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Full Length Research Paper

Hypoglycaemia and improved testicular parameters in *Sesamum radiatum* treated normo-glycaemic adult male Sprague Dawley rats

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The development of a new dietary adjunct with a novel natural antioxidant impact on diabetes mellitus with prevention of its long term deleterious effect on the male fertility in general has been increasingly expressed in recent time. Hence, we aim to evaluate the effects of aqueous extract of *Sesame radiatum* leaves on adult male Sprague Dawley rats' testis using unbiased stereological, biochemical and hormonal studies. Thirty adult male rats were divided into three groups of 10 rats each. The treated groups; 1 and 2 received 28.0 and 14 mg/kg bwt of aqueous extract of sesame leaves via oral garvage, respectively, while the control group received equal volume of 0.9% (w/v) normal saline per day for 6 weeks. Serum follicle-stimulating hormone (FSH), testosterone and blood glucose were assayed. In addition five microns of uniformly random transverse sections of processed testicular tissues were equally analyzed using an un-biased stereological study. The result showed that the mean percentage volume fractions (Vf) of epithelial cells and lumen of the testis were 76% ($P<0.05$) and 22% ($P<0.05$), respectively, in the low dose compared to control. Conversely to the Vf of stroma and glucose level in treated, the mean raw weights of testis and of body weights (g) with the Vf of ST-epithelium in the treated groups were found ($P>0.05$) higher than the control in a dose related manner. Serum testosterone and FSH were significantly higher and lower, respectively, in the high dose sesame when compared to control. Sesame leaves intake improved glucose profile and testicular parameters in a dose related manner via possible improved insulin activity on the cells with a stimulatory impact on sperm production. This also confirmed its folkloric claims.

Key words: Sesame-phytoestrogens, testis, epithelial hyperplasia, stereology, glucose profile.

INTRODUCTION

The development of a new dietary adjunct or a novel natural anti-diabetic agent with a profound therapeutic effect on the long-term complications associated with various metabolic syndromes such as diabetes mellitus (DM) and obesity with the hope of restoring a normal metabolic condition in the body is been envisaged in recent time (Shittu et al., 2007a). Hence, increasing concern is hereby expressed about the need to identify an an ideal medicinal plant with a potent anti-metabolic impact and overall improvement of male infertility, a serious complication associated with diabetes mellitus that is now on the increase recently.

Moreover, there is an increasing preference expressed by many patients in recent time towards the popular use of alternative therapies that include diets, food supplements and herbal/folklore preparations with anti-diabetic activity. This is because of the much scientific evidence available nowadays to support their efficacies in the control of diabetes-related metabolic disorders and long-term complications as equally advocated by World Health

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Organization in their recommendations on DM (Shittu et al., 2007b; WHO, 1980).

In addition, the available synthetic anti-diabetic drugs suffer concomitant side effects and resistance because of their long duration of usage and current survey has equally indicated that cardiovascular mortality was higher in patients with oral hypoglycemics than in those treated with diet and exercise alone or with insulin. Moreover, these medicinal plants are cheap and readily available even in the rural community.

Today, diabetes mellitus just like obesity is no doubt far attaining an epidemic health-needs concern profile affectting all age groups worldwide and recognized many years now to be a clinical syndrome with multiple aetiology (Huerta et al., 2007; Mainous et al., 2007; Shittu et al., 2006a). This condition is characterized by symptoms such as weakness, increased appetite for food, excessive urination of sugar, excessive thirst as well as associated ketonuria and ketosis resulting from altered lipids and proteins metabolisms (Helal and Shahat, 2006).

Two types of DM exist characterized by either the absence of insulin (with associated hyperinsulinaemia) that is NIDDM-Type 1 or relative insensitivity to insulin (with associated hypoinsulinaemia) as in IDDM or type 2 (Mohamed et al, 2002). However, it is a chronic complex metabolic syndrome disease with no cure yet having various genetic determinants and associated with serious long-term morbidity and mortality worldwide.

World Health Organization (WHO) has recognized diabetes mellitus to be among the top global health problem and is the third leading cause of death after heart disease and cancer with about 75% of people estimated to be dying of diabetes-related complications from cardiovascular disease in the United States alone (Vuksan et al., 2001). The rising prevalence of Type 2-DM in children and adolescents is of concern. However, more worrisome nowadays is rising prevalence of undiagnosed (missed) type 2-DM in the general population and making up about 90-95% of all diabetic cases (Zhang et al., 2008).

