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ORIGINAL ARTICLE

Improvement of Sexual Behavior and Ejaculatory Parameters by the Methanolic Extract of *Guibourtia tessmannii* (*Caesalpiniaceae*) in High-Fat-Diet-Fed Sexually Sluggish Male Rats

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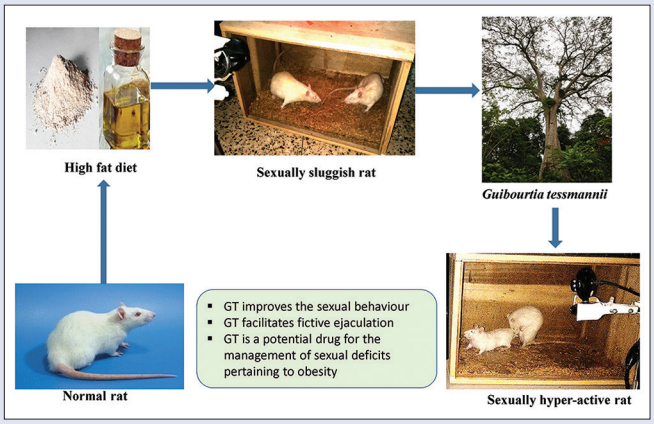
ABSTRACT

Background: *Guibourtia tessmannii* (GT) (*Caesalpiniaceae*) is claimed as a plant having aphrodisiac property as per the African traditional medicine. **Objective:** This study evaluated the effects of the methanolic extract of GT on sexual behavior and fictive ejaculation in high-fat diet (HFD)-induced sexually sluggish male rats. **Materials and Methods:** Male Wistar rats fed either on HFD or standard diet (SD) for 16 weeks were monitored for their growth rate and Lee index. At the end of this period, three consecutive copulatory tests were conducted and HFD-induced sexually sluggish rats were selected. Besides Group 1 as time control (SD), drugs or vehicle (veh) were administered orally everyday into three groups of rats as Group 2: HFD (veh), Group 3: HFD + GT (220 mg/kg), and Group 4: HFD + sildenafil citrate (5 mg/kg). Their copulatory activities were tested on day 1, 7, 14, and 21 and the electromyography of bulbospongiosus muscles was assessed for fictive ejaculation on day 22. **Results:** Treatment with the methanolic extract of GT facilitated sexual behavior by decreasing ($P < 0.001$) ejaculatory latency within 14 days of treatment that sustained till day 21 compared to the control (HFD) and improving sex drive scores ($P < 0.01$). The fictive ejaculation parameters were more pronounced in HFD + GT group compared with the HFD group. For instance, after urethral stimulation, the contraction of the bulbospongiosus muscles was significantly increased in HFD + GT group (12.26 ± 7.25) compared to the control group (6.75 ± 0.25). **Conclusion:** These findings provide robust evidence for the GT treatment in the management of sexual deficits pertaining to obesity.

Key words: Aphrodisiac, fictive ejaculation, *Guibourtia tessmannii*, high-fat diet, sexual behavior

SUMMARY

- *Guibourtia tessmannii* (GT) is a medicinal plant commonly used in traditional medicine as sexual enhancer
- The aim of this study was to evaluate the effects of the methanolic extract of GT on sexual behavior and fictive ejaculation in high-fat-diet-induced sexually sluggish male rats
- GT improves sexual behavior by reducing the ejaculatory latency and increasing the sex drive score
- GT facilitates fictive ejaculation by increasing the number of contraction of the bulbospongiosus muscles after urethral stimulation
- GT is a potential medicinal drug for the management of sexual deficits pertaining to obesity.



Abbreviations used: GT: *Guibourtia tessmannii*; SD: Standard diet; HFD: High-fat diet; ML: Latency of mount; IL: Latency of intromission; EL: Latency of ejaculation; MF: Frequency of mount; IF: Frequency of intromission; PEI: Postejaculation interval; MIII: Mean interintromission; SDS: Sex drive score; EMG: Electromyograms.

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INTRODUCTION

Overweight and obesity constitute a global health problem of increasing prevalence and growing concern.[1] Excessive weight gain can result from intake of high-calorie diet, less physical activity, and genetic predisposition.[2,3] Obesity contributes to sexual dysfunctions such as arousal difficulties, ejaculatory dysfunction, and erectile dysfunctions.[4] Erectile dysfunction is considered as an important sexual disorder which negatively affects sexual performance of obese patients.[5,6] Studies on sexual health issues suggest that many men worldwide have deficits in ejaculatory function.[7] Ejaculation is a complex process wherein coordinated inputs of sympathetic, parasympathetic, and somatic efferent nervous system interact with the pelvi-perineal structures. The timely integration of sensory inputs to the dorsal penile nerve with autonomic and motor outflow during copulation elicits ejaculation.[8]

