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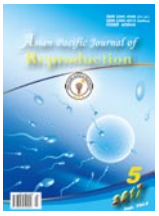

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The methanolic extract of *Guibourtia tessmannii* (Caesalpiniaceae) improves sexual parameters in high fat diet-induced obese sexually sluggish rats

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ABSTRACT

Objective: To evaluate the effects of the methanolic extract of *Guibourtia tessmannii* (*G. tesmannii*) on sperm parameters, lipid profile and testosterone level in obese rats. **Methods:** A total of 193 male Wistar rats were fed either with palm oil diet (n=185) or standard diet (n=8) for 16 wk. At the end of this feeding period, 90 obese rats were selected and randomly divided into 18 groups of five rats each and treated with distilled water (10 mL/kg), vitamin E (75 mg/kg), clomiphene citrate (2 mg/kg) or methanolic extract of *G. tessmannii* (55, 110 or 220 mg/kg) for 7, 21 or 56 d. At the end of each treatment period, sperm parameters, lipid profile and testosterone level were evaluated. Data were analyzed using ANOVA for repeated measures followed by post-hoc Tukey HSD (P<0.05) for multiple comparisons. **Results:** Feeding of rats for 16 wk with palm oil diet significantly damaged sperm parameters. The methanolic extract of *G. tesmannii* improved sperm viability, motility and normality after 21 or 56 d of treatment. The sperm normality increased significantly in rats treated with the methanolic extract of *G. tesmannii* for 7 (110 mg/kg, P<0.01) and 56 d (110 and 220 mg/kg, P<0.05) compared to control group. Triglycerids, total cholesterol, low and very low density lipoproteins cholesterol levels were lower in rats treated with the plant extract for 56 d. *G. tesmannii* also significantly increased the high density lipoprotein cholesterol and testosterone levels in the plasma after 56 d of treatment. **Conclusions:** The methanolic extract of *G. tesmannii* can improve sperm parameters, lipid profile and testosterone level in obese rats. These findings may justify the folkloric use of *G. tesmannii* as a reproductive performance enhancer.

1. Introduction

Obesity is a disease characterized by the accumulation of excessive body fat resulting from intake of high calorie diet, less physical activity and genetic predisposition[1]. Worldwide, the prevalence of obesity has more than doubled since 1980[2], leading to secondary chronic diseases like dyslipidemia, cardiovascular diseases, type 2 diabetes and reproductive problems such as infertility[3,4]. Adverse effects of obesity include low testosterone concentration, altered sperm parameters[5] and hypercholesterolemia[6], which may ultimately affect male fertility.

The treatment of male infertility involves the use of assisted reproductive techniques, synthetic molecules and herbal drugs. In

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developing countries, the investigation for natural products with less side effects and easy availability is on demand. The plant products are used to treat reproductive problems in many countries and are also proven effective in improving fertility in experimental males. For example, *Moringa oleifera*[7], *Panax ginseng*[8], *Ficus asperifolia*[9] and *Allium sativum*[10] have all been reported to have sexual function enhancing effects in male rats. Also known as 'Essingang', *Guibourtia tessmannii* (*G. tessmannii*) is one of such plants. It's a tall tree (40-50 m) extensively found in tropical Africa and southern America in higher rainfall or evergreen forests. The stem barks of *G. tessmannii* are used for the treatment of cardiovascular diseases[11], some cancers[12] and as aphrodisiacs[13,14]. Previously, the authors demonstrated the involvement of dopaminergic receptors in the pro-ejaculatory effects of the aqueous and methanolic extracts of *G. tesmannii* in spinal male rats[15,16]. In animal model, it has been shown that obesity can be induced by genetic, neuroendocrine or dietary changes[17]. The hypercaloric diet used in the present study is the simplest obesity-induction model, and possibly the one that most closely resembles the reality of obesity in humans[18,19]. This study was undertaken to evaluate the beneficial effects of the methanolic extract of *G. tessmannii* on sperm parameters, lipid profile and testosterone level in high fat diet rats. A 15% palm oil diet, which was reported to significantly disrupt the estrus cyclicity in female rats[20] and alter the reproductive performance in male rats[21], was used in this study.

2. Materials and methods

2.1. Reagents

Assay kits for total cholesterol (TC), high density lipoprotein cholesterol (HDL-C), triglycerids (TG) (CORMAY, Łomianki, POLAND) and testosterone (Accubind, Monobind Inc. Lake Forest, USA) were used. Estradiol, progesterone and eosin were purchased from Sigma Aldrich (St. Louis, MO, USA). All other chemicals and reagents used were of analytical grade and purchased from local suppliers. Estradiol and progesterone were dissolved in ethanol and administered in soya oil while other chemicals were freshly prepared in saline solution. The doses used were selected on the basis of previous studies[15,21,22].

2.2. Collection of plant material and preparation of methanolic extract of G. tessmannii

The stem barks of *G. tessmannii* were collected in February 2015 in Ngoumou, located in Central Cameroon. It was identified by Dr. Victor Nana and authenticated with the existing Herbarium Voucher specimen 1037/SRFCA in the Cameroon National Herbarium. After shade-drying, the stem barks were grinded and used to prepare the methanolic extract. The stem barks powder of *G. tessmannii* (300 g) was macerated in methanol (1.5 L) for 72 h. The filtrate was evaporated under reduced pressure to obtain the methanolic extract (34.1 g), with an extraction yield of 11.37%.

