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# Aphrodisiac and male fertility enhancing potentials of *Berlinia grandiflora* (Vahl) Hutch. & Dalziel (Leguminosae) using Wistar rats and *Drosophila melanogaster*

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**Abstract**
Inequitable access to assisted reproductive technologies including *in vitro* fertilization to treat infertility remains a challenge for those living in low and middle-income countries. However, medicinal plants have been employed in traditional medicine as alternative remedies to address the problems of infertility. This study was aimed at investigating the male fertility enhancing activities of *Berlinia grandiflora* stem bark.
The powdered sample of *B. grandiflora* (BG) stem bark was macerated in methanol at room temperature for 72 h, the resulting extract (pilot methanol) was tested for possible phytochemicals and evaluated on Wistar rats for toxicity and male fertility enhancing potentials. Proximate analysis was carried out on the stem bark powder and was also successively macerated into n-hexane, dichloromethane, ethyl acetate, and methanol. The extracts were investigated using Wistar rats (sperm indices and hormonal testosterone) and *Drosophila melanogaster* [survival study, mating latency (ML), copulation duration (CD), fly emergence/% fertility] models. Mesterolone (10 mg/kg) and Sildenafil (2 mg/kg) were used as standards for Wistar rats and Drosophila models respectively. All data were analyzed using Graph pad prism 5.02, level of significance was placed at $p \leq 0.05$.
Results of acute toxicity and survival study using Wistar rats and *D. melanogaster* respectively showed that BG stem bark extracts are safe above 5 g/kg dose level. 600 mg/kg bwt. of BG pilot methanol extract had the most significant sperm count and Testosterone level respectively ($112.92 \pm 8.97 \times 10^6$/mL, $0.51 \pm 0.15$ IU/L) when compared to the untreated group ($83.27 \pm 3.51 \times 10^6$/mL, $0.39 \pm 0.03$ IU/L) and Mesterolone ($107 \pm 5.08 \times 10^6$/mL, $1.13 \pm 0.12$ IU/L). There was a significant increase in sperm motility in the group treated with 600 mg/kg pilot methanol extract and it was dose-dependent. 400 mg/kg of successive dichloromethane (DCM) and methanol extracts of *B. grandiflora* stem bark showed a significant reduction in ML ($p \leq 0.01$) when compared to the untreated group (1 ml of 0.1% Tween 80 per kg diet). DCM extract showed highest % fertility increase ($50.9 \pm 8.2$%) in 400 mg/kg ($p \leq 0.001$) relative to negative control ($0.00 \pm 0.48$). Sildenafil (2 mg/kg) had significant mating latency and copulation duration but a non-significant % increase ($6.10 \pm 2.31$%) infertility
In this study, DCM extract of *Berlinia grandiflora* stem bark was proven to be an effective male fertility enhancer and this has justified its use as a fertility booster in ethnomedicine.

**Keywords:** *Berlinia grandiflora,* DCM extract, *Drosophila melanogaster,* Mating latency, Male fertility enhancer

## Introduction

Infertility is a disease of the reproductive system defined by the failure to achieve a clinical pregnancy after 12 months or more of regular unprotected sexual intercourse (Frey, 2010) [11] and there is no other reason, such as breastfeeding or postpartum amenorrhoea (Singh and Pakhiddey, 2015; WHO, 2018) [29, 35]. While assisted reproduction technologies (ART) have been available for more than three decades, with more than 5 million children born worldwide from ART interventions such as *in-vitro* fertilization (IVF), these technologies are still largely unavailable, inaccessible, and unaffordable in many parts of the world (Dhont *et al.*, 2012) [8], particularly in low and middle-income countries (LMIC). However, medicinal plants have been employed in traditional medicine as alternative remedies to address the problems of infertility at a much-reduced cost and with great accessibility. *Berlinia grandiflora* (Vahl) Hutch. & Dalziel (Leguminosae) ranges in habit from a shrub just 2 - 5 metres tall, to a spreading tree with a dense, rounded, crown that usually grows up to 20 metres tall, exceptionally to 30 metres (Mackinder and Harris, 2006), the bole can be 10 - 70cm in diameter. The tree produces very fragrant, large, conspicuous flowers. The heartwood is pinkish-brown with purple or dark brown veins and frequent resin canals; it is demarcated from the 10 - 15cm wide band of sapwood (Allen and Allen, 1981) [1]. *B grandiflora* is widely distributed in Guinea, Mali, Nigeria, Central Africa, and the Democratic Republic of Congo (Hutchison and Dalziel, 1963) [13]. *B grandiflora* is the most widely spread of the genus and is known to occur within the protected area network. It is called Apado by the Yoruba people and Ububa by the Igbo ethnic groups. *B grandiflora* is well known among herbalists in the Nigerian traditional medicine practice

for managing several disease conditions such as skin infections, haemorrhoids and liver complications (Joseph *et al.*, 2012) [14], cancer (Mike *et al.*, 2010) [19], vermifuge, diarrhea and diabetes (Ode *et al.*, 2013) [20]. Folks use the leafy twigs decoction as a febrifuge, cholagogue, purgative and antiemetic, while the leaf decoction is also drunk as a tonic (Mackinder and Harris, 2006). It was reported that the leaf and stem of the plant are used by herbalists in South-Eastern Nigeria, to treat microbial infections. Sap from its bark is used for the treatment of sores and wounds, while bark decoctions are administered for the treatment of haemorrhoids and liver complaints. The bark is also used to ease labour during childbirth and gastrointestinal disorders (Joseph *et al.*, 2012) [14]. The analgesic activity of stem bark extract was reported by Asuzu *et al.*, (1993) [3], the antihelminthic activity of stem bark and its active principle, betulinic acid (Enwerem *et al.*, 2001) [10] have been reported. Olatunji *et al.*, 2018 evaluated the anti-tuberculosis activity of the n-hexane, ethyl-acetate, methanol, and aqueous extracts of *B. grandiflora* leaves against *M. bovis.* The aqueous extract of *B. grandiflora* seed was found to have saturated and monounsaturated fatty acids. The high concentration of fatty acids in this extract when in contact with the human aqueous system can lead to severe diarrhoea (Duru *et al.,* 2014) [9].