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In developing countries, the investigation for the cost-effective natural products from medicinal plants is in demand, which have the least side effects and easy availability. The plant products are used to treat sexual disorders in many countries, and these are proven effective in improving libido and sexual behavior in male animals. Some plants, e.g., *Psoralea corylifolia*,[9] *Montanoa tomentosa*,[10] *Dracaena arborea*,[11] *Monsonia angustifolia*,[12] *Panax ginseng*,[13] and *Allium sativum*,[14] are reported to have sexual function enhancing effects in male rats. *Guibourtia tessmannii* (GT) is also used as aphrodisiac in Cameroon as per traditional medicine.[15] It is also known as "Essingang" or "Bubinga" which is a tall tree (40–50 m) extensively found in tropical Africa (from Cameroon to the Democratic Republic of Congo) and Southern America in higher rainfall or evergreen forests. Besides, the stem barks of GT are used for the treatment of cardiovascular diseases[16] and some cancers[17] and the prevention of abortion.[18]

The male sexual behavior testing in rat animal model is an established standard procedure to study the effect of drugs on copulatory act, which provides reliable information regarding both the sexual arousal and performance.[19,20] Moreover, the study of fictive ejaculation provides further details on the action of drug on the erectile machinery at the level of penis.[21] In a recent study, we evaluated the proejaculatory effects of the aqueous and methanolic extracts of GT in spinal male rats with the dose 20 mg/kg (intravenous [iv])[22] and illustrated the involvement of D1- and D2-like receptors in ejaculation.[23] However, the effect of oral treatment with GT extracts on reproductive function is still unknown. With the hypothesis that the proejaculatory effects of the bioactive compounds present in GT may restore the ejaculation function of hypoactive rats with late ejaculation, this study was undertaken to evaluate the effects of this plant on sexual behavior and ejaculatory function in high-fat diet (HFD)-fed sexually sluggish rats.

MATERIALS AND METHODS

Animals

Adult male Wistar rats (3 months old, 200–250 g body weight) were obtained from the Animal House of Sree Chitra Tirunal Institute for Medical Sciences and Technology. Each rat was housed individually in polystyrene cages, maintained under standard conditions (12-h light and 12-h dark cycle, 26°C ± 1°C), with free access to food and water. The study was approved by the Institutional Animal Ethics Committee (Reference No. SCT/IAEC-164/JULY/2015/88) and performed in accordance with the guidelines laid down by the Committee for the Purpose of Control and Supervision of Experiments on Animals, India.

Induction of obesity in rats

A hypercaloric diet is an accepted method for the practical induction of obesity in animal models as it bears a close resemblance to obesity in humans.[24] The hypercaloric diet containing high fat (15%) are palatable and induce obesity in rats.[6,25] 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%). 15% of groundnut oil was added to the SD to prepare HFD.[25] The locally available groundnut oil used in this study contained saturated fat (16%), monounsaturated fat (48%), polyunsaturated fat (36%), Vitamin E (0.16%), and 0% of carbohydrate, cholesterol, protein, or sugars. In powder form, chow was mixed with water until it became homogenous in a dough-like consistency. The dough was shaped and used for feeding.

Adult male rats were fed with HFD for 16 weeks. In the control group, rats of the same age were receiving an SD diet for the same duration, and all animals were weighed twice a week. At the end of this period, increase in body weight (more than 15% of initial body weight before HFD) and Lee index (LI, above 0.30) was considered to assess the obesity status of each animal. The LI was calculated using the following formula: LI = (cube root of the body weight [g]/naso-anal length [mm]) × 10. The adiposity index was calculated toward the end of the experiment by taking the sum of epididymal, visceral, and retroperitoneal fat weights divided by body weight × 100.[26]

Preparation of the methanolic extract of *Guibourtia tessmannii*

The stem barks of GT (*Caesalpiniaceae*) 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 bark was ground and used to prepare methanolic extract. The stem bark powder of GT (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%.

Preparation of receptive female for the mating test

The female rats were ovariectomized under ketamine hydrochloride (50 mg/kg intramuscular [i.m.]) and xylazine hydrochloride (5 mg/kg i.m.) anesthesia. After postsurgical recovery, female rats were brought into estrus by the sequential subcutaneous injections of estradiol benzoate (25 μg) and progesterone (1 mg) 48 h and 6 h, respectively.[19,20] Estradiol (25 μg) (Sigma Aldrich) and progesterone (1 mg) (Sigma Aldrich) were dissolved in ethanol and administered in groundnut oil (sildenafil citrate [SC]) while other chemicals were freshly prepared in saline solution. The doses were selected and used based on previous studies.[11,20] They were further screened with nonexperimental vigorous males, and only those exhibiting good sexual receptivity (presence of lordosis position in response to male's stimulation) and no rejection behavior were employed in the tests.