2.3. Animals

A total of 193 adult male Wistar rats (aged 3 months, weigh 200-240 g) were obtained from the Department of Animal Biology of the University of Dschang, Cameroon. Rats were housed 3 per cage and maintained under standard conditions [natural light and dark cycle, food and water available *ad libitum*, (26 ± 1) ℃]. The experiments were done with respect to the internationally accepted standards of ethical guidelines for laboratory animal use and care as described in[23].

2.4. Induction of obesity and animal partition

Control rats were fed on standard diet (SD) consisted of fats (7%-10%), carbohydrate (68%-70%), protein (18%-20%), vitamins (1%-2%) and minerals (1%-2%). About 15% palm oil was added to the SD to prepare the palm oil diet (POD)[20,21]. The locally available palm oil used in this study was characterized by a high amount of saturated fat (56%). The chow was mixed with water until it became homogenous in a dough-like consistency. The dough was shaped and used for feeding.

A total of 185 adult male rats were fed with POD for 16 wk. Other rats of the same age (n=8) received SD for the same time lapse. All rats were weighed twice a week. At the end of 16 wk of POD, increase in body weight (≥15% of initial body weight prior to hyperlipidic diet), hypercholesteremia (≥100 mg/dL) and Lee index (≥300 g) were considered in order to validate the obesity status of each animal[24]. The Lee index was calculated using the following formula: Lee index = [cube root of the body weight (g) / naso-anal length (mm)] × 10[25].

After the onset of obesity, obese rats were selected and used for the sexual behavioral study. At the end of the mating test, only 90 obese rats unable of ejaculating within 15 min in the presence of a receptive female, and they were selected for further studies. They were randomly divided into 18 groups comprising five animals each, and treated with distilled water (10 mL/kg), vitamin E (75 mg/kg), clomiphene citrate (2 mg/kg) or methanolic extract of *G.*

tessmannii (55, 110 or 220 mg/kg) for 7, 21 or 56 d. At the end of each treatment period, rats were sacrificed. Body and sexual organ weights, sperm parameters (motility, viability, normality and count) and sperm morphological abnormalities (abnormal head, abnormal tail, cytoplasmic droplet and tailless head) were evaluated. Lipid profile and testosterone level were also measured in the plasma and testes.

2.5. Sexual behavior study

The sexual behavior was monitored by trained observers in a quiet room with a dim red light, around 7 pm as described by Watcho *et al*[26]. During the study, only estrus female rats (experimentally induced with a subcutaneous injection of 17β-estradiol and progesterone) exhibiting good sexual receptivity (presence of lordosis position in response to male's stimulation) and no rejection behaviour were employed. Tests were ended after completions of first test series [the first post-ejaculatory intromission (EL)], non-occurrence of intromission within 15 min, if EL exceeded 30 min[26].

2.6. Sperm parameters study

At the end of each treatment period, all rats were anesthetized and killed by cervical dislocation. The cauda epididymis was immediately collected, chopped and placed in saline solution (0.9% NaCl, 5 mL). The fluid was incubated in a water bath (37 ℃, 5 min) to allow sperms to leave the epididymal tubules[27].

2.6.1. Sperm count

Sperm count was assayed using Mallassez hemocytometer[28]. Sperm count was expressed as the number of sperms per milliliter of solution.

2.6.2. Sperm motility

Fluid was obtained from the cauda epididymis with a pipette and diluted with Tris buffer solution (2 mL). Immediately after their isolation, sperm motility was evaluated microscopically at 400×magnification as described previously[29]. Sperm forward motility was expressed as a percentage of motile sperms to total sperms counted.

2.6.3. Sperm viability

The ratio of live sperms to dead ones was evaluated using 1% trypan blue staining as previously described[30]. Accordingly, a total number of 200 sperms were counted per slide and the results were expressed as a percentage of the live sperms.

2.6.4. Sperm morphological abnormalities

Percentages of abnormal head, abnormal tail, cytoplasmic droplet and tailless head sperms were determined from a total of 300 sperms per rat in agreement to Bjorndahl *et al*[31]. Sperm morphology was viewed under light microscope (OLYMPUS, 400×). Data were expressed as percentage of morphologically abnormal sperms to total sperm count.

2.7. Collection of tissue and organs

Blood was collected via the abdominal artery for determination of biochemical analysis. The testes, epididymis, vas deferens, ventral prostate and seminal vesicles were removed and their relative weights determined.

2.8. Biochemical analysis

TC[32], HDL-C[32], low density lipoprotein cholesterol (LDL-C)[33], very low density lipoprotein cholesterol (VLDL-C)[33] and TG[34] were estimated using standard colorimetric kits (CORMAY, Łomianki, POLAND)[35] according to the commercial instructions for the kits. The LDL-C and VLDL-C levels were calculated based on Friedewald's equation: LDL = TC – TG/5 – HDL, VLDL = TG/5[33]. The plasma testosterone concentration was quantified using a standard commercial kit (Accubind, Monobind Inc. Lake Forest, USA) following the procedure outlined in the manufacturer's instruction manual.