However, the total number of people with diabetes has been projected to rise from 171 million in 2000 to 366 million in the year 2030 (Wild et al., 2004). The African continent has about 14 million people with diabetes and Nigeria having the highest prevalence with about 1,218,000 people diagnosed (International Diabetes Federation, 2006).

Pituitary hormones under the influence of hypothalamic hormones primarily control testicular function. They include the follicle-stimulating hormone (FSH) that regulates spermatogenesis, and the luteinizing hormone (LH) which controls Leydig cell function (Ward et al., 1991). However, alterations in the male reproductive system and fertility have been widely reported in individuals with diabetes, which is also on the rise and of serious medical concern.

Moreover, studies utilizing both animal and human models of diabetes have suggested that diabetic complications of impotence involved structural lesions in the testis (discrete ultrastructural lesions of the apical Sertoli cell cytoplasm with associated spermatogenic disruption and morphological changes in the interstitial compartment) as part of the overall defect in the pituitary-testicular axis (Don et al., 1985). This is however, thought to be part of microvascular complications, which is peculiar of diabetic metabolic syndrome in general (Don et al., 1985). Further studies have shown that these diabetes-related effects on testicular function may be attributed to the lack of insulin which has been found to have a regulatory action with a direct impact on both the Leydig cells (Khan et al., 1992; Hurtado de Catalfo et al., 1998) and Sertoli cells (Borland et al., 1984; Mita et al., 1985).

Moreover, other studies have shown that streptozotocin (STZ) induced diabetic male rats have decreased testicular testosterone production (Sanguinetti et al., 1995). This may be due to both a decrease in the total number of Leydig cells and in the rhythm of androgen biosynthesis by the remaining functional cells (Hurtado de Catalfo et al., 1998; Orth et al., 1979; Paz and Homonnai, 1979). In addition, other studies have also observed a decrease in the serum levels of pituitary hormones mainly the FSH, LH, prolactin, and growth hormone in diabetic models (Benítez and Pérez-Díaz, 1985; Hutson et al., 1983).

Study on the exact role that insulin plays in the regulation of the male reproductive function was carried out recently by Ballester and co-workers (2004). They showed that there was a significant decreased in serum LH, FSH, and testosterone levels, including a significant ($P < 0.05$) positive correlation between the serum levels of insulin and FSH only with no significant positive correlation between the serum levels of insulin or glucose and LH. They therefore concluded that Leydig cell function and testosterone production decreased because of the absence of the stimulatory effect of insulin on these cells and an insulin-dependent decrease in FSH, which, in turn, reduces LH levels. Moreso, sperm output and fertility were reduced because of a decrease in FSH caused by a reduction in insulin (Ballester et al., 2004).

Sesame diet is the richest food source of lignans, a major type of phytoestrogens (Thompson et al., 1991). It is an important part of some diet in Africa especially in Nigeria because of its nutritive nature and in view of their reported health benefits (Konan et al., 2008; Shittu, 2006; Shittu et al., 2006a; Shittu et al., 2007a, b, c; Shittu et al., 2008). Sesame plant is used in African and Asian folk medicines for the treatment of various ailments (Shittu, 2006; Shittu et al., 2006a) and has different names depending on the areas where they are cultivated, such as ekuku–gogoro (Yoruba name for *Sesamum radiatum*), ridi (Hausa) and beni or gingelly (English) (Gill, 1992).

In Nigeria alone, about 335,000 ha of land is cultivated for sesame with yields of 1.5 to 2.0 ton/ha annually (Ahmed, 2005). However, sesame is generally cultivated in the tropical and subtropical areas on 6.5 million hectares of land worldwide (Laurentin and Karlovsky, 2005). All parts of sesame plant (the leaves, seed and oil) are useful and is locally consumed as staple foods for many years now by rural subsistence farmers in south – west, middle belt and northern areas of Nigeria where they are largely cultivated (Shittu, 2006; Shittu et al., 2006a). Moreover, the seeds are consumed either through its oil, roasted or as local pastes in food. The oil is used for culinary purposes and as carrier ingredient of herbal and synthetic preparations used in pharmaceutical industries (Jayalekshmy et al., 2001; Shittu, 2006). While, the decoction of the leaves and roots serve various traditional and medicinal purposes (Bankole et al., 2007; Shittu, 2006, Shittu et al., 2006a; Shittu et al., 2007b).

