic menu without adverse implication on glycemic control is of utmost concern and such must be critically assessed in the laboratory before recommendation to the diabetics. Adulterated honey are marketed hence care must be taken when recommending honey to the diabetics. Some studies [16,17] however, suggested the co-administration of honey with oral hypoglycaemic agent to achieve good glycemic control. In this regards, the extent of bioavailability and interaction of such drugs with honey must be assessed critically before conclusion is reached.

Testicular histology, Sperm and reproductive hormones analysis in this study depicted the beneficial reproductive effect of honey as evidenced by the elevated level of testosterone, sperm count and enhanced spermatogenesis observed in the histoarchitecture of the testicular tissues in both diabetic and non-diabetic rats on honey diet. These findings agreed with the reports of other studies [18,19,20]. Recommendation of honey-based diet based on these findings may be considered especially in diabetics with reproductive dysfunction in other to enhance their fertility potentials. Results obtained from few studies on the chronic use of honey have been inconsistent. Thus, more investigations involving use of animal and human studies are necessary to determine the extent and rate of honey consumption or otherwise its incorporation in diabetic menu. Moreover, comparative studies on the health benefits of honey by direct oral administration or as part of a meal should be facilitated to ascertain the most suitable and beneficial way of honey consumption.

5. Conclusions

This study demonstrated the beneficial effect of honey-based diet on reproductive potential and the improved tolerance on glycemic control in diabetics when appropriately recommended in right quality and quantity. While further researches are being conducted on the use of honey in the treatment of various ailments, special consideration should be given to the diabetic individuals in terms of glycemic control and profile.

Acknowledgement

This work was carried out in collaboration between the authors. Author MMCA designed, supervised, performed the analysis and wrote the manuscript of the study. Author BJA assisted in provision of essential materials and collection of samples data while MOA prepared and interpreted the histological slides. All the authors read and approved the final manuscript.

Competing Interest

Authors have declare that no competing interest exist.

References

[1] Boivin J, Bunting L, Collins J.A and Nygren K.G, "International estimates of infertility prevalence and treatment-seeking: potential need and demand for infertility medical care". *Human Reproduction*. 22(6). 1506-1512. 2007.

[2] World Health Organization (WHO). "Infertility: A tabulation of available data on prevalence of primary and secondary infertility", *World Health Organization, Geneva, Switzerland*, 1991.

[3] Petrelli, G and Mantovani A, "Environmental risk factors and male fertility and reproduction". *Contraception*. 65. 297-300. 2002.

[4] Plas E, Berger P, Hermann M and Pflűger H, "Effects of aging on male fertility". *Experimental Gerontology*. 35. 543-551. 2000.

[5] Tartibian B and Maleki B.H, "The Effects of Honey Supplementation on Seminal Plasma Cytokines, Oxidative Stress Biomarkers and Antioxidants during 8 Weeks of Intensive Cycling Training". *Journal of Andrology*. 33(3). 2012.

[6] Estevinho L., Pereira A., Moreira L., Dias L. and Pereira E, "Antioxidant and antimicrobial effects of phenolic compounds extracts of Northeast Portugal honey". *Food and Chemical Toxicology*. 46. 3774-3779. 2008.

[7] Bazzocchi R, Grazia M, Bini A, Aurea P, Ussia G, "Bronchoplegic phenomena caused by vagal denervation". Experimental study. *Ann. Ital. Chir*. 60. 303-307. 1989.

[8] Harkness JE, Wagner JE. *Biologia e clínica de coelhos e roedores.* 3rd ed. São Paulo: Roca; 1993. 48-55.

[9] Cheesebrough M. "*Examination of semen. Microbiological tests. In: District laboratory practice in tropical countries"*, part 2, Cambridge University Press, United Kingdom. 2000.130-132.

[10] World Health Organization. "*WHO laboratory manual for the examination of Human semen and sperm cervical inverse interaction"* 4th Edition, Cambridge University Press, Cambridge. 1999.

[11] Watford, M. "Small amounts of dietary fructose dramatically increase hepatic glucose uptake through a novel mechanism of glucokinase activation". *Nutr. Rev*. 60, 253-257. 2002.

[12] Gheldof, N., Engeseth, N. J, "Antioxidant capacity of honeys from various floral sources based on the determination of oxygen radical absorbance capacity and inhibition of in vitro lipoprotein oxidation in human serum samples". *Journal of Agricultural and Food Research*. 50. 3050-3055. 2002.

[13] Born, B. "Carbohydrate malabsorption in patients with non-specific abdominal complaints'' *World Journal of Gastroenterology*, 13(43). 5687-5691. 2007.

[14] Bahrami M, Ataie-Jafari A, Hosseini S, Foruzanfar MH, Rahmani M, Pajouhi M, "Effects of natural honey consumption in diabetic patients: an 8-week randomized clinical trial". Int. J. Food Sci. Nutr. 60. 618-626. 2009.

[15] Erejuwa O.O, "The use of honey in diabetes mellitus: Is it beneficial or detrimental?" Int J Endocrinol. Metab. 10. 444-445. 2012.

[16] Erejuwa O.O, Sulaiman SA, Wahab MS, Sirajudeen KN, Salleh MS, Gurtu S, "Glibenclamide or metformin combined with honey improves glycemic control in streptozotocin-induced diabetic rats". Int. J. Biol. Sci. 7. 244-252. 2011.

[17] Sheriff M, Tukur M.A, Bilkisu M.M, Sera S and Falmata A.S, "The effect of oral administration of honey and glucophage alone or their combination on the serum biochemical parameters of induced diabetic rats". *Research in Pharmaceutical Biotechnology.* 3(9). 118-122. 2011.

[18] Mahaneem M, Sulaiman S.A, Jaafar H and Sirajudeen K.N.S, "Effect of honey on testicular functions in rats exposed to cigarette smoke". *J. ApiProd. ApiMed. Sci*. 3. 12-17. 2011.

[19] Abdul-Ghani AS, Dabdoub N, Muhammad R, Abdul-Ghani R, Qazzaz M, "Effect of Palestinian honey on spermatogenesis in rats". *J. Med. Food*. 11(4). 799-802. 2008.

[20] Salman TM, Alagbonsi IA, Olayaki LA, "Honey increases sperm count in male albino rats by enhancing testosterone production". *Biokemistri.* 25 (2). 39-44. 2013.