## Materials and Methods

### Plant collection, authentication, and preparation

Dried barks and voucher specimen of *B.grandiflora* were collected from Km 7, Iseyin- Ado Awaye road, Oja - Agbe, Iseyin local government area, Oyo State, in July 2016. The sample was authenticated at Forestry Herbarium, Ibadan where a voucher specimen (FHI 113219) was deposited. After collection, the bark was cut into pieces, washed, and air-dried under shade, after which it was ground to powder with a Hammermill using a 5KVA motor. The powdered material was weighed and stored in an air-tight container.

### Proximate analysis on *B. grandiflora* stem bark (BG)

One gram each of *B. grandiflora* stem bark powder was weighed into six porcelain crucibles, heated for 2 hours in an oven at $105^0$c, cooled in desiccators, and weighed. It was heated again at $105^0$c hourly, cooled, and weighed until a constant weight was obtained to determine the water loss (%). The methods adopted for the determination of ash values, and extractive values followed the description given by British Pharmacopoeia (1973) [5].

### Extraction of *B. grandiflora* stem bark (BG)

Two kilograms (1.0 kg) of the pulverized plant material (*B.grandiflora* stem bark: BG) was macerated in methanol for 72 h. It was filtered and the resulting filtrate was concentrated *in vacou* at $36^0$c to obtain 9.36% w/w. The resulting methanol extract (pilot methanol) was used for acute toxicity study and pilot fertility assay. Another batch of the stem bark powder (2.0 kg) was subjected to successive extraction by maceration in n-hexane, dichloromethane, ethyl acetate, and methanol for 72 hours, respectively. Each filtered extract was separately concentrated *in vacuo* to give n-hexane, dichloromethane, ethyl acetate, and methanol extracts which were also used for bioassay.

### Phytochemical analysis of *B. grandiflora* stem bark

All phytochemical procedures were carried out using standard methods as described by Sofowora (1993), and Trease and Evans (1989).

### Toxicity study and fertility study of *B. Grandiflora* stem bark pilot methanol extract on male Wistar rats

Nine rats weighing 130 - 150g were used for the determination of acute toxicity (1st phase of Lorke's method (Lorke, 1983).

Another batch of thirty rats was employed for the evaluation of liver and kidney function, and fertility study. The animals were divided into groups of 6 animals each. Groups 1-3 were administered methanol extract of *B. grandiflora* stem bark orally at 200, 400, and 600 mg/kg. Mesterolone (10mg/kg) (Group IV) and 0.1% Tween 80 (1 mL/kg) (Group V) served as positive and negative control respectively. The administration was carried out at 9.00 am daily for 14 days. Tween 80 (0.1%) was used to dissolve the extract to enhance solubility and this necessitated its use as the negative control.

### Fertility study of *B. grandiflora* stem bark successive extracts on male Wistar rats

The animals were divided into 14 groups of 6 animals each and administered accordingly for 14 days as follows.
Group 1 received 1 ml/kg of 0.1% Tween 80
Group 2 received 10 mg/kg of Mesterolone
Groups 3-5 received 100, 200, and 400 mg/kg, of *B. grandiflora* stem bark n-hexane extract, respectively.
Groups 6-8 received 100, 200, and 400 mg/kg *B. grandiflora* stem bark DCM extract, respectively.
Groups 9-11 received 100, 200, and 400 mg/kg *B. grandiflora* stem bark ethyl acetate extract, respectively
Groups 12-14 received 100, 200, and 400 mg/kg *B. grandiflora* stem bark methanol extract, respectively.

### Blood collection and semen analysis

On the 15th day i.e. a day post final extract administration, blood was collected through the orbital sinus of each of the animals in all the groups. The animals were sacrificed by cervical dislocation. The organs such as testes,

kidneys, liver, and epididymis were harvested and weighed. The blood collected was centrifuged at 3000 rpm for 10 minutes to obtain serum which was tested for Testosterone, alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Alkaline phosphatase (ALP), Blood Urea Nitrogen (BUN) and Serum Creatinine levels (Reitman *et al.,* 1957). Testosterone level was determined at Madonna University Teaching Hospital (MUTH) using ELISA random Kit while the toxicity profile (EIA, England) was assayed at the Chemical Pathology Department, Faculty of Veterinary Medicine, University of Ibadan. The right epididymis was removed and the caput was lacerated on a glass slide using a warm (27°C) sterile lancet to release the semen (Oyeyemi *et al*., 2000). The semen was examined for sperm motility, count, and abnormalities.

**Sperm motility**

Drops of normal saline and eosin stain were added to affect the full motility of the spermatozoa. Average gross motility was scored under the microscope x 40 objectives, **%** life-death ratio and caudal epididymis volume were determined (Aweda *et al*., 2010) [10].