Phytochemically, the sesame plant is rich in phenolic compounds (phenols, sterol, lignans and flavonoids), non-protein amino acids, cyanogenics glycosides, alkaloids, polyunsaturated fats and lipids, mucilage, phospholipids, and vitamins B_1, B_2, C and E. Others include trace elements/minerals such as calcium, iron, magnesium, zinc, copper and phosphorus (Konan et al, 2008; Obiajunwa et al, 2005; Shittu, 2006; Shittu et al., 2006a). In addition, GC/MS, HPLC and other extractive processes of sesame plants (seed, oil and leaves) have shown the presence of lignans mainly sesamin, sesamolin, sesaminol, sesamolinol, pinorescinol and sesamol (Jayalekshmy et al., 2001; Laurentin and Karlovsky, 2005; Namiki, 1995; Nesbitt et al., 1999; Penalvo et al, 2005; Shittu et al., 2006a; Shittu et al., 2007c).

Recent study on bioavailability of sesame plants intake in humans showed that plant lignans undergo extensive metabolism in the intestine depending on the individual characteristics of the intestinal microflora to produce mammalian lignans-enterolactone. Sesame lignans undergo entero-hepatic circulation, conjugated in the liver via absorption from the lateal system (lymphatic duct) to reach peak in less than 2 h with half life of less than 6 h in the blood. Moreover, plasma lignans concentrations do show a linear correlation with urinary excretion of lignans (Penalvo et al., 2005). Previous studies have shown that aqueous *S. radiatum* leaves extract may acts via aromatase inhibition, estrogen receptors binding with transactivation of androgen receptors (Shittu, 2006; Shittu et al., 2007b; Shittu et al., 2008), as well as free radical scavenging/antioxidant property (Bankole et al., 2007; Shittu et al., 2006a; Shittu et al., 2007a; Shittu et al., 2008).

Due to paucity of knowledge, we aim to confirm the folkloric claim and evaluate the impact of aqueous sesame leaves extract consumption on the testicular tissues of a non-diabetic adult male Sprague dawley rats using biochemical, hormonal and stereological techniques.

MATERIALS AND METHODS

Preparation of aqueous extracts of sesame leaves

Sesame plants (*Sesamum radiatum*, Schum and Thonn – Pedaliaceae family) were bought from a vendor in Agege market, Lagos in May 2005. The plant was authenticated by the herbarium section of Forestry Institute of Research (FRIN) with FHI # 107513 on the 5^{th} of August, 2005 (Shittu et al., 2006a). Voucher specimens were deposited in Botany Departments of University of Ibadan and Lagos State University.

The leaves of the plants were air dried for 2 weeks and powdered. Then, 100 g of the powdered leaves added to 1.0 litre of distilled water at a ratio of 1:10 in a beaker and allowed to boil to boiling temperature after intermittent stirring on a hotplate for one hour. The decoction was filtered into another clean beaker using a white sieve clothing material and the filtrate evaporated at 50^oC to dryness in a desiccator to produce a black shinning crystal residue form with a yield of 83% w/w of the extract. The crude extract was kept in the refrigerator (4^oC) before being reconstituted and used for the *in vivo* study.

Animal experiment

All procedures involving animals in this study conformed to the guiding principles for research involving animals as recommended by the Declaration of Helsinki and the Guiding Principles in the Care and Use of Animals and approved by the Departmental Committee on the ethics and research American Physiological Society (2002).

Thirty mature and healthy adult male Sprague-Dawley rats weighing 120 to 250 g were procured from the Animal House of Lagos State University, College of Medicine, Ikeja and housed in a well ventilated wire-wooden cages in the departmental Animal House. They were maintained under controlled light schedule (12 h Light: 12 h Dark) at room temperature (28^oC) and with constant humidity (40-50%). The animals were acclimatized for a period of 14 days before the start of treatment. During this period, they were fed with standard rat chows/pellets supplied by Pfizer Nigeria Ltd and water *ad libitum*. Ear tag was used for the individual identification of the entire group animals.

The rats were randomly divided into three groups of 10 rats each. The treated groups received 28.0 and 14 mg/kg body weight of aqueous extract of sesame leaves via oral garvage. While the control group received equal volume of 0.9% (w/v) normal saline per day for a period of 6 weeks. All procedures involving animals in this study conformed to the guiding principles for research involving animals as recommended by the Declaration of Helsinki and the Guiding Principles in the Care and Use of Animals and approved by the Departmental Committee on the ethics and research.

