Aphrodisiac properties of *Craterispemum schweinfurthi* Leaf Extract in Lead Induced Testicular Toxicity in Male Wistar Rats

**SARONEE, F; DAN-JUMBO, D; AZOSIBE, P**

**ABSTRACT:** The current study was designed to evaluate the aphrodisiac properties of *Craterispemum schweinfurthi* leaf extract in lead induced testicular toxicity in male Wistar rats using appropriate standard techniques. Adult male Wistar rats were divided into 7 groups and daily treated with different concentrations of extract and phytosterol only. Compared to group 1 (Control) rats, significantly higher values of mount, intromission, ejaculatory latencies as well as post ejaculatory interval and decreased values of mount, intromission and ejaculatory frequencies were observed amongst group 2 (2.25mg/kg Lead only) rats (p<0.05), following treatment with 2.25mg/kg body weight of lead acetate; suggesting a possible anti-fertility effect of lead in male Wistar rats. However, significantly lower values of mount, intromission, ejaculatory latencies and post ejaculatory interval and higher values of mount, intromission and ejaculatory frequencies were observed following the administration of the leaf extract of *Craterispemum schweinfurthi* at 250mg/kg, 500mg/kg and 750mg/kg body weight doses amongst groups 3, 4 and 5 rats compare to group 2 (2.25mg/kg Lead only) rats (p<0.05), indicating a possible mitigating effect of the extract against lead induced toxicity in male Wistar rats. *Craterispemum schweinfurthi* improves appetitive and precopulatory components of sexual behavior like sexual excitement, arousal and libido; indicating intense and sustained sexual activity.

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multifarious applications in folklore medicine. Ethnobotanical survey of *Craterispermum schweinfurthi* shows that almost every morphological part of the plant is used in traditional medicine for managing various ailments and for a wide variety of applications (Saronee et al., 2023; Saronee et al., 2024). The seed, leaves, and inner bark have been traditionally reported to have beneficial efficacies in ulcer, infertility, diabetes, anemia and fever (Sofwora, 1993; Saronee et al., 2023; Saronee et al., 2024; Saronee et al., 2024). Guided by the above described benefits associated with *Craterispermum schweinfurthi*, the current study aims to investigate the aphrodisiac properties of *Craterispermum schweinfurthi* in lead induced testicular toxicity in male Wistar rats.

**MATERIALS AND METHODS**

**Collection, Identification and Extraction of Plant Materials:** Fresh leaves of *Craterispermum schweinfurthi* were obtained from the University of Port Harcourt Botanical Garden. Dr. Chimezie Ekeke of the Department of Plant Science and Biotechnology, University of Port-Harcourt, Nigeria identified and authenticated the specimen and assigned a reference code; UPH/V/296. Voucher specimen was subsequently deposited in the University Herbarium for future reference. The plant leaves were gathered, and all extraneous materials carefully removed. The leaves were air dried at room temperature for a minimum of 7 days after which it was pulverized into powder and the weighed quantity of 670.6g dissolved using Soxhlet device in 390ml of water-methanol mixture (25:75% v/v BDH) for three days in a jar. It was filtered and concentrated using a rotary evaporator at 40°C and the yield was 73%. Obtained extract was preserved in airtight containers and stocked at room temperature prior administration.

**Procurement and Handling of Experimental Animals:** Wistar rats weighing between 100-250g were used for the study. Animals were acquired from the Department of Physiology Animal House, Faculty of Basic Medical Sciences, College of Health Sciences, University of Port Harcourt, Nigeria. Rats were placed in different compartments, one for each experimental group and cared for under standard laboratory conditions. Wood shavings and beddings were changed on a daily basis to prevent any infection due to unkept beddings. The animals were acclimatized for two weeks and subsequently grouped for the study.

**Ethical Approval and Acute Toxicity Studies:** The study protocol was approved by our Institutional Ethical Committee vide a communication dated 23rd November, 2021 with the protocol code: UPH/CEREMAD/REC/MM82/024. The acute toxicity of the hydromethanol extract of *Craterispermum schweinfurthi* leaves was determined using Karber’s method as modified by Aliu and Nwude, (1982). Lethal dose (LD50) of the extract was found to be 3968mg/kg body weight. The study was conducted in accordance with the guidelines for the care and use of laboratory animals (US NRC, 2019).