Copulatory test (male sexual behavior test)

Each male rat was submitted to three consecutive mating tests with primed receptive female on 3 different days for confirmation of consistent sexual behavior. The sexual behavior of male rats was monitored by a trained observer and also recorded in a sound-attenuated air-conditioned room with dim red light at 7 pm. Single male rat was placed in a test cage (40 cm × 50 cm × 40 cm) for 5 min for habituation. After this, receptive female was introduced in the cage, and the copulatory test was started. The latencies and frequencies of the events (pursuit, mount, intromission, and ejaculation) were registered manually by pressing the assigned keys in a computer program (the male sexual behavior program). The computer did timing operation, using its internal clock. The following parameters of sexual behavior were measured: latencies of mount, intromission (IL), and ejaculation (EL); frequencies of mount (MF) and intromission (IF); postejaculation interval (PEI); mean interintromission (MIII); and sex drive score (SDS). Tests were terminated immediately after completions of first test series (the first postejaculatory intromission), nonoccurrence of intromission within 15 min if EL exceeded 30 min.[19]

Experimental protocol

At the end of the feeding period of 16 weeks (fed on SD or HFD), three consecutive sexual behavior tests were monitored on 3 different days through pairing of each rat with same receptive female. The HFD-induced sexually sluggish rats were those having high ejaculatory latency (EL ≥9 min) and low (SDS ≤7). Altogether 48 rats were used in the study (18 HFD and 6 SD fed male rats and 24 primed female rats). The rats receiving SD constituted Group 1: SD (control) and the HFD fed rats were randomly distributed into three groups of six rats each and treated as follows: Group 2: HFD (HFD-induced sexually sluggish rats) receiving vehicle for 21 days; Group 3: HFD + GT (HFD-induced sexually sluggish

rats) receiving the methanolic extract of GT (220 mg/kg/day) for 21 days; Group 4: HFD + SC, HFD-induced sexually sluggish rats receiving SC (5 mg/kg) on day 1 and 21 that served as a positive control [Figure 1]. In Groups 2, 3 and 4, HFD was continued during 3 weeks of testing. The dose of GT was based on our pilot study (unpublished).

Since SC has a maximum effect 4 h after administration,[27] an oral suspension of it at a dose of 5 mg/kg was given to rats in Group 3 on day 1 and 21, about 1 h before the mating behavior assessment. Copulatory activities were recorded on day 1, 7, 14, and 21 follow-up by fictive ejaculation on the subsequent day in Groups 1, 2, and 3.

Procedure to test fictive ejaculation

Under urethane-anesthesia (1.2 g/kg intraperitoneally), perineum was incised to expose the bulbospongiosus muscles of penis. A catheter (PE-50, 0.9 mm o.d.) was inserted into the pelvic urethra through a bladder incision. Two insulated electrodes with exposed tip (EL 452, 12 mm) were inserted into the bulbospongiosus muscles to record electromyograms (EMG). At the end of the surgical approach, the spinal cord was blunt transected around T6 spinal level and prepared for recording.

Two consecutive ejaculatory motor patterns were evoked at 3 min intervals by the injection of saline solution (0.2 ml/min) through a PE-50 catheter (0.9 mm o.d.) inserted into the pelvic urethra (urethral stimulation) or by tactile stimulation of the penis using two forceps (penile stimulation). After urethral or penile stimulations, the EMG of the bulbospongiosus muscles were recorded for 5 min, by registering to a polygraph (Biopac MP 150). The latency of contractions was noted as the time elapsed from the application of a test stimulus until the first contraction of the bulbospongiosus muscles.[28]

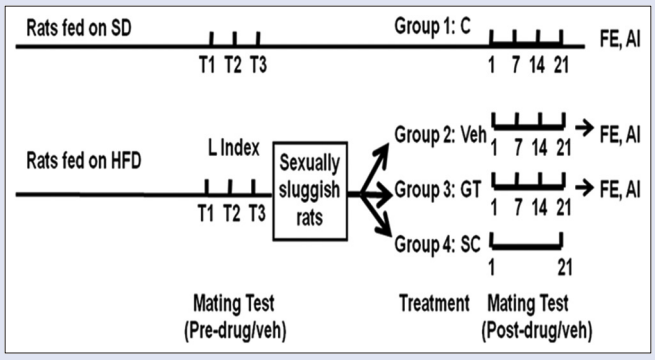

Figure 1: Experimental protocol of the study. T1–T3 denotes three mating tests before drug/veh treatment, and 0, 7, 14, 21 designates days after drug/veh treatment. No drug/Veh treatment was given to SD fed Group 1 rats. SD: Standard diet; HDF: High-fat diet; Veh: Vehicle; GT: *Guibourtia tessmannii;* SC: Sildenafil citrate; FE: Fictive ejaculation; AI: Adiposity index

Collection of tissue and organs

At the end of fictive ejaculation study, anesthetized rats were killed by cervical dislocation. Adipose tissue from the epididymal, visceral, and retroperitoneal areas was isolated and weighed. The testis, epididymis, vas deferens, ventral prostate, and seminal vesicle were removed and their weights (relative to body weights) were determined.

Statistical analysis

The data obtained were reported as the mean ± standard error of the mean. The statistical evaluation was performed using STATISTICA version 8.0 (StatSoft, Inc., Tulsa, USA). Significance was calculated by ANOVA repeated measure followed with *post hoc* Tukey's HSD test for multiple comparisons. Significance level was set at $P < 0.05$.