The rats were anaesthetized after an overnight fasting using previously described method (Shittu et al., 2007b). Weekly weighing of the animals was carried out all through the experimental period.

Biochemical Indices

Blood glucose levels were determined by glucose oxidase method as described by Trinder (1969). The estimation of serum testosterone and FSH were carried out using the procedure enclosed with the specific commercial kit purchased from Amersham International Plc. Buckinghamshire, United Kingdom) as previously described in our study (Shittu et al., 2007b; Shittu et al., 2008).

Testes

The testes were initially dissected out whole via midline abdominal incision, cleared of fats and blotted dry. Their weights were measured on a sensitive digital balance with volume measured by water

Table 1. Summary of body and testicular weights of animals.

Group	Pre-experimental body weight (g) (mean ± SD)	Final body weight (g) (mean ± SD)	Testicular weight (g) (mean ± SEM)	Testiculo-somatic weight (g/100 g bwt) (mean ± SEM)
Control	127.3 ± 5.55	185.2 ± 11.05*	0.55 ± 0.03*	0.67 ± 0.01
High sesame dose	206.2 ± 6.45	248.2 ± 14.40*	0.76 ± 0.01*	0.46 ± 0.02*
Low sesame dose	186.3 ± 1.99	219.8± 4.47*	0.57 ± 0.02*	0.52 ± 0.02*

N = 10 rats per group. *P < 0.05 was considered significant statistically.

displacement using a 10 ml measuring cylinder. Later, the sizes (length and width) were recorded by use of a sliding gauge (d=0.1) before eventually fixed in freshly prepared 10% formol saline solution as earlier described (Shittu et al., 2007b). The two testes of each rat were measured and the average value obtained for each of the parameters was regarded as one observation.

Light microscopy

Serial paraffin sections of 5 µm were obtained from fixed processed testicular tissues blocks and stained with Haematoxylin and Eosin (H and E) stains as prepared and previously described in our earlier studies (Shittu et al., 2006b; Shittu et al., 2007b).

Morphometric parameters

The following morphometric parameters: testicular volume and weight; diameter; and cross-sectional area of the seminiferous tubules, were determined. The diameter (D) of seminiferous tubules with profiles that were round or nearly round for each animal was estimated. A mean, D, was taken as the average of two diameters, D1 and D2 (D1 is the short axis while D2 is the long axis; both D1 and D2 are perpendicular to each other). D1 and D2 were considered only when ratio of D1:D2 or D1/D2 > 0.85 (Form factor) (Shittu et al., 2006b).

The cross-sectional areas (A_C) of the seminiferous tubules were determined from the formula $A_C = \pi D^2/4$, (where π is equivalent to 3.142 and D = the mean diameter of the seminiferous tubules) (Shittu et al., 2006b).

Determination of stereological parameters

Serial transverse sections of 5 µm of the H and E-stained specimens prepared were subjected to un-biased stereological techniques modified from previous report (Mouton, 2002; Shittu et al., 2006b). Each image of the seminiferous tubules at a magnification of 400 X was projected and drawn on a 16-point grid, completely counted in six different fields to make a total of 96 point-test grid for each of the systemic randomly selected section such that 5 sections/rat were taken from each group. Manual point intercept counting methods consist of a counting grid made up of a series of crosses in a regular and uniform square array. The density of crosses was such that one cross represented an area of 4 cm^2 on the counting grid. The total number of crosses (circled or otherwise) falling on each structure per each section of specimen was counted and then added-up to get the final estimated result.

Using this procedure, the volume density of the stroma, epithelia lining and tubular lumen of seminiferous tubules were estimated as previously described by Weibel and Gomez (1962). The percentage volume density was determined by multiplying the volume density by 100.

Statistical analysis

The weight data were expressed in mean ± SD (standard deviation) while other data were expressed as mean ± SEM (standard error of the means). Comparisons between groups were done using the student's *t*-test and non-parametric Mann-Whitney U test. All the data were inputted into SPSS 12 software Microsoft computer (SPSS, Chicago, Illinois). Statistical significance was considered at p<0.05.

