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A Comparative Study of the Effects of Ethanolic Extract of *Sesamum indicum* Seeds and Mesterolone on Some Testicular Morphometry of Adult Male Wistar Rats Secondary to Ketoconazole-Induced Damage

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Abstract

Aim: The study aimed at comparing the effects of ethanolic extract of *Sesamum indicum* seeds with mesterolone on some testicular morphometry following ketoconazole induced damage in Wistar rats.

Methods: Thirty-six (36) adult male Wistar rats were divided into six (6) groups of six rats each. Groups 1,2,3,4,5 and 6 were administered 1 ml of normal saline, 100 mg /kg body weight (bwt) of Ketoconazole only, 500 mg/kg bwt of Ethanolic Extract of *Sesamum indicum* (EESI) seeds then 100 mg/kg bwt of Ketoconazole, 100 mg/kg bwt of Ketoconazole then 250 mg /kg body weight of EESI seeds, 100 mg/kg bwt of Ketoconazole then 500 mg/kg bwt of EESI, 100 mg/kg body weight of Ketoconazole, respectively. All administrations were carried out orally and once per day. The histological slides were prepared for morphometric analyses.

Results: No observed significant differences in the morphometry of the groups administered EESI and Mesterolone. However, the morphometry of the group administered EESIS and Mesterolone were significantly increased when compared with group 2 alone ($P \leq 0.05$).

Conclusions: The effects of the administration of ethanolic extract of *Sesamum indicum* seeds on the morphometry of the testis appeared to be indifferent with Mesterolone; and both EESIS.

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Mesterolone also appeared to have minimize the distortion of the normal dimensions of the testis caused by ketoconazole which may be due to their androgenic properties.

Keywords

Evaluation; Sesamum; Morphometry; Testicular; Ketoconazole; Rats

Introduction

Adverse effects of drugs among other factors are known to affect normal body functions and most especially reproductive health [1]. One of such drugs is Ketoconazole, an antifungal drug [2].

Ketoconazole is packaged pharmaceutically in the form of tablets, creams and injections and has been reported to inhibit the stimulation of testicular testosterone production by Human Chorionic Gonadotropin (HCG) in a dose dependent manner [3]. Steroids such as dihydrotestosterone and estradiol are equally, selectively displaced from serum-binding globulin by ketoconazole [4]. The suppression of testicular testosterone synthesis and the displacement of oestrogen from sex hormone binding globulin may decrease the androgen/oestrogen ratio of the blood and contributes to the development of gynaecomastia that has being reported in some ketoconazole treated patients [4]. Administration of ketoconazole alters sperm indices.

Over the years, there has been increased scientific research to minimize the health hazards potentiated by some toxins and this was done on phytochemicals extracted from plant species [5]. In an effort to stem the contraindications of some of these synthesized drugs, plant medicine (phyto-medicine) has been used as alternatives in many parts of Africa and the rest of the world [6].

One of such phyto-medicine is *Sesame indicum*. Sesame seeds are reported to contain high level of unsaturated fatty acids such as Oleic acid-38.84% and linolenic acid-46.26 % [6]. Trace elements such as Calcium, Iron, Magnesium, Zinc, Copper and Phosphorous were also reported to be contained in it [7]. Sesame seeds are also reported to be rich in phyto estrogenic lignans which is an important phytochemical known to man [8]. The entire sesame plant is very valuable and serves as staple food in most ethnic groups in North-Central and South-Western part of Nigeria [8].

Materials and Method

Seed procurement and identification

Amount of 470 g of *Sesamum indicum* seeds were obtained from Samaru market- Zaria, Kaduna State, Nigeria and authenticated in the herbarium of the Department of Biological Sciences, Ahmadu Bello University Zaria and assigned voucher no. 4. The seeds were macerated using 95% ethanol to obtain the extract with a yield weight of 46.42 g in solvent form.

Drugs and Reagents

Two hundred (200) mg of ketoconazole tablets and 25 mg of Tablet Provirone (Mesterolone) manufactured by Divine New Favour Pharmaceuticals (Anambra State) and Bayers Pharmaceuticals (United Kingdom) with batch Nos. 82339737 and 400 8500 060056 and NAFDAC Nos. A4-2186 and 04-1300, respectively, were purchased from Micro novo Pharmaceutical Company Plc. Kano, Kano State, Nigeria.