RESULTS

Effects of high-fat diet on body weight and Lee index after 16 weeks of treatment

Rats fed with HFD and SD showed a net body weight gain which was time dependent. No significant change was observed at any time point in both the groups [Figure 2a]. Similarly, the LI in HFD groups was only slightly increased compared to SD group without reaching statistical significance after 16 weeks of treatment [Figure 2b].

Effects of the methanolic extract of *Guibourtia tessmannii* on body weight, relative sexual organ weights, and adiposity index after 21 days of treatment

When compared with HFD group, the methanolic extract of GT decreased the body weight by 10.81% after 21 days of treatment [Figure 3a]. Abdominal fat ($P < 0.05$), epididymal fat ($P < 0.001$), and adiposity index ($P < 0.01$) were significantly increased in the rats in HFD group compared to those fed with SD The treatment with the methanolic extract of GT induced a significant decrease ($P < 0.05$) in epididymal fat

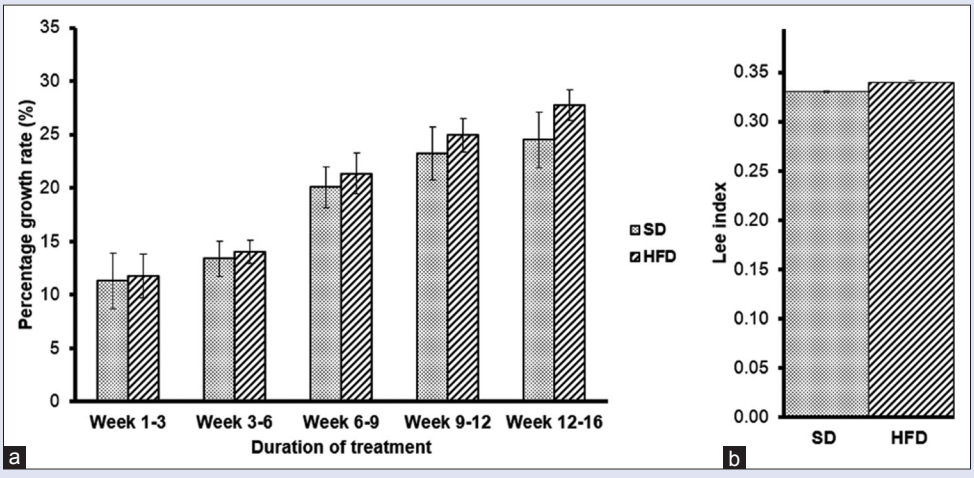

Figure 2: Body weight (a) and Lee index (b) of SD and HFD-rats during 16 weeks of treatment. All values are expressed as mean ± SEM ($n = 5$). SD: Standard diet; HFD: High-fat diet; SEM: Standard error of mean

compared with HFD group [Figure 3b and d]. There was no statistical change in the relative sexual organ weights in all groups. Meanwhile, in the rats treated with the plant extract, the relative weights of testis and seminal vesicle were increased by 5.23% and 26.59%, respectively, compared to HFD group [Figure 3c].

Effects of the methanolic extract of *Guibourtia tessmannii* on sexual behavior in high-fat diet-rats after 1, 7, 14, and 21 days of treatment

The HFD-induced sexually sluggish rats were those having high ejaculatory latency (EL ≥9 min) and low (SDS ≤7) before starting any treatment. On day 1 (1st day of treatment), compared to SD group (5.43 ± 1.03 min), the values of EL were significantly higher in HFD (11.23 ± 1.09 min; $P \leq 0.01$), HFD + GT (11.88 ± 1.14 min; $P \leq 0.001$), and HDF + SC (9.14 ± 0.86 min; $P \leq 0.05$) groups [Figure 4a]. On days 7 and 14, the methanolic extract of GT (220 mg/kg) exhibited a significant decrease in EL compared to HFD group. On day 21, in rats treated with SC (5 mg/kg), the EL was significantly lowered ($P \leq 0.001$) compared with HFD group. In animals treated with the plant extract, compared to the 1st day of treatment (day 1) (11.89 ± 1.14 min), a significant decrease in EL was observed after 14 ($P \leq 0.001$) and 21 ($P \leq 0.05$) days of treatment. When compared with SD, the EL in HFD group remains higher during the treatment periods. The maximum pro-ejaculatory effect of GT was observed after 14 days of treatment [Figure 4a].

On day 1, the SDS was significantly lowered in HFD, HFD + GT, and HFD + SC groups compared with SD group. The methanolic extract of GT (220 mg/kg) and SC (5 mg/kg) increased SDS