2.9. Statistical analysis

Data were expressed as mean±SEM. The statistical evaluation was performed using STATISTICA (data analyses software system, version 8.0). Significance was calculated by ANOVA for repeated measures followed by the *post-hoc* Tukey HSD test for multiple comparisons. Significance level was set at $P<0.05$.

3. Results

3.1. Effects of POD on rats after 16 wk of treatment

As expected, POD rats showed a net body weight gain which was time-dependent (Figure 1A). At the end of the 16 wk of POD, there was a significant increase in the Lee index ($P<0.001$) and total cholesterol ($P<0.01$) concentration (Figure 1B and D). Except the

relative weights of the testis (P<0.001) and epididymis (P<0.001), no statistical changes in the weights of other reproductive organs were recorded after 16 wk of diet exposure (Figure 1C). After the feeding period, 66.67% of rats were declared obese, among which 69.83% were unable to ejaculate within 15 min in the presence of a receptive female. These sexually sluggish rats were selected for further experiments.

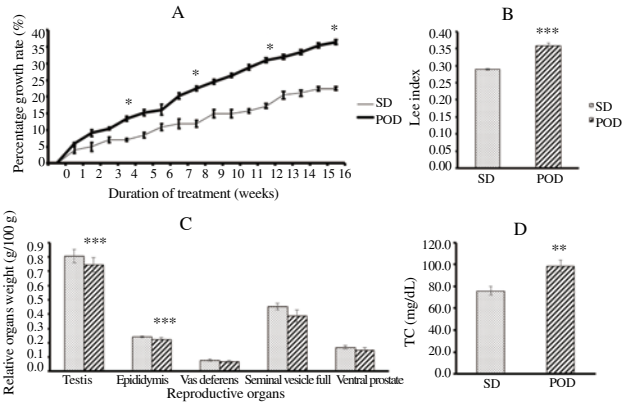

Figure 1. Effects of SD and POD on obese rats after 16 wk (n=5).

A: body weight; B: Lee index; C: reproductive organ weights; D: TC. $^{*}P$<0.05, $^{**}P$<0.01, $^{***}P$<0.001 significantly different compared with SD group.

3.2. Effects of POD on sperm parameters and sperm morphological abnormalities

In POD rats, the sperm parameters were negatively affected with the most harmful effects recorded for sperm motility, sperm viability and sperm normality. These animals also significantly exhibited sperm abnormal heads, abnormal tails and cytoplasmic droplets. No significant differences were observed in sperm counts and tailless heads (Table 1).

3.3. Effects of methanolic extract of G. tessmannii on body weight

No statistical change in body weight was observed in rats treated with the methanolic extract of *G. tessmannii* for 7 and 56 d. At the end of 21 d of treatment, the methanolic extract of *G. tessmannii* at the dose 220 mg/kg induced a significant increase (P<0.05) in body weight compared with distilled water group. On the contrary, when compared with distilled water or vitamin E group, a significant decrease (P<0.05) in the body weight was observed in rats treated with clomiphene citrate after 56 d (Table 2).

Table 2

Effects of vitamin E, clomiphene citrate and methanolic extract of *G. tessmannii* on body weight in obese rats (g) (n=5).

Treatments	Dose	7 d	21 d	56 d
Distilled water	10 mL/kg	1.021 ± 0.031	1.451 ± 0.217	0.526 ± 0.173
Vitamin E	75 mg/kg	0.069 ± 0.638	0.327 ± 0.285	-8.210 ± 0.459
Clomiphene citrate	2 mg/kg	-3.188 ± 1.799	-3.617 ± 1.817	-19.329 ± 4.910$^{***,\alpha\alpha\alpha,\mu\mu\mu,\circ\circ\circ}$
G. tessmannii	55 mg/kg	0.529 ± 0.228	3.884 ± 1.721	2.750 ± 0.848
	110 mg/kg	0.425 ± 0.463	3.954 ± 1.959	2.077 ± 0.473
	220 mg/kg	1.221 ± 0.016	8.108 ± 0.111$^{*,\mu}$	-0.602 ± 0.212

$^{*}P$<0.05, $^{***}P$<0.001 significantly different compared with distilled water group in the same column. $^{\alpha\alpha\alpha}P$<0.001 significantly different compared with vitamin E group. $^{\mu}P$<0.05 significantly different compared with the *G. tessmannii* treatment of 7 d. $^{\mu\mu\mu}P$<0.001 significantly different compared with the clomiphene citrate treatment of 7 d. $^{\circ\circ\circ}P$<0.001 significantly different compared with the clomiphene citrate treatment of 21 d.

3.4. Effects of methanolic extract of G. tessmannii on genital organ weights

The relative weights of testis and epididymis were significantly (P<0.001) decreased in POD rats compared to SD rats (Figure 1C). After 7 d of treatment, no change in the reproductive organ weights was observed in all groups, except the epididymis which was significantly increased (P<0.05) in rats treated with clomiphene citrate (compared with distilled water and vitamin E groups) and lowered in rats treated with the methanolic extract of *G. tessmannii* (compared with clomiphene citrate group). After 21 d of treatment, the methanolic extract of *G. tessmannii* (110 mg/kg) induced a significant increase in the epididymis weight (P<0.01), compared with the treatment of 7 d (Figure 2B). After 56 d of treatment, the relative weights of the testes, epididymis and seminal vesicles were significantly lowered in rats treated with clomiphene citrate (Figure 2A, C and D). Moreover, the methanolic extract of *G. tessmannii* significantly increased the relative weight of epididymis (doses 55, 110 and 220 mg/kg), vas deferens (doses 110 and 220 mg/kg) and ventral prostate (dose 55 mg/kg) after 56 d of treatment (Figure 2B).