Methods

Extraction of Sesame Seeds

Amount of four hundred and seventy (470) g of sesame seed was macerated in a maceration apparatus. The sesame seeds were poured into 2 ml maceration apparatus (Pyrex) and the seeds coats were manually removed. The macerated seeds were later transferred into a three and half (3.5) lt beaker containing 95% v/v of ethanol to cause the removal of flavanoids and lignans. It was stirred and left for six hours to allow the extraction of polar substances. The supernatant was sieved off using a metallic sieve and the filtrate was collected into a separate beaker. The filtrate was collected into an evaporating dish with the use of a spatula and placed on a water bath, heated to a temperature of 60°C to completely remove the ethanol. The extract was kept for eighteen hours to allow for evaporation to take place. The yield weight of the extract was 46.42 g in solvent form.

Experimental Procedure

The LD₅₀ the ethanolic extract of *Sesamum indicum* seeds in wistar rats was researched and found to be above 5000 mg/kg bwt meaning that the extract is safe.

The equivalent dose of the drugs and the sesame seed extract; in ml (milliliters) were derived using the formulae:

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$$\text{Volume (ml)} = \frac{\text{Dose} \times \text{weight (kg)}}{\text{Stock concentration}}$$

The equivalent dose in ml of ethanolic extract of *Sesamum indicum* seeds was calculated as follows: 500 mg/kg bwt = 500 mg = 1000 g, the average weights of the rats at the time of commencement of the experiment was 180 g; therefore, the administrable dose (x) for a rat that weighed 180 g was calculated as:

$$\text{Volume} = \frac{500 \text{ mg/kg} \times 0.180 \text{ kg}}{128.3 \text{ mg/ml}}$$

0.70 ml was obtained as the volume for a dose of 500 mg/kg bwt which was the high dose group. Similar method was used to calculate the volume of the low dose group (250 mg/kg bwt) and the value 0.35 ml was obtained. Tablet mesterolone was prepared as 25 mg and it was the recommended dose for an adult person (Average weight = 70 kg).

From above, the administrable dose per 1 kg was calculated as shown below

25 mg (mesterolone) = 70 kg = 70000 g; therefore, the dose (x) for every 1 kg = 1000 g is calculated as follows:

$$x = \frac{25 \text{ mg} \times 1,000}{70,000} = 0.36 \text{ mg/kg bwt}$$

From above, the administered dose for a rat that weighed 180g was calculated as:

$$\frac{0.36 \text{ mg} \times 180 \text{ g}}{1000 \text{ g}} = 0.065 \text{ mg}$$

Earlier, it was stated that the packaged form of mesterolone is 25 mg per tablet.

The tablet was dissolved in 10 ml of distilled water to obtain a concentration of 2.5 mg/ml. from above, 2.5 mg is contained in 1 ml, x ml will contain 0.065 mg. By calculation,

X = 0.065/2.5 = 0.026 ml, therefore, 0.026 ml which contains 0.065 mg of mesterolone tablet was measured using a micropipette and was administered to the rats.

Animal Sacrifice

The animals were put to death via perfusion technique, the left testis was excised and immersed in a solution of 10% formalin and prepared histologically for the determination testicular morphometric parameters.

Statistical Analysis

All data obtained were expressed as mean $\pm$ Standard Error of Mean, (SEM). One way Analysis of Variance (ANOVA) was used to compare the mean difference between and within the groups and a P- value of ≤ 0.05 was considered statistically significant, a post-Hoc was carried out to indicate where significant difference lie. Statistical analysis was carried out using Statistical Package for Service Solution (SPSS) Version 20.