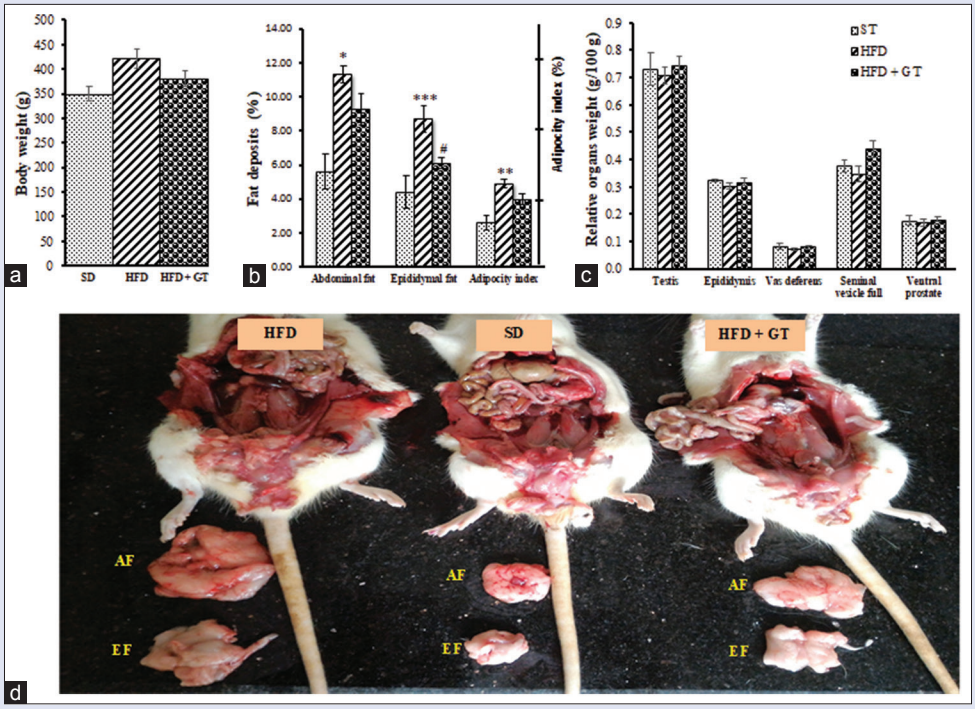

Figure 3: Effects of the methanolic extract of *Guibourtia tessmannii* (220 mg/kg) on body weight (a); fat deposits (b and d) and relative organs weight (c) after 21 days of treatment. All values are expressed as mean ± SEM ($n = 5$). *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$: Significantly different compared with SD group. #$P < 0.05$: Significantly different compared with HFD group. SD: Standard diet; HFD: High-fat diet; AF: Abdominal fat; EF: Epididymal fat; SEM: Standard error of mean

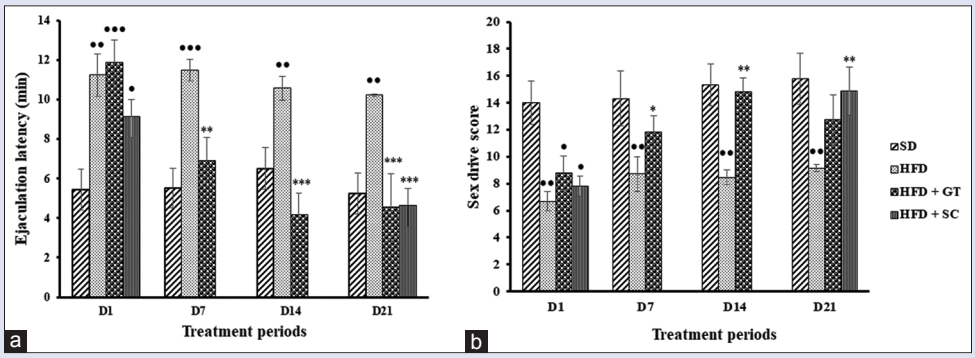

Figure 4: Effects of the methanolic extract of *Guibourtia tessmannii* (220 mg/kg) on ejaculation latency (a) and sex drive score (b) in HFD-rats after 1, 7, 14 and 21 days of treatment. HFD: High fat diet (15%); SC: sildenafil citrate (5 mg/kg); GT: *Guibourtia tessmannii* (220 mg/kg). All values are expressed as mean ± SEM ($n = 5$). ·$P < 0.05$; ··$P < 0.01$; ···$P < 0.001$ significantly different compared with SD, of respective test day. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$ significantly different compared with HFD. SEM: Standard error of mean

significantly ($P < 0.05$) on day 14 (14.78 ± 2.79) and 21 (14.87 ± 1.76), respectively, compared to HFD group (6.68 ± 1.72). However, the SDS was similar in SD group during all test days. The maximum effect of GT on SDS was observed after 14 days of treatment [Figure 4b].

The effects of GT and SC on latencies, MIII, and PEI are summarized in Table 1. In all groups, no statistical change was observed after 7, 14, and 21 days of oral treatment even though the trend for higher values of MIII and PEI in the HFD group compared to SD group on all tested days was evident which reduced after treatment of GT in the HFD + GT group after 14–21 days. GT decreased IF gradually from day 1 to 21 of treatment without affecting the PF, MF, and IF [Table 2]. Based on the EL and SDS, the maximum pro-ejaculatory effect of the methanolic extract of GT was observed after 14 days of treatment.