Table 1

Effects of SD and POD on sperm parameters and sperm abnormalities after 16 wk of feeding (n=5).

Groups	Sperm parameters				Sperm morphological abnormalities			
	Motility (%)	Viability (%)	Normality (%)	Sperm count (million/mL)	Abnormal head (%)	Abnormal tail (%)	Cytoplasmic droplet (%)	Tailless head (%)
SD rats	39.250±8.000	24.480±6.950	81.790±2.850	182.380±8.860	1.140±0.810	4.350±1.200	2.620±1.180	10.600±2.390
POD rats	7.275±1.577***	2.354±0.748***	19.964±2.723***	147.429±12.811	7.848±2.211*	47.709±3.543***	29.715±2.838***	12.925±2.536

$^{*}P$<0.05, $^{***}P$<0.001 significantly different compared with SD group.

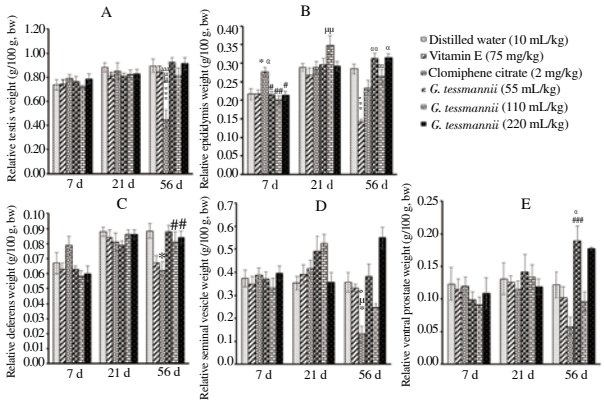

Figure 2. Effects of vitamin E, clomiphene citrate and methanolic extract of *G. tessmannii* on genital organ weights of obese rats (n=5).
A: relative testis; B: epididymis; C: vas deferens; D: seminal vesicle; E: ventral prostate. $^{*}P$<0.05, $^{**}P$<0.01, $^{***}P$<0.001 significantly different compared with distilled water group. $^{\alpha}P$<0.05, $^{\alpha\alpha}P$<0.01, $^{\alpha\alpha\alpha}P$<0.001 significantly different compared with vitamin E group. $^{\#}P$<0.05, $^{\#\#}P$<0.01 significantly different compared with clomiphene citrate group. $^{\mu}P$<0.05, $^{\mu\mu}P$<0.01 significantly different compared with the treatment of 7 d. $^{\circ}P$<0.05, $^{\circ\circ}P$<0.001 significantly different compared with the treatment of 21 d.

3.5. Sperm parameters of obese rats receiving methanolic extract of G. tessmannii

3.5.1. Sperm viability, motility, normality and density

In animals submitted to POD for 16 consecutive wk, the sperm parameters were seriously damaged when compared to SD values (Table 1). However, treatment of POD rats with the plant extract significantly (P<0.01) improved the sperm normality, count, motility, and viability at different time points of treatment. Vitamin E and clomiphene citrate produced similar effects (Figure 3).

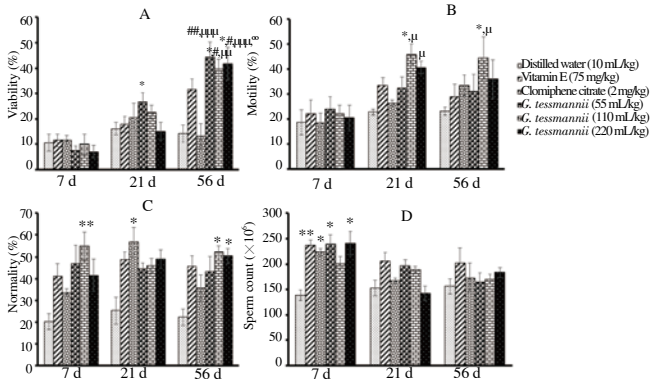

Figure 3. Effects of vitamin E, clomiphene citrate and methanolic extract of *G. tessmannii* on sperm parameters of obese rats (n=5)
A: viability; B: motility; C: normality; D: count. $^{*}P$<0.05, $^{**}P$<0.01 significantly different compared with distilled water group. $^{\#}P$<0.05, $^{\#\#}P$<0.01 significantly different compared with clomiphene citrate group. $^{\mu}P$<0.05, $^{\mu\mu}P$<0.01, $^{\mu\mu\mu}P$<0.001 significantly different compared with the treatment of 7 d. $^{\circ\circ}P$<0.01 significantly different compared with the treatment of 21 d.

3.5.2. Sperm morphological abnormalities

The rats in the POD group showed sperm abnormal head, abnormal tail, and cytoplasmic droplet (Table 1).