**Sperm count**

The sperm concentration was examined using Neubauer Haemocytometer. (Aweda *et al*., 2010; Osuchukwu *et al*., 2016) [10, 24]

**Sperm abnormalities**

Different types of abnormalities found in the sperm cells were analyzed using Smith *et al*., (1977) method. Two drops of a vital stain eosin-negrosin were added to the semen sample on the slide mixed and a semen smear was prepared on a new clean glass slide. The slide was then scored for such abnormalities as rudimentary tail, bent tail, curved tail, coil tail, tailless head, and headless tail. The scoring was done under x 100 magnification using an Olympus microscope.

**Hormonal assay**

Blood was collected through the orbital sinus of the Wistar rats using a capillary tube. The blood was thereafter centrifuged, and the serum was separated and used for hormonal assay (ELISA, England) according to the manufacturer's instructions.

**Drosophila collection and preparation**

Adult *Drosophila melanogaster* was obtained from the Department of Biochemistry, *Drosophila* laboratory, University of Ibadan, Nigeria in 2018. The flies were transported in a controlled environment of 15 to 25$^0$c to Madonna University where they were bred at the Pharmacognosy laboratory extension, Madonna University. They were fed with freshly prepared Corn agar media using the procedure of Guruprasad *et al.,* 2010 [12] with some modifications. After 8.5 days of the life cycle, the eggs on the diet metamorphosized into imago. Newly emerged flies were used for the experiment.

**Survival study on successive extracts of *B. grandiflora* stem bark using Drosophila**

Fourteen groups of thirty-five (35) flies each of both sexes from the population were transferred into treatment vials and used for the study. The groups were categorized as follows:

Group 1-3 received 100, 200, and 400 mg/kg *B. grandiflora* stem bark n-hexane extract, respectively in addition to the diet.

Groups 4-6 received 100, 200, and 400 mg/kg, *B. grandiflora* stem bark DCM extract, respectively in addition to the diet.

Groups 7-9 received 100, 200, and 400 mg/kg *B. grandiflora* stem bark ethyl acetate extract, respectively in addition to the diet.

Groups 10-12 received 100, 200, and 400 mg/kg *B. grandiflora* stem bark methanol extract, respectively in addition to the diet.

Groups 13 – untreated group; received diet only (negative control).

Group 14 received Sildenafil citrate (positive control).

The flies were carefully transferred at intervals of four days into freshly prepared diets containing the corresponding extracts. The same was done on the untreated group only that it was not fed with the extracts. Each group was carried out in replicates of five. The number of dead flies was counted and recorded daily for 28 days. Any death occurrence at day zero was counted as death due to handling while deaths from day one to the end of the study were termed as death due to tested extract(s). The percentage of surviving flies was calculated based on the number of flies (35) at the onset of the study.

**Effect of *B. grandiflora* stem bark extracts on mating latency, copulation duration, and fly emergence of *D. melanogaster***

The procedure of Suchira and Shakunthala (2014) [32] was followed with some modifications. Both sexes were aged for 3 days during which the following procedures were undertaken. The flies were separated into virgin females and bachelor males before 8-10 hours of their emergence when the neurons for mating would have been

activated. The males were divided into groups of 5 each and starved for 8 hours. They were then fed for 64 hours with different concentrations of *B. grandiflora* stem bark extracts (100, 200, and 400 mg/kg diet) of n-hexane, dichloromethane, ethyl acetate, and methanol extracts. The females were also divided into groups of five each but they were not fed with the extracts. After 64 hours of treatment of the male flies with the extracts of which both sexes of flies must have been 3 days old, a pair of both sexes was introduced into the mating chamber and observed for a maximum of 1 hour to determine mating latency and copulation duration. After copulation, male and female fruit flies were transferred from the mating chamber to fresh diets (without extract) for 24 hours before laying eggs. At 24 hours intervals, they were transferred into new vials containing a freshly prepared diet for five days. All the vials in which the flies have been transferred within five days were left for observation to enable the eggs laid to develop into adult flies. The newly emerged flies were summed up in comparison to the untreated group to determine percentage fertility. Sildenafil citrate (2 mg/kg) was used as the reference standard.

**Data analysis**

Data were analyzed using one-way analysis of variance (ANOVA) employing GraphPad Prism version 5.02 for Windows (Graph pad software, San Diego California, USA, www.graphpad.com) at $P < 0.05$. Dunnet multiple comparison test at 95% Confidence Interval of difference was considered significant.