Effects of saline solution, urethral and penile stimulations on the contraction of the bulbospongiosus muscles, and expression of corpus spongiosum pressure in a spinal rat

In all rats, the iv injection of saline solution (2 ml) failed to trigger the rhythmic contraction of the striated bulbospongiosus muscles [Figure 5a, d, g and Table 3]. On the contrary, urethral (US) and penile (PS) stimulations induced rapid rhythmic contractions of the bulbospongiosus muscles in SD (US = 7.83 ± 1.01; PS = 9.50 ± 0.76), HFD (US = 6.75 ± 0.25; PS = 9.75 ± 1.75) and HFD + GT (US = 12.25 ± 7.25; PS = 10.75 ± 1.25) rats [Figure 5b-f, h, i and Table 3]. These ejaculatory motor responses were sometimes followed by penile movements, erection, and emission of the seminal plugs. In HFD + GT group after urethral stimulation, the number of contraction of the ejaculatory muscles was found to be significantly ($P < 0.05$) higher compared with HFD group. In SD, HFD, and HFD + GT groups, the latency of contraction of the ejaculatory muscles was significantly ($P < 0.001$) lowered after penile stimulation, compared with urethral stimulation [Table 3]. Based on the latency of response, in all groups, penile stimulation appeared to be more efficient than urethral stimulation in stimulating rhythmic ejaculatory motor pattern.

DISCUSSION

The present study conducted on the HFD-induced sluggish rats clearly showed that treatment with the methanolic extract of GT improved the sexual behavior and fictive ejaculation within 2 weeks. The facilitation of male sexual behavior was primarily achieved by improvement in

Table 1: Effects of the methanolic extract of *G. Tessmannii* (220 mg/kg) on pursuit latency, mount latency, intromission latency, mean interintromission intervals, and post-ejaculation interval in high-fat diet -rats after 7, 14 and 21 days of treatment

Groups	Periods	PL (min)	ML (min)	IL (min)	MIII (min)	PEI (min)
SD	D1	0.05±0.01	0.48±0.27	0.55±0.30	0.34±0.07	4.58±0.25
HFD	D1	0.10±0.05	0.11±0.01	0.19±0.02	0.62±0.04	5.45±0.12
HFD + GT	D1	0.06±0.02	0.45±0.33	0.55±0.32	0.56±0.11	5.62±0.19
HFD + SC	D1	0.18±0.01	2.00±1.11	2.24±1.15	0.57±0.04	5.61±0.60
SD	D7	0.04±0.01	0.12±0.03	0.31±0.11	0.39±0.09	4.94±0.47
HFD	D7	0.04±0.04	0.29±0.24	0.35±0.23	0.63±0.17	6.40±0.28
HFD + GT	D7	0.04±0.09	0.20±0.08	0.42±0.04	0.48±0.14	5.86±0.23
SD	D14	0.04±0.01	0.12±0.04	0.18±0.03	0.38±0.09	5.05±0.62
HFD	D14	0.05±0.01	0.35±0.17	0.57±0.23	0.59±0.05	6.01±0.24
HFD + GT	D14	0.04±0.01	0.09±0.02	0.36±0.21	0.36±0.08	5.92±0.27
SD	D21	0.04±0.01	0.12±0.05	0.55±0.05	0.37±0.07	5.27±0.76
HFD	D21	0.23±0.19	0.13±0.00	0.22±0.03	0.66±0.10	6.69±0.46
HFD + GT	D21	0.06±0.02	0.89±0.66	0.48±0.21	0.53±0.17	5.74±0.33
HFD + SC	D21	0.04±0.00	0.33±0.23	2.86±1.78	0.34±0.05	5.53±0.32

PL: Pursuit latency; ML: Mount latency; IL: Intromission latency; MIII: Mean-interintromission intervals; PEI: Poste ejaculatory interval. Data are expressed as mean±SEM (n=5). SD: Standard diet; HFD: High fat diet (15%); SC: Sildenafil citrate (5 mg/kg); GT: *Guibourtia tessmannii* (220 mg/kg); SEM: Standard error of mean