After 7 d of treatment, sperm abnormal tail was significantly lowered in the rats treated with clomiphene citrate or methanolic extract of *G. tessmannii* at all doses (Figure 4B). Vitamin E induced a significant decrease in sperm abnormal head, abnormal tail and cytoplasmic droplet (Figure 4A, B and C).

After 21 d of treatment, sperm abnormal tails were significantly lowered in rats treated with vitamin E, clomiphene citrate or methanolic extract of *G. tessmannii* at the dose 220 mg/kg (Figure 4B).

At the end of 56 d of treatment, the methanolic extract of *G. tessmannii* induced a significant decrease in sperm abnormal tail (55 and 110 mg/kg, P<0.05) and tailless head sperm (110 mg/kg, P<0.05). Similar effects were observed with vitamin E and clomiphene citrate (Figure 4B and C).

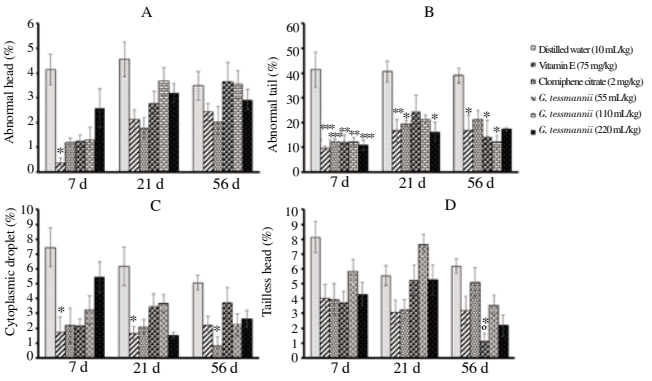

Figure 4. Effects of vitamin E, clomiphene citrate and methanolic extract of *G. tessmannii* on sperm morphological abnormalities in obese rats (n=5).
A: abnormal head; B: abnormal tail; C: cytoplasmic droplet; D: tailless head. $^{*}P$<0.05, $^{**}P$<0.01, $^{***}P$<0.001 significantly different compared with distilled water group. $^{*}P$<0.05 significantly different compared with the treatment of 21 d.

3.6. Biochemical analysis of obese rats receiving methanolic extract of G. tessmannii

3.6.1. Plasmatic lipids

After 7 d of treatment, no change in lipid profile was observed in all groups. On the contrary, at the end of 21 d of treatment, TC was significantly lowered in rats treated with clomiphene citrate compared to those receiving distilled water (P<0.05). Vitamin E and methanolic extract of *G. tessmannii* (220 mg/kg) induced a significant decrease in LDL-C compared to the control group treated with distilled water (P<0.05) (Table 3).

Table 3

Effects of vitamin E, clomiphene citrate and methanolic extract of *G. tessmannii* on plasmatic TC, TG, HDL-C, LDL-C and VLDL-C in obese rats (n=5)

Treatments	Doses	Time (d)	TC (mg/dL)	TG (mg/dL)	HDL-C (mg/dL)	LDL-C (mg/dL)	VLDL-C (mg/dL)
Distilled water	10 mL/kg	7	54.444 ± 6.455	160.762 ± 25.096	19.689 ± 3.319	4.477 ± 0.474	28.285 ± 5.019
		21	52.273 ± 0.455	120.000 ± 10.857	20.252 ± 1.947	6.691± 1.664	24.000 ± 2.171
		56	52.576 ± 5.606	140.286 ± 19.143	19.603 ± 0.130	4.916 ± 1.907	28.057 ± 3.829
Vitamin E	75 mg/kg	7	53.232 ± 5.060	116.000 ± 4.927	26.699 ± 3.544	3.333 ± 1.319	23.200 ± 0.985
		21	42.727 ± 2.018	78.095 ± 11.359	25.834 ± 0.417	1.274 ± 0.134*	15.619 ± 2.272
		56	42.616 ± 2.169	67.619 ± 8.940$^{*,\mu\mu}$	28.171 ± 1.332***	0.922 ± 0.816	13.524 ± 1.788*
Clomiphene citrate	2 mg/kg	7	54.343 ± 9.724	135.429 ± 30.465	24.795 ± 9.596	2.462 ± 1.742	27.086 ± 6.093
		21	39.545 ± 1.364*	101.143 ± 20.571	15.968 ± 2.856	3.349 ± 0.105	20.229 ± 4.114
		56	50.303 ± 2.431	97.524 ± 10.403	26.094 ± 0.653**	4.705 ± 0.872	19.505 ± 2.081
G. tessmannii	55 mg/kg	7	56.364 ± 8.706	105.143 ± 7.258	23.411 ± 6.904	6.895 ± 2.642	26.057 ± 0.262
		21	47.475 ± 1.313	117.905 ± 11.915	21.939 ± 3.190	1.954 ± 0.056$^{\mu}$	23.581 ± 2.383
		56	41.515 ± 3.406	62.857 ± 19.454$^{*,\mu\mu}$	26.180 ± 0.377**	2.764 ± 0.487	12.571 ± 3.891$^{*\mu}$
	110 mg/kg	7	48.788 ± 6.385	111.619 ± 18.014	24.146 ± 2.967	2.318 ± 1.450	22.324 ± 3.603
		21	45.354 ± 3.189	88.952 ± 16.536	25.488 ± 2.497	2.075 ± 0.486	17.790 ± 3.307
		56	44.747 ± 1.578	85.714 ± 5.752	24.449 ± 0.780*	3.155 ± 1.129	17.143 ± 1.150
	220 mg/kg	7	50.202 ± 8.806	116.190 ± 1.008	23.930 ± 1.946	3.034 ± 1.142	23.238 ± 0.202
		21	42.121 ± 2.063	104.038 ± 7.877	19.776 ± 3.722	1.538 ± 0.228*	20.808 ± 1.575
		56	47.348 ± 3.848	83.000 ± 5.322	27.587 ± 2.872**	3.162 ± 0.573	16.600 ± 1.064