**Experimental Design:** 35 adult male Wistar rats weighing between 100-250g were randomly divided into 7 groups of 5 rats each after acclimatization. Testicular toxicity was induced following an oral daily administration of 2.25mg/kg of lead acetate at 09:00am, as was previously described by Falana and Oyeyipo, (2012) in all rat groups except groups 1 and 7. Mating test was performed on day 28 of the study. The rats were daily treated as follows for 28 days;

- Group 1: Control group; rats in this group received extract vehicle only
- Group 2: Untreated Lead acetate toxicity rats
- Group 3: Low extract dose group; rats in this group received 250mg/kg of the leaf extract of *Craterispermum schweinfurthi*
- Group 4: Medium extract dose group; rats in this group received 500mg/kg of the leaf extract of *Craterispermum schweinfurthi*
- Group 5: High extract dose group; rats in this group were given 750mg/kg of the leaf extract of *Craterispermum schweinfurthi*
- Group 6: Lead acetate toxicity + Phytosterol (2000mg/kg)
- Group 7: Phytosterol only (2000mg/kg)

**Determination of Sexual Behavior:** The method previously described by Nwafor et al., (2021) was adopted for the determination of sexual behavior. A total of 24 female Wistar rats and 32 male Wistar rats weighing between 100-250g were used for the determination of sexual behavior. The rats were exposed to red dim light for three days before commencement of the study. Oestrus was induced in all female rats by administration of 1ml solution (equivalent to 0.5mg estradiol) consisting of 2mg of estradiol dissolved in 4ml of tween 80 solvent and 1ml solution (equivalent to 2.27mg progesterone) consisting of 25mg progesterone dissolved in 11ml of distilled water. These solutions were administered separately 48 and 4 hours respectively prior commencement of sexual behavior studies. The female rats where then placed together with the male rats: 3 female rats per male rat group.

Male rats excluding the ones described above were mated with the female rats to confirm female receptivity before the actual determination of sexual
behavior. Female rats that failed to exhibit receptivity were excluded. Only the most receptive female rats were therefore used. The test was conducted at 20:00 h in the animal house. The receptive female rats were subsequently paired with the male rats as described above. Mating observation lasted for two consecutive mating attempts. The test was suspended if the male rats failed to demonstrate optimum sexual desire.

The following male sexual behavior parameters were measured as described previously by Agmo, (1997), Gauthaman et al. (2002) and Zanoli et al., (2003): Mount latency (ML): time from the introduction of the female until the first mount; Intromission latency (IL): time from introduction of the female to the first intromission (vaginal penetration); Ejaculation latency (EL): time from the first intromission to ejaculation:

Post-ejaculatory interval (PEI): time from ejaculation to the first intromission of the second copulatory series: Mount frequency (MF): number of mounts preceding ejaculation; Intromission frequency (IF): number of intromissions preceding ejaculation: Ejaculation frequency (EF): number of ejaculations in a copulatory series.

Statistical Analysis: Results are as presented in Tables 1 and 2 as Mean ± Standard Error of Means (SEM). Significant differences were determined using one-way ANOVA and LSD Post Hoc test. A p value of less than 0.05 was considered statistically significant.

RESULTS AND METHODS
Values of mount and intromission parameters in lead acetate induced testicular toxicity following extract and phytosterol treatment: Compared to group 1 (Control) rats in table 1, significantly higher values of mount and intromission latencies and decreased values of mount and intromission frequencies were observed amongst group 2 (2.25mg/kg Lead only) rats (p<0.05), following treatment with 2.25mg/kg body weight of lead acetate; suggesting a possible anti-fertility effect of lead in male Wistar rats.

However, significantly lower values of mount and intromission latencies and higher values of mount and intromission frequencies were observed following administration of the leaf extract of Craterispermum schweinfurthi at 250mg/kg, 500mg/kg and 750mg/kg body weight doses amongst groups 3, 4 and 5 rats compare to group 2 (2.25mg/kg Lead only) rats (p<0.05), indicating a possible mitigating effect of the extract against lead induced toxicity in male Wistar rats.

Administration of 2000mg/kg body weight of phytosterol to animals in groups 6 and 7 shows similar results. However, a comparison of the effect of the extract amongst group 5 rats and the effect of phytosterol amongst groups 6 and 7 rats, shows that the extract of Craterispermum schweinfurthi at the dose administered to group 5 rats, significantly decreased the values of mount and intromission latencies and increased the values of mount and intromission frequencies compared to groups 6 and 7 rats respectively (p<0.05): suggesting a possible higher potency of the extract.

Values of ejaculatory parameters in lead acetate induced testicular toxicity following extract and phytosterol treatment.: In table 2, Administration of lead to animals in group 2, caused a significant decrease in ejaculatory latency, ejaculatory frequency and increased the values of post ejaculatory interval compared to group 1 (Control) rats, demonstrating a possible prolong recovery period from exhaustion after the first series of mating.