RESULTS AND DISCUSSION

There was evidence of significant (p<0.05) weight gain was observed in all the animals. The raw testicular weight was significantly (p<0.05) higher while, the relative testicular weight decreased significantly (p<0.05) in the sesame-treated group as compared to the control in a dose dependent manner as reflected in Table 1. There is a significant (p>0.05) increase in testosterone level and a significant (p<0.05) decrease in FSH level of the high dose sesame-treated compared to control. However, the low dose showed significantly lower hormonal values difference from the control as shown in Table 2.

Blood glucose level in the sesame treated animals were significantly (p<0.05) lower than the control in adose-dependent manner. However, these values were within the normal range for adult rats as shown in Table 2.

The percentage mean volume density of the tubular lumen increased (p<0.05) by 225%, interstitial activity increased (p<0.05) by 107% while that of the epithelial height deceased by 48% (p<0.05) in the high sesame-treated rats when compared to the control (Table 3). The mean diameter of the seminiferous tubules of sesame-treated groups was significantly higher (p< 0.05) than that of the control in a dose dependent manner as shown in Table 3.

There is increasing role of sesame lignans research contribution to medicine and sesame, and being rich in antioxidant lignans (phytoestrogens) have the ability of improving fertility potential of the male reproductive tract as shown in our previous studies (Shittu et al., 2007; Shittu et al., 2008). Sesame contains abundant lignans such as lipid-soluble lignans (sesamin and sesamolin), sterol and water-soluble lignan glycosides (sesaminol triglucoside and sesaminol diglucoside) with varied potent antioxidative activity (Jayalekshmy et al., 2001; Shittu et al., 2006a, Konan et al., 2008).

Table 2. Serum hormonal profile and blood glucose profile of the animals.

Hormone	Control group	High dose sesame group	Low dose sesame group
FSH (IU)	11.0 ± 2.3*	6.3 ± 0.6*	2.1 ± 0.2*
Testosterone (ng/ml)	0.8 ± 0.03	0.9 ± 0.2*	0.1 ± 0.0*
Blood Glucose (mg/100 ml)	105.0 ± 1.51	72.0 ± 32.0*	94.8 ± 7.15*

N = 10 rats per group. *P < 0.05 was considered significant statistically.

Table 3. Effects of *Sesamum radiatum* leaves extract on volume fraction (Vf) of testicular tissues profile and diameter of seminiferous tubule of Sprague-Dawley rats.

Group	Interstitium	Surface epithelium	Tubular lumen	Seminiferous tubular diameter (µm)
Control	0.14 ± 0.06	0.82 ± 0.06	0.04 ± 0.01	295.5 ± 5.0
High dose Sesame	0.29 ± 0.04*	0.49 ± 0.04*	0.13 ± 0.04*	311.7 ± 10.4*
Low dose Sesame	0.05 ± 0.01*	0.84 ± 0.04*	0.09 ± 0.03*	315.6 ± 5.1*

N = 10 rats per group. *P < 0.05 was considered significant statistically.

However, exogenous phytoestrogens have been shown to be generally less potent in its estrogenic activities than their endogenous counterparts, hence their environmental exposure has been regarded as non harmful and even beneficial (Whitten and Naftolin, 1998). In addition, phytoestrogens have constituted the staple diets in Eastern populations for hundreds of years now with no documented toxicity (Adlercreutz et al., 1991; Shittu, 2006). Studies have shown that decoction process removes poisons/toxins from plant making them to have better phamacological activity than the ones obtained with maceration, such was the case where ethanolic and methanolic extracts are more effective in their anti-microbial activities than the aqueous extract (Ahmed et al., 2007; Bankole et al., 2007; Mohamed et al., 2002; Shittu et al., 2007a).

We have earlier reported the anti-lipidaemic effect of aqueous crude leaves extract of sesame in a non-obese rat (Shittu et al., 2007c). Moreover, sesame plant consumption is used traditionally for treating and countering the symptoms of diabetes mellitus (Atkinson 1979; Palaiseul, 1983). In addition, sesamum is popularly used in folk medicine of the Southern Nigeria to treat diabetes. This study reports for the first time the scientific basis for its use in diabetic impotent and low sperm management.