$^{*}P$<0.05, $^{**}P$<0.01, $^{***}P$<0.001 significantly different compared with distilled water group in the same column. $^{\mu}P$<0.05, $^{\mu\mu}P$<0.01, $^{\mu\mu\mu}P$<0.001 significantly different compared with same treatment group of 7 d.

After 56 d of treatment, vitamin E and methanolic extract of *G. tessmannii* (55 mg/kg) induced a significant decrease in triglyceride level compared to distilled water. HDL-C was significantly increased in rats treated with vitamin E, clomiphene citrate and methanolic extract of *G. tessmannii* at the dose 55 mg/kg, 110 mg/kg and 220 mg/kg. When compared with distilled water, VLDL-C was observed to be significantly low in groups treated with vitamin E and *G. tessmannii* (55 mg/kg) (Table 3). It is noteworthy mentioning that the improvement of lipid parameters was more effective after 56 d of continuous oral treatment. For instance, the HDL-C was significantly increased in all groups.

3.6.2. Effects on testicular lipids

At the end of 7 d of treatment, HDL-C level was high in rats treated with the methanolic extract of *G. tessmannii* (55 mg/kg) and vitamin E compared with distilled water (Table 4). After 21 d of treatment, HDL-C level was also increased in rats treated with the methanolic extract of *G. tessmannii* (55 mg/kg).

Vitamin E, clomiphene citrate and methanolic extract of *G. tessmannii* decreased triglycerides and increased HDL-C level after 56 d of treatment. Moreover, VLDL-C was significantly decreased in rats treated with the methanolic extract of *G. tessmannii* (Table 5).

3.6.3. Effects on plasmatic testosterone

At the end of 21 d of treatment, the methanolic extract of *G. tessmannii* (110 mg/kg) significantly increased plasmatic testosterone level compared to distilled water, clomiphene citrate and vitamin E groups (Table 4). It was observed that *G. tessmannii* increased plasmatic testosterone gradually from day 7 to 56 of treatment (Table 5).

Table 5

Effects of vitamin E, clomiphene citrate and methanolic extract of *G. tessmannii* on plasmatic testosterone level in obese rats (ng/mL) (n=5).

Treatments	Doses	7 d	21 d	56 d
Distilled water	10 mL/kg	1.300±0.100	0.925±0.525	1.100 ± 0.100
Vitamin E	75 mg/kg	1.600±0.300	1.050±0.150	0.333 ± 0.088
Clomiphene citrate	2 mg/kg	1.625±0.175	0.450±0.050	0.767 ± 0.233
G. tessmannii	55 mg/kg	2.737±0.518	0.833±0.033	0.517 ± 0.219
	110 mg/kg	1.350±0.500	2.700±0.346$^{**,\#,\alpha\alpha}$	12.333±0.333$^{***,\#\#\#,\alpha\alpha\alpha,\mu\mu\mu,\sim\sim}$
	220 mg/kg	1.567±0.717	0.550±0.176	4.125 ± 0.175$^{**,\#,\alpha\alpha,\mu\mu,\sim\sim}$

$^{**}P$<0.01, $^{***}P$<0.001 significantly different compared with distilled water group in the same column. $^{\#}P$<0.05, $^{\#\#\#}P$<0.001 significantly different compared with clomiphene citrate group in the same column. $^{\alpha\alpha}P$<0.01, $^{\alpha\alpha\alpha}P$<0.001 significantly different compared with vitamin E in the same column. $^{\mu\mu}P$<0.01, $^{\mu\mu\mu}P$<0.001 significantly different compared with the same treatment of 7 d. $^{\sim\sim}P$<0.01 significantly different compared with the same treatment of 21 d.

4. Discussion

The present study was undertaken to investigate the effects of the methanolic extract of *G. tessmannii* on sperm parameters, lipid profile and testosterone level in high fat diet-induced obese rats. The high fat diet used in this study (POD) was effective in promoting obesity, as demonstrated by the significant increase in the growth rate (P<0.05), Lee index (P<0.001) and total cholesterol concentration (P<0.05). After 16 wk of POD exposure, 60.67% of rats were declared obese while 33.33% failed to respond. This difference in response of animals from the same husbandry could be justified by unknown factors including the intraspecific response among those animals[36]. This success in POD-induced obesity is in accordance with previous studies[20,21,37,38]. The hyperlipidic diet used in this study essentially comprised of palm oil (15%) was commonly available in local market. This palm oil contained high percentage

Table 4

Effects of vitamin E, clomiphene citrate and methanolic extract of *G. tessmannii* on testicular TC, TG, HDL-C and VLDL-C in obese rats (n=5).