**Results and Discussions**

The percentage moisture content of *B. grandiflora* does not fall in the specified range for a powdered herbal product by British Pharmacopoeia, (1973) [5]. This means *B. grandiflora* stem bark may be susceptible to microbial infection. Other physicochemical analyses are within Pharmacopeia standards as revealed in Table 1. The phytochemicals of *B. grandiflora* are shown in Table 2 and they are similar to the observation of Olatunji *et al*., (2018) [23] in their work on Phytochemical evaluation of the same plant. The presence of phytochemicals in plants account for medicinal uses in folklore remedies for the treatment of various ailments (Odoh *et al*., 2020) [21]. There was no significant difference in body weight of animals treated with *B. grandiflora* stem bark extracts (Tables 3 and 4). The organs' weights were not negatively affected in all the groups treated with *B. grandiflora* stem bark extracts unlike in Mesterolone which elicited a reduction in organ weights as shown in Fig 1. Also, all the biochemical indices were normal, the concentrations of Aspartate aminotransferase, Alanine aminotransferase, Alkaline phosphate, Bilirubin urea nitrogen and Creatinine were not significantly different from the control group indicating that the extracts at the tested doses did not have any hazardous effect on the liver and the kidney (Table 5). These biochemical indices, if altered, will impair the normal functioning of the organs (Appidi *et al*., 2009) [2]. The pilot methanol extract showed a dose dependent increase in percentage sperm motility with significant activity at 600 mg/kg (Fig 2) but livability was not affected and there was no significant difference in sperm motility of animals treated with the successive extracts of *B. grandiflora* (Fig 3). A significant increase in sperm motility is an indication of a beneficial effect on the spermatozoa and will enable easy transport of sperm cells into the ovule (Oladeinde *et al*., 2007) [22]. A significant 36% increase in sperm count was produced by pilot methanol extract (600 mg/kg) compared to the negative control (0.1 % Tween 80) and this was higher than that obtained for the positive (29%) control (10 mg/kg Mesterolone). A significant increase in testosterone level was also observed at 400 and 600 mg/kg of the pilot methanol extract with the highest percentage increase of 31% in 600 mg/kg but was lower than that obtained in 10 mg/kg Mesterolone (190%) as represented in Table 6. The successive extract showed no abnormalities in spermatozoa (Table 7). A significant increase in sperm count was also recorded for DCM 400 mg/kg, 200, and 400 mg/kg of methanol extracts among the groups treated with *B. grandiflora* successive extracts (Table 8). A significant increase in sperm count corresponds to improve fertility (Adeleke *et al*., 2022) [15].

A developmental study is a means of investigating the level of toxicity of the test substance. A fruit fly has an average life span of about 50 days (Taylor *et al.,* 2013) [33]. The result of survival study showed no significant difference between the groups treated with different concentrations of the extracts of *B. grandiflora* stem bark (Fig 4) in comparison to the negative control group. This implies that the extracts are not toxic to the flies at the concentrations investigated (Ehigiator *et al*., 2021) [7]. In Drosophila species, successful mating depends on male activity and female receptivity, and courtship/mating latency is one of the parameters which indicates the vigor of males. It represents the time between the introduction of male and female flies into the observation chamber and the initiation of mating (Mathew and Krishnamurthy, 2018) [18]. A male with high vigor reacts quickly in the presence of a female while a male with less vigor reacts slowly. The mating latency and copulation duration here indicate the aphrodisiac property (Adeleke *et al*., 2022) [15] of *B. Grandiflora* stem bark (Fig 5), it was observed that Sildenafil citrate, 400 mg/kg of DCM, and 400 mg/kg diet of methanol extracts showed a significant reduction in mating latency ($p \leq 0.01$) and prolonged copulation duration but it was not significantly different from the untreated group (0 mg/kg diet). A decrease in mating latency means an increase in the vigor of males (Singh and Singh, 2014) [28], the present study showed that DCM and methanol extracts arouse the mating ability of male Drosophila flies. The extended duration of copulation allows enough time for the male to introduce more sperms into the female flies (Pankaj *et al*., 2011) [26] as evident in percentage fertility. The percentage fertility of successive extracts of *B. grandiflora* stem bark was also determined. The results obtained revealed a 400 mg/kg diet of DCM and methanol extracts as significantly better concentrations among others. 400 mg/kg of DCM and methanol extracts elicited % fertility increases of 50.9% ± 8.2 and 48.2% ± 3.2 respectively at $p \leq 0.01$ when compared to (negative control) untreated group (0.0 ± 4.8). Sildenafil citrate (2 mg/kg) had a significant

aphrodisiac effect but a non-significant % fertility increase (6.1 ± 8.4) as represented in Fig 7. Increased fly emergence implies that the flies are under the influence of the investigated extracts.

*B. grandiflora* DCM and methanol stem bark extracts were proven to be effective male fertility enhancers. This study provides scientific data to back up the use of the resins secreted from *B. grandiflora* stem bark in the treatment of male infertility by traditional healers.

## Conclusion

The use of *B. grandiflora* stem bark in the management of infertility was justified and since it is relatively non-toxic, it can serve as a lead to the discovery of new drug(s) in the treatment of male infertility. However, further studies are required to isolate the bioactive constituents with male fertility-enhancing agents.

**Table 1:** Proximate analysis of powdered *B. grandiflora* stem bark

| Parameters | Values (%) |
|---|---|
| Moisture content | 15.80±1.20 |
| Total ash | 12.00±0.20 |
| Acid insoluble ash | 1.00±0.01 |
| Water-soluble ash | 5.37±0.04 |
| Alcohol extractive value | 8.79±0.80 |
| Water extractive value | 4.99±0.23 |

**Table 2:** Phytochemical analysis of *B. grandiflora* stem bark

| Test | Inference |
|---|---|
| Saponins | + |
| Tannins | + |
| Anthraquinones | - |
| Terpenoids | + |
| Alkaloids | + |
| Flavonoids | + |
| Cyanogenic glycosides | - |
| Steroids | + |

Key: + = Present, - = Absent

**Table 3:** Effect of pilot methanol extracts (PME) of *B.grandiflora* stem bark on body weight of Wistar rats per week

| Weeks | Distilled water (0.9ml/kg)) | 10 mg/kg mesterolone | Extract (200mg/kg) | Extract (400mg/kg) | Extract (600mg/kg) |
|---|---|---|---|---|---|
| 0 | 141.20±5.46 | 145.60 ± 3.20 | 165.20 ± 3.22 | 173.81± 5.49 | 167.43± 3.61 |
| 1 | 170.80±3.82 | 160.40 ± 3.60 | 178.76 ± 4.33 | 184.52± 1.37 | 195.25± 2.51 |
| 2 | 185.40±5.10 | 156.40 ±1.76 | 191.52± 3.28 | 213.06±3.41 | 209.58± 4.34 |