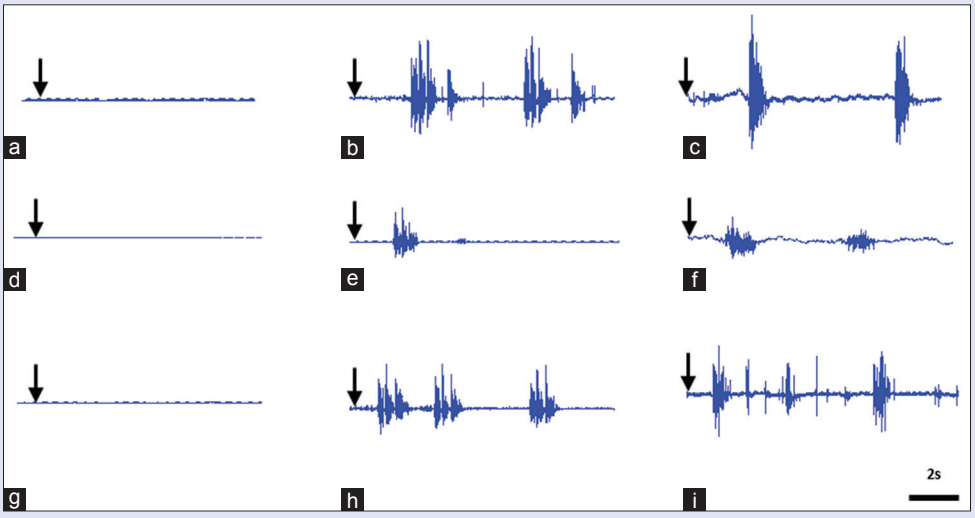

Figure 5: Polygraphic EMG tracings showing the effects of saline solution (a, d, g), urethral stimulation (b, e, h) and penile stimulation (c, f, i) on bulbospongiosus muscles. Figure a-c = SD rats; d-f = HFD rat; g-i = HFD + GT. Arrows indicate the time of injection. SD: Standard diet; HFD: High-fat diet; GT: *Guibourtia tessmannii*

sexual drive and performance as evident from reduction in the EL and an increase of SDS scores. The fictive ejaculation study involving urethral stimulation also showed increased number of contraction of the bulbospongiosus muscles after treatment with GT in the HFD rats, thus corroborating our finding that GT is a potential medicinal drug for recovering sexually lethargic rats.

It is pointed that in the current study, the HFD prepared with groundnut oil was not too effective in promoting obesity after 16 weeks of treatment, as demonstrated by a nonsignificant variation in the growth rate and LI (criteria to mark obesity in rat) compared to the SD group, even though an increase in visceral fat was evident. We used hyperlipidemic diet essentially comprised locally available refined groundnut oil (15%) that contained high percentage of mono and polyunsaturated fats (total of 82%) and relatively lesser saturated fat (16%). Probably, the presence of anti-oxidants in the form of Vitamin E (0.16%) and the high amount of mono- and poly-unsaturated fats would have slowed down the emergence of obesity. However, unrefined crude oil commonly used in less developed nations would definitely elicit obesity much faster.[23,25] In fact, HFD rich in unsaturated fatty acids prevents accumulation of body fat and are considered less deleterious for human health than those rich in saturated fat.[29] Nevertheless, long-term use of HFD with this groundnut oil gave rise to increased fat accumulation even though the relative sexual organs weight did not show any distinct differences between HFD and SD groups after 16 weeks of diet intake. In Wistar rats, an HFD model of obesity, obese animals show no difference in reproductive organs weights, excepting the relative weight of empty seminal vesicle after 45 weeks of diet exposure, compared with control rats.[30] Similarly, diet-induced obesity male mice exhibited no changes in the average weight of the testis or epididymis.[31] These data are in accordance with the results of the present study.

Our study also draws attention to the fact even though various HFD has been used for inducing obesity in a variety of mammals including nonhuman primates, dogs, pigs, hamsters, squirrels, mice, and rats,[32] the effectivity and harmful effects would be dependent on dietary fat quality and composition.[33,34] Increasing prevalence of sedentary lifestyles and dietary changes cannot be denied for obesity which is contributing to male infertility.[35]

It is evident that prolonged consumption of the HFD in rat model induced lethargy with reduced sexual behavior as they displayed prolonged EL and reduced SDS score throughout the test period. Improvement in SDS after treatment with GT indicated an overall recovery of sexual performance in the sexually lethargic rats. The male sexual behavior consists of two distinct behavioral components, sexual arousal, and performance.[36] The slight reduction in IL and PEI deciphered increased sexual arousal, whereas reduction in EL and MIII after treatment with GT indicated stimulation and persistence of sexual drive to perform. This effect on EL was more pronounced than the effect induced by SC (5 mg/kg), the most common drug used in clinic to treat erectile dysfunction (Burnett 2004).[37] The reduction in EL after treatment with GT marks increase in sexual performance.[37] In addition, the effects of GT on sexual behavior was established not only through a reduction in the IF preceding ejaculation but also by significantly increasing the SDS. Similar pro-ejaculatory effects have been reported with the aqueous extract of *Turnera diffusa* (10 mg/kg)[38] and aqueous and methanolic extracts of *Aframomum melegueta*.[39]

In our study, the fictive ejaculation in spinally and urethane-anesthetized male rats was induced effectively by sensory and pharmacological intervention as evident from the recording of the rhythmic motor pattern of ejaculation.[39] Mechanically (urethral and penile stimulations) or pharmacological induction (by systemic injection of dopamine) at the spinal generator is already proved by our research group[11,39] and is considered as the robust model for the study of the spinal pattern generator of ejaculation.[28] The urethral and penile stimulations induced fictive ejaculation in all spinal rats in the present study, characterized by rhythmic contraction of the bulbospongiosus muscles. These findings are in corroboration with previous studies.[6,23] Even though the number and latency of contractions of the bulbospongiosus muscles in SD group rats were not different in HFD and HFD + GT groups after penile stimulation, improved fictive ejaculation response after treatment with GT evident from increased number of contractions of the ejaculatory muscles

Table 2: Effects of the methanolic extract of *Guibourtia tessmannii* (220 mg/kg) on the frequency of sexual behavior parameters in high-fat diet rats after 7, 14, and 21 days of treatment