Treatments	Dose	Time (d)	TC (mg/g)	TG (mg/g)	HDL-C (mg/g)	VLDL-C (mg/g)
		7	53.030 ± 4.035	434.095 ± 47.416	1.376 ± 0.450	86.819 ± 9.483
Distilled water	10 (mL/kg)	21	49.192 ± 2.879	400.762 ± 19.143	0.584 ± 0.195	71.600 ± 3.829
		56	49.667 ± 8.939	426.857 ± 32.000	1.299 ± 0.260	85.371 ± 6.400
		7	49.596 ± 6.697	400.571 ± 78.055	4.630 ± 1.576*	80.114 ± 15.611
Vitamin E	75 (mg/kg)	21	35.455 ± 8.447	334.667 ± 39.372	0.692 ± 0.385$^{\mu}$	66.933 ± 7.874
		56	32.525 ± 2.632	232.762 ± 13.500$^{**,\mu}$	4.847 ± 0.637$^{*,\circ}$	46.553 ± 2.700
		7	42.121 ± 9.555	323.809 ± 58.756	2.509 ± 0.610	64.762 ± 11.751
Clomiphene citrate	2 (mg/kg)	21	38.333 ± 10.455	322.571 ± 66.571	2.142 ± 1.493	64.514 ± 13.314
		56	55.152 ± 6.502	252.000 ± 40.390*	6.924 ± 1.138$^{**,\circ}$	50.400 ± 8.808
		7	49.192 ± 10.660	493.143 ± 92.017	5.193 ± 3.205*	98.629 ± 18.403
G. tessmannii	55 (mg/kg)	21	50.808 ± 5.522	407.809 ± 47.919	3.419 ± 0.189$^{*,\alpha}$	81.562 ± 9.584
		56	47.879 ± 11.404	257.524 ± 14.866$^{*,\mu,\circ}$	5.019 ± 0.241*	51.505 ± 2.973
		7	55.253 ± 10.292	440.190 ± 60.548	2.899 ± 0.826	88.038 ± 12.110
	110 (mg/kg)	21	38.788 ± 7.275	328.762 ± 20.028	2.683 ± 0.043	65.752 ± 4.006
		56	37.979 ± 7.530	241.715 ± 17.818$^{**,\mu}$	4.889 ± 0.173*	48.343 ± 3.564
		7	65.758 ± 4.795	502.286 ± 35.231	2.726 ± 0.783	100.457 ± 7.046
	220 (mg/kg)	21	40.202 ± 7.667	324.571 ± 44.619	2.034 ± 0.385	64.914 ± 8.924
		56	38.636 ± 4.396	154.143 ± 17.875$^{***,\mu\mu}$	4.316 ± 0.840*	30.829 ± 3.545$^{\mu\mu}$

$^{*}P$<0.05, $^{**}P$<0.01, $^{***}P$<0.001 significantly different compared with distilled water group in the same column. $^{\alpha}P$<0.05 significantly different compared with vitamin E group in the same column. $^{\mu}P$<0.05, $^{\mu\mu}P$<0.01 significantly different compared with the treatment of 7 d in the same column. $^{\circ}P$<0.05 significantly different compared with the treatment of 21 d in the same column.

of saturated fats (56%). In fact, a high fat diet rich in saturated fat facilitates accumulation of body fat and is considered more deleterious for human health than that rich in unsaturated fat[39]. Clomiphene citrate and vitamin E were selected as positive controls because of their androgenic[40] and antioxidant[41,42] potentials, respectively. When compared with distilled water, significant decreases in the body and reproductive organs (testis, epididymis, vas deferens and seminal vesicles) weights were observed in rats treated with clomiphene citrate for 56 d. Clomiphene citrate, a weak estrogen receptor antagonist, is a good drug for obese patients. The beneficial effects of clomiphene citrate in the improvement of sperm parameters are due to its ability to increase testosterone level[43]. At the level of the hypothalamus, clomiphene citrate competes with circulating estradiol for the estrogen receptors and inhibits the normal negative feedback mechanism, resulting in the high production of GnRH[44]. The high level of GnRH increases the production of FSH and LH by the pituitary gland, leading to the improvement of steroidogenesis and spermatogenesis[40]. It was also observed that the methanolic extract of *G. tessmannii* significantly increased the weights of sexual organs (epididymis, vas deferens and seminal vesicles) after 56 d of treatment. Similar results were found in male rats treated with extracts of *Xylopia aethiopica*, *Psidium guajava* and *Syzygium aromaticum*[45-47]. The weight of reproductive organs was particularly regulated by androgens through an increase in the synthesis of proteins and subsequently muscle mass[48]. Androgens, especially testosterone, thereby contribute to the increased volume and weights of the testes, epididymis and seminal vesicles by stimulating protein synthesis as observed in the present study[49]. The increase observed in these organs could be correlated with the androgenic properties of *G. tessmannii*.