Data are expressed as Mean ± SEM (n=6)

**Table 4:** Effect of successive extracts of *B.grandiflora* stem bark on body weight of treated rats

| Grps/ wks | 0 | 1 | 2 |
|---|---|---|---|
| Dist water | 128.4±4.5 | 146.5±6.3 | 167.8±5.1 |
| Mest 10 mg/kg | 126.6±6.5 | 141.3±8.3 | 159.2±4.7 |
| N-H 100 | 131.1±4.2 | 147.4±5.9 | 158.2±3.8 |
| N-H 200 | 127.2 ± 3.7 | 141.3 ± 3.2 | 164.9 ± 4.6 |
| N-H 400 | 128.4±2.9 | 141.0±3.9 | 158.2±6.6 |
| DCM 100 | 130.4±4.9 | 153.9±4.1 | 169.7±7.2 |
| DCM 200 | 131.4±7.1 | 147.2±4.7 | 168.4±3.4 |
| DCM 400 | 131.0±7.8 | 142.9±4.8 | 164.5±5.3 |
| EtOAc 100 | 129.2±4.8 | 146.7±8.9 | 157.5±6.5 |
| EtOAc 200 | 129.6±4.2 | 146.2±8.2 | 154.9±5.6 |
| EtOAc 400 | 126.4±6.4 | 143.7±7.3 | 152.8±8.8 |
| MeOH 100 | 127.3±4.9 | 140.1±6.4 | 143.9±6.2 |
| MeOH 200 | 127.5±3.1 | 143.6±2.7 | 149.3±6.9 |
| MeOH 400 | 125.7±4.7 | 138.2±5.3 | 148.9±8.2 |

Data expressed as Mean ± SEM (n=6)

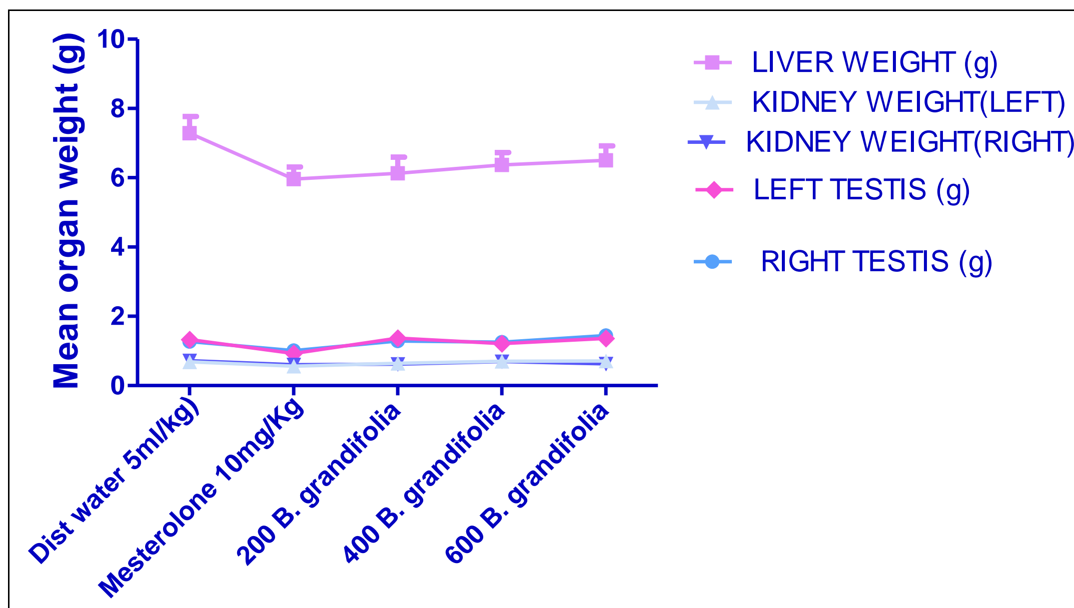


**Fig 1:** Effect of *B.grandiflora* pilot menthanol stem bark extract on organ weight of animals after 14 days administration

**Table 5:** Effects of pilot methanol extracts of *B.grandiflora*stem bark on liver and kidney parameters of the treated animals

| Liver/kidney function test (IU/L) | Distilled water | Mesterolone 10 mg/kg | MeOH 200 mg/kg | MeOH 400 mg/kg | MeOH 600 mg/kg |
|---|---|---|---|---|---|
| AST | 37.7±0.7 | 40.7±0.3 | 35.9±0.6 | 36.7±0.2 | 38.3±0.8 |
| ALT | 28.7±0.3 | 30.0±0.0 | 27.8±0.3 | 27.1±0.6 | 28±0.9 |
| ALP | 116.0±1.0 | 119.3±0.7 | 111.0±8.2 | 116.7±7.3 | 118±4.7 |
| BUN | 11.1±0.1 | 14.0±0.1 | 11.5 ±0.1 | 12.3±0.8 | 12.8±0.4 |
| CREATININE | 0.8±0.0 | 1.0±0.0 | 0.7±0.1 | 0.7±0.2 | 0.8±0.2 |

Data expressed as Mean ± SEM (n=6)