Diabetes mellitus as a chronic metabolic disease affects virtually every organ or system in the body such that the heart, pancreas, kidneys and eyes among others are the most severely affected organs especially seen during early stage of the disease (Alberti and Press, 1982). Nevertheless, widespread pathological changes such as thickening of capillary basement membrane, increase in vessel wall matrix and cellular proliferation have in no doubt become the sequel of chronic hyperglycemia in diabetes. In addition, all these pathological changes culminated into development of both micro- and macro-vascular complications. Although, this vascular complication is mediated via increased oxidative stress activity, it thus constitutes an important aetiopathogenetic factor responsible for most of the associated symptoms and complications found in diabetes, which include lumen narrowing, early atherosclerosis, myocardiac infarction, sclerosis of glomerular capillaries, retinopathy, neuropathy and peripheral vascular insufficiency (Jakus, 2000). It is this peripheral vascular insufficiency involving the testicular vessels among other factors that may be responsible for the ultrastructural testicular damages seen in diabetic male patients (Don et al., 1985).

Studies have equally suggested that chronic hyperglycemia in diabetes leads to excess production of reactive oxygen species (ROS), lipid peroxidation (LPO) caused by glucose auto-oxidation, glycol-oxidation, and protein glycation , thereby leading to severe tissues damages (Hunnt et al., 1999; Perkiner et al., 2002). Such may involve the testicular tissues, for example. Thus, antioxidants have also been found to play a crucial role to protecting the body against damage by reactive oxygen species and their role in diabetes have been equally evaluated, such that it has now been established that many plant extracts and their products possess significant antioxidant activity (Babu et al., 2006; Shittu, 2006a).

Most of the biochemical damaging effect of diabetes only happens when the endothelial linings of the cellular membrane/endothelium are damaged, which is usually mediated through various mechanisms such as nitric oxide action produced during ROS activity. Sesame lignans are known to counteract oxidative damage induced by reactive oxygen species during Fe^{2+}-induced oxidative stress in the body (Hemalatha et al., 2004). Konan and co-workers (2008) have demonstrated that the protective mechanism, that is, the vasoprotective and cardioprotective activities of aqueous sesame leaves extract are via cyclo-oxygenase pathway with enhanced potassium and nitric oxide activity in an intact endothelial lining of the cardiovascular system. Sesame lignans have also been shown to increase tissue tocopherol levels by inhibition of cytochrome P450 3A-dependent n-hydroxylasse pathways of tocopherol catabolism and/or regeneration of oxidized tocopherol (Hemalatha et al, 2004).

In this present study, we have used matured adult male rats because morphometric study by light microscopy is best performed when the organ has reasonably developed to a sizable dimension (Shittu et al., 2006b). In addition, rat appeared to be a suitable animal model for studying the roles of the androgenic hormones within the male reproductive system, in that it operates on a two-way androgen model of sexual differentiation (DHT and testosterone). Unlike, in mouse model which is dependent on testosterone action alone for the differentiation of the male urogenital tract (Shittu et al., 2006b). We now know that the combination of a well characterized animal model with stereological techniques always allow for proper quantitative study of any hormonal impact on male reproductive system (Shittu, 2006, Shittu et al., 2006b). In this study, we had applied stereological techniques on the adult matured rat testis with hormonal assay including blood glucose estimation done in order to appreciate differential impact of sesame leaves extracts on normo-glycaemic rats.

All the animals showed evidence of weight gained in a dose dependent manner compared to control with no obvious signs of toxicity observed. This is similar to our previous findings (Shittu et al., 2007b; Shittu et al., 2008). The sesame treated-animals have significant increased in the raw testicular weight. However, there was a significant reduction in the relative testicular weight as compared to control in a dose dependent manner. These differential changes in the weight of testes were well correlated with the seminiferous tubular profile of the testes for each group of animals as observed in our previous study (Shittu, 2006b).

It is clear that the tested aqueous leaf extract of sesamum induced mild significant ($p<0.05$) dose-dependent reductions in fasting blood glucose concentrations in the normo-glycemic rats. This effect is also observed in another medicinal plant, *Artemisia dracunculus* with demonstrable anti-diabetic effects (Ribnicky et al., 2006). A similar study has shown that a moderate significant reduction in insulin and/or glucose concentrations in normal animals could cause hypoglycemia, a common side effect of diabetic therapy especially with synthetic oral hypoglycaemia (Davidson, 1998). This is not the case in this present study and it is therefore noteworthy that sesamum leaves did not severely affect blood glucose levels or insulin concentrations by bringing it to a level that is above the lower limit of normal range for a normo-glycaemic rats.