Morphometric Analyses

Luminal and Epithelial Dimensions of the Seminiferous Tubules

The longitudinal and transverse dimensions of the lumen of the seminiferous tubules were measured with an ocular micrometer and a stage micrometer. The ocular micrometer made up of vertical and horizontal grid lines was inserted in a $\times 400$ objective lens and aligned with the calibrated stage micrometer. The length of the un-calibrated ocular micrometer was estimated from that of the stage micrometer after alignment. The stage micrometer was replaced with slides containing tissue sections and the luminal dimensions were obtained from the estimated distances on the ocular micrometer. The part of the lumen of the seminiferous tubule that corresponded with the horizontal axis of the grid line on the ocular micrometer was considered as the transverse dimension and the part that corresponded with the vertical axis was considered as the longitudinal dimension. The same procedure was used in measuring the epithelial height as shown in Fig. 1. The height of the epithelium of each seminiferous tubule was determined with an ocular micrometer and the mean for each group was obtained, Fig. 2-7.

Figure 1: Histogram representing drug administration.

(NS: Normal Saline, KTHD: Ketoconazole High Dose, SS: Sesamum Seeds, KTLD: Ketoconazole Low Dose Group, MST: Mesterolone).

Figure 2: A section of the testis obtained from group 1. (Arrow indicates luminal dimensions (Longitudinal dimension LD; 2.75 μ m, Transverse dimension LD; 2.92 μ m and epithelial height EH 2.92 μ m (Masson Trichrome stain $\times$ 250).

Figure 3: A section of the testis obtained from group 2. (Longitudinal dimension LD; 6.17 μ m, Transverse dimension LD; 4.83 μ m and epithelial height EH 2.92 μ m (Masson Trichrome stain $\times$ 250).

Figure 4: A section of the testis obtained from group 3. (Longitudinal dimension LD; 2.00 μm , Transverse dimension LD; 8.17 μm and epithelial height EH 2.92 μm (Masson Trichrome stain $\times 250$).

Figure 5: A section of the testis obtained from group 4. (Longitudinal dimension LD; 4.33 μm , Transverse dimension LD; 7.00 μm and epithelial height EH 2.92 μm (Masson Trichrome stain $\times 250$).

Figure 6: A section of the testis obtained from group 5. (Longitudinal dimension LD; 3.92 μm , Transverse dimension LD; 4.17 μm and epithelial height EH 2.92 μm (Masson Trichrome stain $\times 250$).

Figure 7: A section of the testis obtained from group 6. (Longitudinal dimension LD; 3.67 μm , Transverse dimension LD; 6.75 μm and epithelial height EH 2.92 μm (Masson Trichrome stain $\times 250$).

Results

Luminal Dimensions

The results of the transverse dimension of the lumen of the seminiferous tubules of the testes are shown in Table 1 and 2. It was observed that no significant differences in the transverse dimension of the lumen of the testis exist between group 2 and groups 5 and 6 respectively. No observed significant difference between groups 5 and 6. However, groups 1 and 2 were observed to be significantly increased when compared with group 3.

S/N	Groups	Dosage/Duration of Administration
1	Negative Control	1 ml of Normal Saline daily for the experimental period.
2	Positive Control	Ketoconazole (100 mg/ kg bwt) once daily for five (5) days (14 th -18 th day of experiment).
3	Prophylactic Group	500 mg/kg bwt of ethanolic extract of <i>Sesame indicum</i> seeds for (13 days) + 100 mg/kg bwt of ketoconazole for (5) five days.
4	Therapeutic Low Dose Group	100 mg/kg bwt of Ketoconazole for (5) five days + 250 mg/kg bwt of ethanolic extract of <i>sesame indicum</i> seeds once daily for five (5) days and thirteen (13) days respectively.
5	Therapeutic High Dose Group	100 mg/ kg bwt of Ketoconazole for (5) five days + 500 mg/kg bwt of ethanolic extract of <i>sesame indicum</i> seeds for thirteen days.
6	Ketoconazole and Mesterolone	Ketoconazole (100 mg/ kg bwt) for five days + 0.36 mg/kg bwt of mesterolone once daily for thirteen days.

Table 1: Groups of the animals and their administrations.
(All administrations were carried out orally).