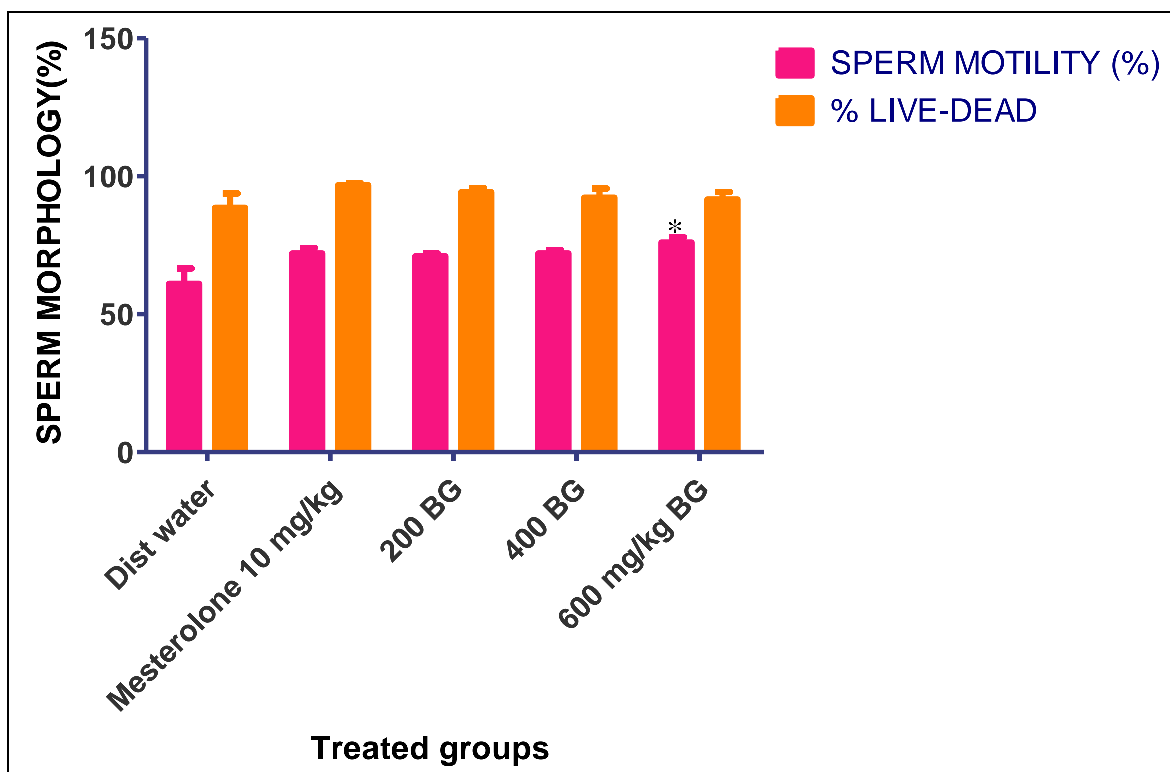


**Fig 2:** *B.grandiflora* pilot methanol stem bark extracts on sperm morphology of animals after 14 days administration. Data expressed as Mean ± SEM (n=6), * = $p \leq 0.05$

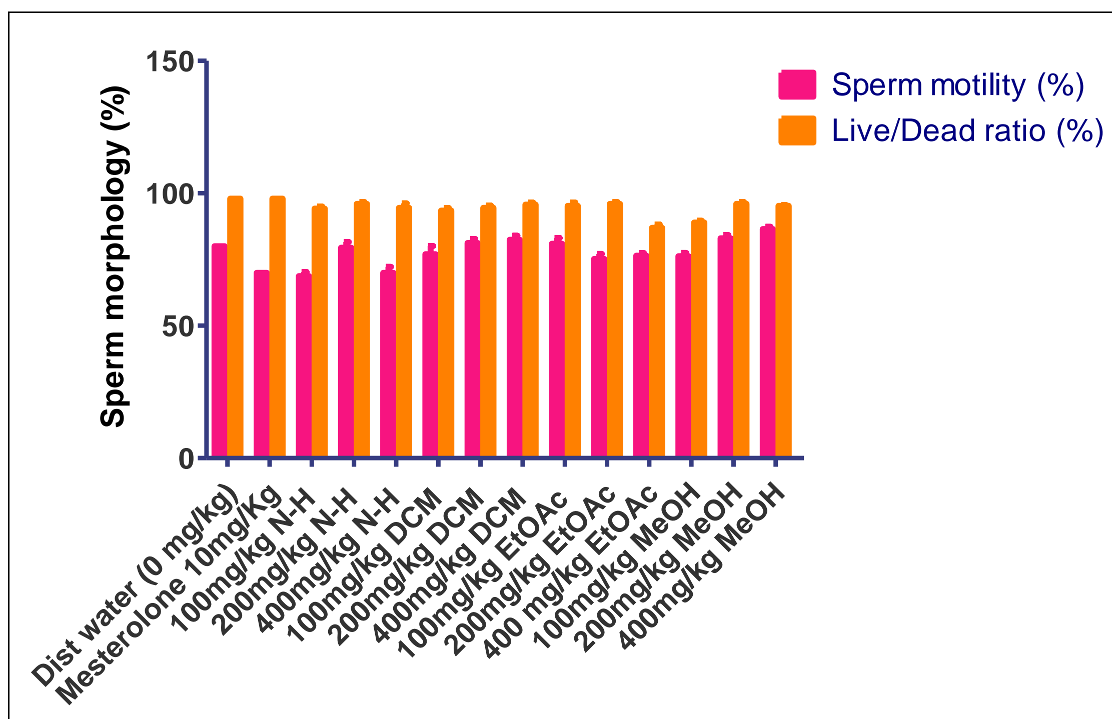


**Fig 3:** Effect of *B. grandiflora* stem bark successive extracts on the sperm morphology, N-H: Normal hexane, DCM: Dichloromethane, EtOAc: Ethyl acetate, MeOH: methanol. Data expressed as Mean ± SEM (n=6).