This effect of induced insulin usage by sesame in the SD rats is most likely be due to the active phyto-estrogenic lignans (with potent antioxidative property) present in sesame leaves extract which resulted in increase in the effectiveness of the insulin (decrease in insulin resistance), thereby enhancing glucose uptake into peripheral tissues (that is, muscle or adipose) and decreasing glucose output. This may suggest potentially multiple modes of action for sesame leaves extract when compared to other known synthetic anti-diabetic drugs such as troglitazone and metformin, which increase insulin sensitivity rather than insulin concentration as seen in other studies (Stumvoll and Haring, 2002).

Sesame seed contain about 50-60% oil and 2.7 to 6.7% dietary fibre, which is even higher in the defatted seed powder (Beckstrom-Sternberg and Duke, 1994; Kato et al., 1998). However, fibers present in the seeds are known to slow down gastric transit time, thereby slowing the rate and extent of glucose and cholesterol absorption in the gut and contributing to the hypoglycemic benefits of sesame (Kochar and Nagi, 2005; Shittu et al., 2007b). Also, sesame oil is shown to be beneficial in improving the blood glucose, glycosylated hemoglobin, lipid peroxidation, and antioxidant levels in streptozotocin (STZ) induced diabetic female rats (Ramesh et al., 2005).

Study has shown that tissues-sections usually are compressed by 83% of their original dimension during processing, thereby necessitating the need of a correction factor. However, volume density parameters are not affected by this compression (Mouton, 2002) and thus, more suitable for stereological analysis in the present study as previously applied in an earlier study (Shittu et al., 2006b). For stereological and morphometric analyses of a normal Sprague dawley rat semini-ferous tubules, evidence has shown that changes involving the diameter of the seminiferous tubules as well as the volume of the seminiferous tubules and epithelium occurred from stage to stage during the spermatogenesis cycle. However, from stage V to stage VII, the volume of the seminiferous tubule lumen dramatically increased at the expense of the volume of the semini-ferous epithelium (per unit length of the tubule), which peaked at stage V of the next cycle (Wing and Christensen, 1982). This is similar for the control in the present study as shown in Table 3. We found that the percentage volume densities of the seminiferous tubular lumen significantly increased in the sesame treated groups in a dose dependent manner. The volume densities of the tubular interstitium of high dose and low dose sesame-treated groups were significantly higher and lower, respectively, than the control group. However, the reverse was the case in the volume densities of surface epithelial height of sesame treated groups such that low dose has significantly higher epithelial height and activity than the controls as shown in Table 3.

Thus, it is not surprising that spermatogenic activity and sperm output were significantly higher and numerous over 100% compared to the control in our previous studies (Shittu et al., 2007b; Shittu et al., 2008).

In diabetic rats, there are evidences of a reduction in seminiferous tubule diameter, increased thickening of the basement membrane in seminiferous tubules, severe gem cells depletion or degeneration and Sertoli-cell vacuolization. This is usually preceded by primary Leydigs cell dysfunction (Guneli et al., 2008; Murray et al., 1983). In this study we found that the seminiferous tubular diameters in the sesame treated groups were significantly higher than in the control group in a dose dependent manner. This implied enhancement of spermatogenic activity in the cells and the positive impact of sesame leaves consumption in reversing the delete-rious effects of diabetes.

The large tubular diameter observed in this study also confirmed that the low dose sesame impact is more pronounced on the testis than the epididymides, which are predominantly ERα sites (Shittu et al., 2007b). However, both α and β estrogen receptors (ERs) do co-exist naturally. Because sesame are exogenous phytoestrogens, they tend to promote aromatization of testosterone to estrogen (E2). This is such that the low dose sesame makes less endogenous estrogens avail-able and compete less, that is may serve as non-competitive substrate to endogenous estrogens. This is the reverse for the high dose sesame, which will cause more E2 production and compete more if possible as anti-estrogens. Moreover, low dose E2 is needed for spermatogenesis than the high dose (Shittu, 2006). There is an increase interstitial space and hyper-chromatic Leydig cell seen in high dose sesame, unlike in the low dose sesame that has a narrower interstitial space with scanty Leydig cells. This may have accounted for the significantly lower testosterone level (0.1± 0.0*) in the low dose sesame group compared to the control group (0.8 ± 0.03) as shown in Table 2.