Group	N	Transverse dimension (μm)	Longitudinal dimension(μm)	Epithelial dimension (μm)
		Mean $\pm$ SEM	Mean $\pm$ SEM	Mean $\pm$ SEM
1	6	4.58 $\pm$ 0.27	2.75 $\pm$ 0.25	2.92 $\pm$ 0.33*bce
2	6	4.58 $\pm$ 0.37	6.17 $\pm$ 0.79*de	4.83 $\pm$ 0.48*b
3	6	1.83 $\pm$ 0.31*a	2.00 $\pm$ 0.26*acd	8.17 $\pm$ 0.65*d
4	6	4.33 $\pm$ 1.12	4.83 $\pm$ 0.48	7.00 $\pm$ 0.26
5	6	4.17 $\pm$ 0.42	3.92 $\pm$ 0.33	5.42 $\pm$ 0.49
6	6	3.75 $\pm$ 0.31	3.67 $\pm$ 0.67	6.75 $\pm$ 0.54
*abcde = 1, 2, 3, 4, 5 & 6 ($P \leq 0.05$).				

Table 2: Indices of the seminiferous tubule of animals in the experimental groups.

Epithelial Height

The result of the study of the epithelial height of the seminiferous tubules of the testes in the experimental animals are shown in Table 2.

The result showed that, no significant difference in the epithelial height of the seminiferous tubules exist between group 2 and groups 5 and 6. No significant difference in the epithelial height was also observed between groups 5 and 6 ($P \leq 0.05$). However, groups 5 and 3 were significantly increased when compared with group 2 ($P \leq 0.05$).

Discussion

The luminal dimension of the seminiferous tubules of the Groups treated with ethanolic extract of *Sesamum indicum* seeds and mesterolone (Groups 3, 4, 5 and 6) were observed to be decreased in longitudinal dimension when compared with the Group treated with ketoconazole alone (Group 2) as most of the matured sperm cells and spermatids that lined the lumen of the seminiferous tubules in the ketoconazole treated group were degenerated. Osinubu et al., (2005) had a contrasting view when they observed that certain endocrine disruptors demonstrated a continuous decrease in the mean testicular volume, diameter and cross sectional area of the seminiferous tubules [7].

The continuous decrease in mean testicular morphometry, of seminiferous epithelium in rats administered ketoconazole an endocrine disruptor, suggests that the morphology of germinal epithelium responsible for unique composition of luminal fluids is compromised. This unique composition of luminal fluid is an essential prerequisite for the process of spermatogenesis [7]. It may therefore be suggested that endocrine disruptors like ketoconazole can also compromise

the process of spermatogenesis but the mean luminal dimension of the lumen of the seminiferous tubule did not decrease in the present research and therefore did not agree with the work of Osinubu et al., (2005). It has been reported that endocrine disruptors are wide spectrum ion blockers. This also suggests that ketoconazole disrupted the ion channel leading to the disturbance in ionic equilibrium across membrane and ultimately to a disruption or distortion in the cellular morphology. From the present study, it was observed that disruption of the tubular morphology was also accompanied by general destruction of testicular interstitium.

From the observations made, the ethanolic extract of *Sesamum indicum* seeds and Mesterolone have decreased the luminal dimension of the seminiferous tubules especially in the prophylactic Group (3) when compared with the Control Groups 1 and 2 and also increased the epithelial dimensions of the testes of rats in other experimental experimental Groups (5 and 6). The influence exhibited by the ethanolic extract of *Sesamum indicum* seeds and Mesterolone could be attributed to their antioxidant activities and enzyme (cytochrome P-450 and desmolase 17, 20) boosting effects.

Conclusion

The morphometry of the seminiferous tubules was enhanced as the epithelial height of the seminiferous tubules in rats administered EESI before ketoconazole were significantly increased compared with rats in the Control Group 2 more so, the transverse and longitudinal dimensions of the seminiferous tubules in rats administered EESI before ketoconazole were reduced significantly when compared with rats in the Control Group 2.

Significance of the Study to Humans

Scientific research has proved that the DNA composition of Wister rats (Rat models) body metabolism and physiology only differs from that of humans by 0.5%. This implies that, whatever result is obtained from the rat models, there is a 99.5% chance that the same result will be obtained in humans.

The results obtained from this research therefore, means that, intake of ethanolic extract of *Sesamum indicum* seeds in a proportionate dose (mg/kg body weight) with what was administered to the rat models in humans medicating on ketoconazole for certain ailments can help minimise its adverse effects as seen on the micrographs and table of testicular morphometry that was analysed.

Recommendation

A hormonal assay should be conducted in further studies to ascertain the level of increase in testosterone.

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