**Table 6:** Effect of *B. grandiflora* stem bark pilot methanol extract on sperm indices and testosterone level of treated animals.

| Treatment/Parameters | Sperm Count ($10^6$/ml) | Sperm volume (ml) | Testosterone( IU/ml) |
|---|---|---|---|
| Dist water + 0.1ml | 83.27±3.51 | 0.18±0.02 | 0.39 ± 0.03 |
| Mest 10mg/kg | 107.00 ± 5.08* (29%) | 0.16±0.02 | 1.13 ± 0.12*** (190%) |
| 200 mg/kg BG | 85.78 ± 7.10 (3%) | 0.18 ± 0.02 | 0.38 ± 0.20 (-3%) |
| 400 mg/kg BG | 111.57 ± 4.32* (34%) | 0.16 ± 0.02 | 0.45 ± 0.11* (15%) |
| 600 mg/kg BG | 112.92 ± 8.97* (36%) | 0.18 ± 0.00 | 0.51 + 0.15** (31%) |

Data expressed as Mean ± SEM (n=6), *P≤0.05, **P≤0.01, *** P≤ 0.001, Mest: Mesterolone

**Table 7:** Effects of *B. grandiflora* stem bark successive extracts on Total sperm abnormalities. N-H: normal hexane, DCM: dichloromethane, EtOAc: ethyl acetate, MeOH: methanol. Data expressed as Mean ± SEM (n=6)

| Treatment groups | Total sperm abnormalities |
|---|---|
| Distilled water | 402.50 ± 0.64 |
| Mesterolone | 401.25 ± 0.77 |
| N-H 100 mg/kg | 402.68±0.37 |
| N-H 200 mg/kg | 402.41±0.62 |
| N-H 400 mg/kg | 402.86±0.46 |
| DCM 100 mg/kg | 402.57±0.27 |
| DCM 200 mg/kg | 402.68±0.37 |
| DCM 400 mg/kg | 402.41±0.62 |
| EtOAc 100 mg/kg | 402.86±0.46 |
| EtOAc 200 mg/kg | 403.32±0.52 |
| EtOAc 400 mg/kg | 403.43±0.49 |
| MeOH 100mg/kg | 402.41±0.62 |
| MeOH 200mg/kg | 402.86±0.46 |
| MeOH 400 mg/kg | 402.57±0.27 |

**Table 8:** Effect of *B. grandiflora* stem bark successive extracts on Sperm count of Wistar rats. N-H: Normal hexane, DCM: Dichloromethane, EtOAc: Ethyl acetate, MeOH: methanol, Mest: Mesterolone, BG: *B. grandiflora.* Data expressed as Mean ± SEM (n=6), * = p ≤ 0.05

| Treatment Groups | Sperm count ($10^6$/mL) | % fertility increase/reduction relative to control |
|---|---|---|
| Distilled water (control) ml/kg | 97.5± 3.3 | - |
| 10mg/kg Mest. | 108.0± 3.6 | 10.8 |

| | | |
|---|---|---|
| 100 N-H BG | 90.3±3.8 | -7.4 |
| 200 N-H BG | 92.3±3.6 | -5.3 |
| 400 N-H BG | 95.0±1.1 | -2.6 |
| 100 DCM BG | 102.2± 4.1 | 4.8 |
| 200 DCM BG | 96.0±3.7 | -1.5 |
| 400 DCM BG | 116.3± 6.2* | 19.3 |
| 100 EtOAc BG | 98.0±3.6 | 0.5 |
| 200 EtOAc BG | 90.3±2.5 | -7.4 |
| 400 EtOAc BG | 93.0±3.5 | -4.6 |
| 100 MeOH BG | 111.2± 2.5 | 14.1 |
| 200 MeOH BG | 120.5± 3.4* | 23.6 |
| 400 MeOH BG | 118.0± 5.4* | 21.0 |