The low testosterone level observed in the low dose sesame could also be as a result of the fact that some T were irreversibly aromatized to E2 and or converted to DHT by the aromatase and reductase enzymes, respectively. Although, significantly higher testosterone level is found in the high dose sesame (0.9 ± 0.2*) compared to control (0.8 ± 0.03) as shown in Table 2. Studies have shown that both FSH and testosterone play a role in the spermatogenic process as observed in the present study. For example, FSH is involved in increasing spermatogonial number and the maturation of spermatocytes, including meiosis. While, spermatid maturation is essentially testosterone-dependent, a step that cannot be completed in-spite of high FSH. However, testosterone and FSH are known to act synergistically in spermatogenesis. It is possible that as FSH begins to rise, it gets to a peak during the cycle after which any further increase as seen in the control group would have no impact on spermatogenetic activity (Shittu, 2006; Shittu et al., 2008).

Moreover, it was found that residual spermatid maturation occurred despite the presence of testosterone level is below that needed for the spermatid maturation in testosterone-estradiol treated rats, which was the case in the low-dose sesame group with testosterone value at 0.1 ± 0.0 as shown in previous studies (Meachem et al., 1997, Shittu et al., 2007b; Shittu et al., 2008).

Study has also shown that estrogen is responsible for maintaining the differentiated epithelial morphology through an unknown mechanism .Thus, estrogen or its receptor is important for normal functioning of the male reproductive tract in numerous animal species including humans (Hess and Carnes, 2004). Since sesame is a known phytoestrogens that bind to the estrogen receptors in the testis, one expects that it would stimulate spermatogenesis via the following mechanisms such as increased epithelial proliferation and tubular diameter; increased luminal diameter, especially from stages V to VII of spermatogenesis (Wing and Christensen, 1982). In addition, mild reduction in the interstitial space and activity as shown in Table 3 and in other similar studies (Shittu et al., 2007b; Shittu et al., 2008). The findings of Oliveira et al. (2003) where ER-α was selectively blocked with estrogen inhibitor resulting in testicular atrophy and infertility are in sharp contrast to what was observed in this present study.

The low glucose level attained in the sesame treated groups implied that there is stimulation of insulin or insulin action being produced by its active ingredients including phytoestrogens present in the sesame leaves extracts. This similar effect was equally obtained in sesame oil treatment on blood glucose in STZ diabetic female rats (Ramesh et al., 2005). When the dose of sesame was doubled (from 14 to 28 mg/kg body wt.), the level of glucose significantly reduced from 94.8 ± 7.15 mg/100 ml to 72.0 ± 32.0 mg/100 ml, which implies a corresponding increase in insulin level according (Wadood et al., 2007; Punitha et al., 2006). FSH level also significantly increased from 2.1 ± 0.2 IU to 6.3 ± 0.6 IU and since insulin is positively correlated with FSH (Ballester et al., 2004) and contrary to their findings, we found increase in insulin activity with increase in FSH and leydig cells stimulation in this present study. It is possible that sesame stimulation on pancreatic β-cells (probably by supplying necessary elements like Mg^{2+}, Ca^{2+}, Cu^{2+} for proper functioning) led to increase secretion of insulin (decrease glucagon and glucose level) which in-turn stimulated the HPO axis to produce more FSH and/or LH. Both the FSH and LH interacted with the sertoli cells and leydig cells, respectively, to produce an increase in testosterone secretion with enhanced spermatogenesis as seen in the present study.

This positive effect of sesame leaves may also be attributed to its positive influence on the hypothalamic pituitary-testicular (HPO) axis (Shittu et al., 2007b; Shittu et al., 2008) and antioxidative activity of its phytoestrogenic lignans constituents, among others (Jayalekshmy et al., 2001; Hemalatha et al., 2004). It is also possible that sesame extract reduces the peripheral utilization of glucose and/or reduce intestinal absorption of glucose, or enhance stimulation of glucogenogenesis and/or inhibittion of the glycogenolysis process in the liver (Mohamed et al., 2002). However, its antioxidant activity stabilizes the cellular membranes of cells (Hemalatha et al., 2004; Konan et al., 2008; Shittu et al., 2006a). This is usually the target sites for most ROS activities (released from impaired metabolism of glucose) via the production of various biochemical substances among which is the nitric oxide, responsible for most of the damaging effects of diabetes mellitus especially at bio-molecular levels. Further study is on to appreciate the particular active elements in sesame that is responsible for the positive activity in the diabetic rats.

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