A study of sperm parameters is an important criterion to evaluate the underlying cause of male infertility[50]. It has been reported that obesity impaired male fertility by decreasing sperm motility, viability and normality as well as increasing sperm morphological abnormalities[5]. In the present study, the methanolic extract of *G. tessmannii* induced a significant increase in sperm viability and motility after 21 and 56 d of treatment. When compared with distilled water group, the methanolic extract of *G. tessmannii* at the dose 110 mg/kg (7 and 56 d) and clomiphene citrate (7 and 56 d) induced a significant increase in the percentage of sperm normality. In addition, sperm count was significantly higher in rats treated with vitamin E, clomiphene citrate or *G. tessmannii* for 7 d. Sperm morphology analysis is an important technic to evaluate male fertility because of its ability to clarify cytotoxic events[51,52]. In this study, significant decreases in sperm abnormal head, abnormal tail, tailless head and cytoplasmic droplet were observed in rats treated with vitamin E, clomiphene citrate and methanolic extract of *G. tessmannii*. These results are in agreement with Saez Lancellotti, *et al*[53] who reported that olive oil improved sperm parameters in high fat diet rats. In addition, Curcumin and Kolaviron isolated from Curcuma longa and Garcia kola respectively are reported to improve sperm motility and decrease sperm abnormalities by preventing peroxidative changes in the sperm and testicular membranes[54]. It has been reported that the beneficial effect of vitamin E on the male reproductive system is mainly due to its ability to increase sperm count[55], sperm motility[56], sperm viability[57] and fertilizing capacity[56]. The beneficial effect of clomiphene citrate on sperm parameters observed in the present study is in line with the literature reports since it is used to improve sperm parameters in obese individuals with hypoandrogenism by increasing GnRH and LH levels, resulting in an increase in testosterone production[40].

Lipogenesis up-regulation in POD-induced experimental obesity

leads to increase serum (LDL-C and VLDL-C) concentrations[58], decrease HDL-C level and affect sperm function in obese rats[59]. Such reproductive complications of obesity could be lowered when serum lipid concentrations are reduced by hypocholesterolemic drugs. In the present study, the effects of vitamin E, clomiphene citrate and methanolic extract of *G. tessmannii* on plasma and testicular lipids were more pronounced after 21 and 56 d of treatment. The methanolic extract of *G. tessmannii* induced a significant decrease in triglyceride level in the plasma and testis after 56 d of treatment. As well, LDL-C and VLDL-C were observed to be significantly lowered in rats treated with vitamin E and methanolic extract of *G. tessmannii* in the same period. Moreover, HDL-C was significantly higher in rats treated with vitamin E, clomiphene citrate and methanolic extract of *G. tessmannii* for 56 d. Similarly, the aqueous extract of *Eugenia caryophyllus*[60], ethanol extract of *Terminalia paniculata* bark[61], polyphenol-rich hydroethanolic extract of *Tetrapleura tetraptera* spice and *Sasa borealis* stem extract[62] reduced all lipoproteins except the HDL in high fat diet-fed rats.

The increase fat deposit observed in obese patients not only amplifies hypercholesterolemia, but also leads to decrease testosterone concentration[6] and induce oxidative stress[63]. These changes may alter testicular functions and subsequently male fertility by oxidizing membrane spermatozoa[64] and inducing lipid peroxidation, which may affect acrosome reaction and induce infertility[65]. The improvement of sperm parameters by the methanolic extract of *G. tessmannii* could be justified by the presence of phenols, flavonoids, tannins and terpenoids[66,67]. Moreover, spectroscopic analysis of the stem bark of *G. tessmannii* revealed the presence of a dihydrochalcone glucoside 'Asebotin'[68]. The antioxidant properties of Asebotin could be responsible for the beneficial effect of *G. tessmannii* on sperm production. Apart from the antioxidant properties of *G. tessmannii*[68,69], its androgenic properties could give more information about its fertilizing effects.

Testosterone, the main androgen produced by Leydig cells[70], controls (at a certain concentration) the initiation and maintenance of spermatogenesis as well as the growth and function of the prostate gland and seminal vesicles[71]. The reduction of sperm concentration and reproductive organ weights in high fat diet-induced obese rats indicate the impairment of male fertility[21]. It has been reported that the first criteria to evaluate spermatogenesis is the size of the testes because the seminiferous tubules and germinal elements constitute about 98% of the total mass of the testis[72]. In the present study, the methanolic extract of *G. tessmannii* induced a significant increase in plasma testosterone level after 21 and 56 d of treatment. This increase could be attributed to a direct effect of the plant extract on the gonadal tissues or on the hypothalamo-pituitary-testis axis. Present results are correlated with those reported by Anderson *et al*, Srikhanth *et al* and Pahlen[73-75].

On the basis of the results obtained in the present study, the therapeutic effect of the methanolic extract of *G. tessmannii* in POD-induced obese rats may be due to the potency and efficacy of the phytoconstituents present in it. These phytochemical molecules may act synergistically to attain potent biological efficacy. Future studies may lend support for the evidence of biological potency of isolated active phytoconstituents like Asebotin present in the methanolic extract of *G. tessmannii*.

It can be concluded that the methanolic extract of *G. tessmannii* improves male fertility by increasing testosterone production and ameliorating sperm parameters (density, viability, normality and motility) in obese rats. The fertility properties of the methanolic extract of *G. tessmannii* could therefore justify its folkloric use as a reproductive performance enhancer.

Conflict of interest statement

The authors declare that they have no competing interest.

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