Upon the administration of graded doses (250mg/kg, 500mg/kg and 750mg/kg body weight) of the extract to groups 3, 4 and 5 rats, a significant and dose dependent increase in ejaculatory frequency and decrease in the values of ejaculatory latency and post ejaculatory interval were observed compared to group 2 rats; suggesting a possible reversal of the harmful effect of lead in Wistar rats. Similar results were observed amongst groups 6 and 7 rats following phytosterol treatment.

Table 1: Values of mount and intromission parameters in lead acetate induced testicular toxicity following extract and phytosterol treatment.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mounting Latency (Sec.)</th>
<th>Mounting Frequency (Sec.)</th>
<th>Intromission Latency (Sec.)</th>
<th>Intromission Frequency (Sec.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>60.60±0.812 ×</td>
<td>20.00±0.707 ×</td>
<td>82.00±0.707 ×</td>
<td>5.80±0.374 ×</td>
</tr>
<tr>
<td>2.25mg/kg Lead only</td>
<td>82.80±0.860 ×</td>
<td>15.20±0.374 ×</td>
<td>92.60±0.812 ×</td>
<td>3.40±0.400 ×</td>
</tr>
<tr>
<td>250mg/kg Lead</td>
<td>73.00±0.894 ×</td>
<td>17.80±0.374 ×</td>
<td>82.80±0.1019 ×</td>
<td>5.20±0.374 ×</td>
</tr>
<tr>
<td>500mg/kg Lead</td>
<td>61.60±1.208 ×</td>
<td>20.60±0.244 ×</td>
<td>73.00±0.509 ×</td>
<td>4.60±0.244 ×</td>
</tr>
<tr>
<td>750mg/kg Lead</td>
<td>48.60±0.509 ×</td>
<td>22.60±0.244 ×</td>
<td>61.40±0.979 ×</td>
<td>7.60±0.254 ×</td>
</tr>
<tr>
<td>2000mg/kg Phytosterol</td>
<td>78.00±0.706 ×</td>
<td>16.40±0.254 ×</td>
<td>86.40±0.244 ×</td>
<td>4.60±0.254 ×</td>
</tr>
</tbody>
</table>

Values are shown as Mean ± SEM; n=5; * Significant at P<0.05 compared with Control (Group 1). † Significant at p<0.05 compared with 2.25mg/kg Lead only (Group 2).

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In the current study, *Craterispermum schweinfurthi* extract was tested in animal experimentation for its aphrodisiac properties in lead induced toxicity in male Wistar rats. The study showed that the extract of *Craterispermum schweinfurthi* possesses sexual enhancing functions and potentials as observed amongst rats in the treatment groups. Results of sexual behavior test (Mating test) showed that the extract increased Mounting Frequency, Intromission Frequency, Ejaculatory Frequency, and decreased Mounting Latency, Intromission Latency and Post Ejaculatory Interval in all the test groups. Mounting Frequency and Intromission Frequency are known indices of libido and potency. Therefore, the extract apparently affects libido and potency at the administered doses. Mounting and intromission latencies are inversely proportional to sexual motivation and desire (Beach, 1956). So, the affectation of these indices in the present study is suggestive that at the administered doses, the extract improved sexual desire and motivation. However, the extract was observed to significantly decrease ejaculatory latency and increase ejaculatory frequency. The mating potential of male rats is determined by the number of ejaculations within a time-limited sexual behavioral performance or the number of ejaculations prior to sexual satiety attainment (Sachs and Meisel, 1988). The significant reduction in ejaculatory latency and consequent increase in ejaculatory frequency in all the test groups after 28 days of administration suggest that the hydromethanol leaf extract of *Craterispermum schweinfurthi* causes a reduction in the duration of coitus, which is indicative of an intense and sustained sexual activity and therefore enhances copulatory activity. Post Ejaculatory Interval which is a known index of potency and libido, shows the rate of recovery from exhaustion after the first series of mating exercise (Tajuddin et al., 2004). Post Ejaculatory Interval was found to be significantly reduced in a dose dependent fashion in all rat groups in the present study, suggesting that the extract could cause a quick recovery after an exhausting mating exercise, indirectly stimulating an increase in mitochondrial activity (Breitbart et al., 1984; Randel et al., 1992). Male sexual behavior, including both appetitive and consummatory divisions, is regulated by gonadal hormones secreted from the testes of male animals (Hull et al., 2006; Saronee et al., 2024).

In conclusion, the current study showed that in male Wistar rats, *Craterispermum schweinfurthi* improves appetitive and precopulatory components of sexual behavior like sexual excitement, arousal and libido; indicating intense and sustained sexual activity which reflects possible enhancement of copulatory performance. It causes quick recovery after an exhausting mating activity. These results suggest that the extract may have similar effects in humans by improving sexual desire (libido) and copulatory functions.

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