Groups	Periods	PF	MF	IF
SD	D1	5.20±1.20	8.00±0.55	16.00±2.09
HFD	D1	6.67±0.88	7.33±1.76	12.67±0.33
HFD + GT	D1	13.00±2.86	10.00±1.47	17.50±2.50
HFD + SC	D1	10.75±2.18	13.00±4.14	16.00±0.58
SD	D7	5.00±1.00	10.20±1.02	15.20±2.27
HFD	D7	9.67±2.03	13.67±0.88	21.00±5.29
HFD + GT	D7	9.00±1.78	7.00±2.12	16.50±2.33
SD	D14	7.80±1.56	10.40±2.29	17.60±3.04
HFD	D14	10.00±3.00	12.67±2.33	18.33±2.85
HFD + GT	D14	5.75±1.55	4.57±0.63	13.00±3.94
SD	D21	8.80±0.97	8.00±1.45	15.00±2.81
HFD	D21	10.00±0.58	16.67±2.85	16.00±2.08
HFD + GT	D21	5.75±1.11	8.00±0.91	12.00±3.58
HFD + SC	D21	8.75±1.55	11.50±6.95	13.25±1.38

PF: Pursuit frequency; MF: Mount frequency; IF: Intromission frequency. Data are expressed as mean±SEM (n=5). SD: Standard diet; HFD: High-fat diet (15%); SC: Sildenafil citrate (5 mg/kg); GT: *Guibourtia tessmannii* (220 mg/kg); SEM: Standard error of mean

Table 3: Effects of intravenous administration of saline solution (0.2 ml), urethral and penile stimulations on the number and latency of contractions of the bulbospongiosus muscles in rats receiving standard diet, high-fat diet, and methanolic extract of *Guibourtia tessmannii* (220 mg/kg)

Groups	Treatments	Number of contractions	Latency of contractions (s)	Ejaculatory motor response
SD	Saline injection	0	ND	-
	Urethral stimulation	7.83±1.01***	73.64±40.85	+
	Penile stimulation	9.50±0.76***	0.366±0.07$$$	+
HFD	Saline injection	0	ND	-
	Urethral stimulation	6.75±0.25***	11.21±2.88	+
	Penile stimulation	9.75±1.75***	0.525±0.03$$$	+
HFD + GT	Saline injection	0	ND	-
	Urethral stimulation	12.26±7.25*,#,***	23.31±17.66	+
	Penile stimulation	10.75±1.25***	0.41±0.14#,$$$	+

***P≤0.001; significantly different compared with saline solution; $$$$P$<0.001; significantly different compared with urethral stimulation (SD); *P≤0.05; #P≤0.01; significantly different compared with urethral stimulation (HFD); All values are expressed as mean±SEM (n=5). ND: Not determined; GT: *Guibourtia tessmannii*; SD: Standard diet; HFD: High-fat diet; +: Present; -: Absent; SEM: Standard error of mean

indicated its pharmacogenetic effects. Not only that, but GT-treated rats also displayed reduced latency of contraction after urethral stimulation in comparison to penile stimulation. However, based on the result obtained, penile stimulation was more efficient in promising ejaculatory motor pattern. This observation is similar in previous studies.[28,40] Besides modulation of male rat sexual behavior and ejaculation by high structures of central nervous system,[41] a spinal pattern generator located within the lumbosacral cord controls emission and expulsion phases of ejaculation and the penile sexual reflexes.[42] It is under the influence of supraspinal sites of the brainstem and hypothalamus.[21,42]

It is evident from these results that GT has the potential of improving the male sexual performance primarily by acting on the ejaculatory circuit. Considering the fact the ejaculation is a complex process, GT emerges as a promising pharmacogenic therapeutic herbal product for the management of reduced sexual behavior that is becoming prevalent in current society amid present lifestyle. The reduction on visceral fats after treatment with GT also point to a probable metabolic regulatory effects.

The pro-sexual effect of GT could be attributed to the various active ingredients present in the methanolic extract. In fact, previous phytochemical analysis of GT revealed the presence of phenols, flavonoids, tannins, and terpenoids.[43] Bioactive compounds of GT have not been isolated yet except for asebotin (4,2'-dihydroxy-4'-methoxy-6'-β-gluco pyranosyloxydihydrochalcone) that was recently reported.[44] Phenols, flavonoids, as well as tannins possess pro-sexual properties. Terpenoids have an implication in the triggering of penile erection as well as in the improvement of the sexual performances.[45] These bioactive components would have an effect on the central nervous system by the activation of neurotransmitters[10] or the periphery by stimulation and release of NO by the nerve endings located at the level of cavernous body.[46] Further studies may be required in the aged rats to address the competency of GT on sexual functions later in life.

CONCLUSION

It emerged from all these investigations that GT facilitated sexual activity by improving sexual drive, performance and fictive ejaculation in HFD rats. Our study provided strong scientific evidence on the potential therapeutic property of this plant which justifies its use as aphrodisiac by indigenous populations and strengthens the conventional traditional curative claims.

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Conflicts of interest

There are no conflicts of interest.

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