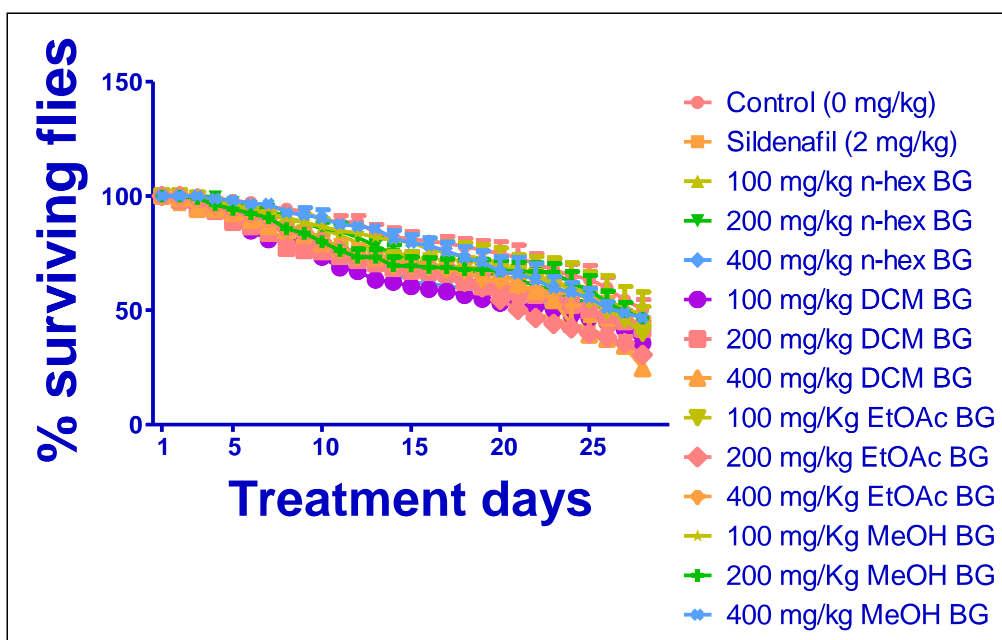


**Fig 4:** Effect of *B. grandiflora* stem bark successive extracts on 28 days survival study using *Drosophila melanogaster,* n-hex: Normal hexane, DCM: Dichloromethane, EtOAc: ethyl acetate, MeOH: methanol

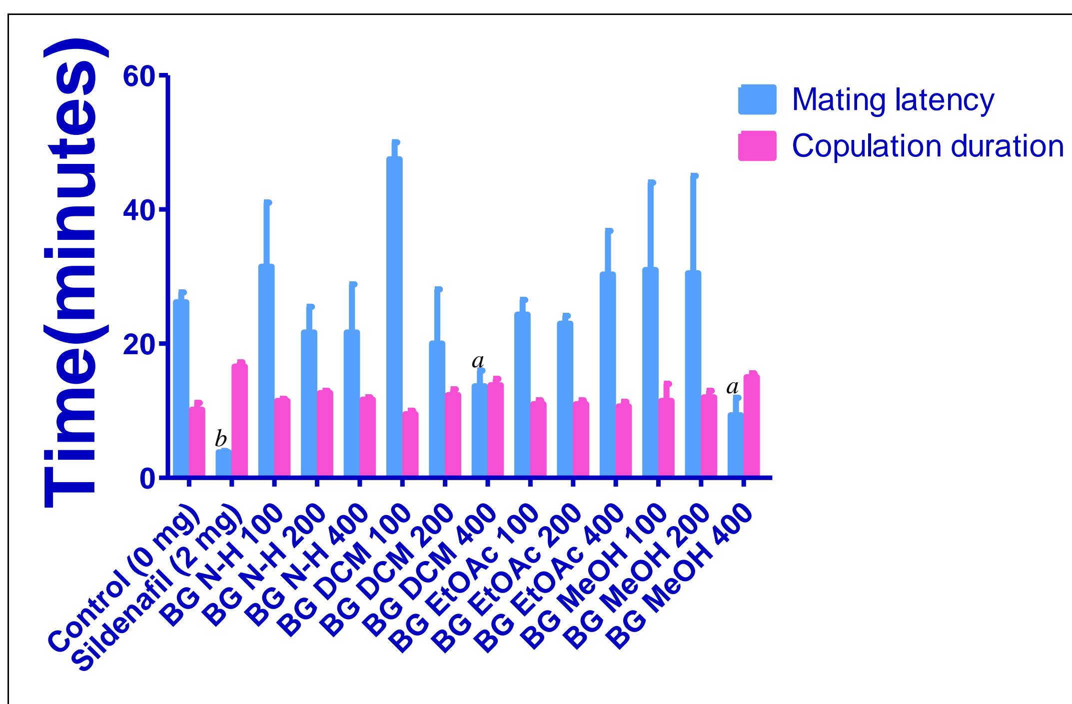


**Fig 5:** Effect of *B. grandiflora* stem bark successive extracts on mating latency and copulation duration of *D melanogaster* (fruit flies), N-H = normal hexane, DCM = Dichloromethane, EtOAc = ethyl acetate, MeOH = methanol, a = P ≤ 0.01, b = p≤ 0.001

**Table 9:** Effect of *B. grandiflora* stem bark extracts on fertility using *D. melanogaster* (fruit flies) emergence. N-H= n-hexane, DCM = dichloromethane, EtOAc = ethyl acetate, MeOH = methanol, ** = P ≤ 0.01 malenogaster. Behav Genet., 7: 359–372.

| **Treatment Groups** | % Emergence of fly | % Fertility increase/ reduction |
|---|---|---|
| Distilled water (0 mg/kg) | 100.0 ±4.8 | 0.0 ±4.8 |
| Sildenafil (2 mg/kg) | 106.1 ± 2.3 | 6.1 ± 2.3 |
| N-H 100 | 84.4 ± 3.6 | -15.6 ± 3.6 |
| N-H 200 | 87.3 ± 4.1 | -12.7 ± 4.1 |
| N-H 400 | 135.7 ± 3.8 | 35.7 ± 3.8 |
| DCM 100 | 121.7 ± 3.0 | 21.7 ± 3.0 |
| DCM 200 | 148.1 ± 14.4** | 48.1 ± 14.4** |
| DCM 400 | 150.9 ± 8.2** | 50.9 ± 8.2** |
| EtOAc 100 | 110.8 ± 6.8 | 10.8 ± 6.8 |
| EtOAc 200 | 135.2 ± 14.0 | 35.2 ±14.0 |
| EtOAc 400 | 112.9 ± 1.4 | 12.9 ± 1.4 |
| MeOH 100 | 75.1 ± 1.4 | -24.9 ± 1.4 |
| MeOH 200 | 114.5 ± 3.8 | 14.5 ± 3.8 |
| MeOH 400 | 148.2 ± 3.2** | 48.2 ± 3.2** |

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