APHRODISIAC STUDIES OF DIHERBAL MIXTURE OF ZANTHOXYLUM LEPRIEURII GUILL. & PERR. AND PIPER GUINEENSE SCHUMACH. & THONN. ON MALE WISTAR RATS

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ABSTRACT

The effects of 50, 100 and 150 mg/kg body weight of ethanol extract of diherbal mixture of Zanthoxylum leprieurii and Piper guineense on aphrodisiac potentials and hormonal levels was investigated. A total of twenty healthy, sexually experienced Albino male rats (Rattus norvegicus), weighing between 190–230g were randomly divided into four groups (A–D) of five rats each, group A were orally administered once daily with 1 ml of distilled water (vehicle), while groups B, C and D received 50, 100 and 150 mg/kg body weight of the extract in 1ml of the vehicle. After 21 days of treatment the animals were studied for mating behaviour and hormonal assay, the mating behaviour monitoring showed that mount latency, intromission latency and post ejaculatory intervals were significantly decreased (P < 0.05), mount frequency, intromission frequency, ejaculatory frequency and ejaculatory latency were all significantly increased (P < 0.05), however, only the 150 mg/kg body weight dosage produced significant increase in copulatory efficiency. Hormonal assay showed that all doses produced significant increase (P < 0.05) in testosterone, luteinizing hormone and follicle stimulating hormone, similarly all doses produced significant increase (P < 0.05) in progesterone except 50 mg/kg, prolactin was decreased significantly (P < 0.05) by all dose level. The effects of these extracts on the mating and hormonal studies were dose dependent and the findings validated the acclaimed use of this herbal product as an aphrodisiac in men.

KEYWORDS: Aphrodisiac, Diherbal mixture, Zanthoxylum leprieurii and Piper guineense

Cite this Article

INTRODUCTION

The incidence of male sexual dysfunction is on the increase globally (Kaiser, 1999). It is reported as having a prevalent rate of 10% across all ages (Lauman et al., 1999), and because sexual dysfunction is an inevitable process of aging, the prevalence is over 50% in men between 50 and 70 years of age (Rendell et al., 1999).

Male sexual dysfunction is classified as a disorder of desire that is persistent absence of sexual fantasy and desire for sexual activity (Kandeel et al., 2001), erectile dysfunction that is persistent inability to develop and maintain a penile erection that is for intercourse and ejaculation in 50% or more of attempts (Kandeel et al., 2001), disorder of ejaculation that is problem with expulsion of semen at the climax of the sexual act, disorder of orgasm that is delay in or absence of orgasm after a normal sexual excitement phase during sexual activity (Rosen and Lieblum, 1995), failure to detumescence that is prolonged (> 4 hours duration) and extreme painful erection unaccompanied by sexual desire (Kandeel et al., 2001).

The causes of male sexual dysfunction include factors like psychological disorders (performance anxiety, strained relationships, depression, stress, guilt, fear of sexual failure), hormonal condition (androgen deficiency, hyperprolactinemia), chronic medical conditions (diabetes, hypertension), penile disease (priapism, smooth muscle dysfunction), neurological disorder (Parkinson disease, stroke, cerebral trauma, alzheimer’s disease, spinal cord or nerve injury), drug side effect (antihypertensive, psychiatric medications, antiulcer and antidepressants), lifestyle (chronic alcohol abuse, cigarette smoking), aging and systemic disease (cardiac, hepatic, renal, pulmonary, metabolic), post organ transplant (Kandeel et al., 2001; Guay et al., 2003; Yakubu et al., 2007).

Despite the medical advancements in treatment and treatment facilities for male sexual dysfunction (Lim et al., 2005), most sufferers often shy away from these treatment options and these could be attributed to its sensitivity and social stigma attached to male sexual dysfunction in the African context (Lim et al., 2005), these treatment options are equally very expensive, not easily accessible to the poor and rural dwellers and are often associated with some serious side effects, consequently medicinal plants with marked pharmacological activities are readily available all year round, cheap and accessible and often with minimal side effects (Tilburt and Kaptchuk, 2008; Ufelle et al., 2011; Yadav and Agarwala, 2011), and are being explored globally as panacea. All through history many preparation from plants have been used and reputed to have sex invigorating (aphrodisiac) properties and these include Yohimbine, Gingseng, Massularia acuminata, Montanoa tomentosa (Ang et al., 1997; Baljinder et al., 2010; Yakubu and Akanji, 2011).

The composite extract of the bark of Zanthoxylum leprieurii Guill. & Perr. and the seeds of Piper guineense Schumach. & Thonn. are two of such sex tonics that is largely used by the people of Niger Delta region of Nigeria, but with no scientific footing. Z. leprieurii belong to the family Rutaceae and locally called Prickly ash or toothache tree, it is an aromatic, spiny, thicket forming deciduous shrub or tree. The alternate branches are armed with strong brown prickles about 2–3 cm long, cone shaped with a broad base and found irregularly throughout the tree (Todd, 2008). Ethnomedically, it is used in the treatment and management of muscle spasm, varicose vein, raynauld disease, arthritis, rheumatism, neuralgia, flu, fever, toothache and gum diseases (Tiloston, 2011). P. guineense commonly called climbing pepper is a slender climber up to 12 m high with prominent nodes and clasping roots, the leaves are elliptic in shape about 15 cm long and 7 cm broad, the flowers are red and turns black when dry (Iwu, 1988). Traditionally the seeds are used as spices and as sex invigorator in men.
The present study was therefore undertaken to evaluate the aphrodisiac property of the composite ethanol extract of the stem bark of *Zanthoxylum leprieurii* and the seeds of *Piper guineense* using a dose level of 50, 100 and 150 mg/kg body weight with a view to validate the acclaimed use of this sexual invigorator.

**MATERIALS AND METHODS**

The plant samples were bought in a local herb market in Warri, Delta State, Nigeria. Both the species were identified and confirmed at the Herbarium of the Department of Plant Science and Biotechnology of the University of Portharcourt, Rivers State.

**Preparation of Plant Extract**

The bark *Z. leprieurii* and the seeds of *P. guineense* were thoroughly washed with distilled water to remove debris and contaminants, they were then dried in an oven at 40°C until a constant weight was reached, and then pulverized using an electric blender (Blender, 462 Nakai Japan). 200 g of the powdered mixture (i.e 100 g each of *Z. leprieurii* and *P. guineense*) was extracted in 600 ml of absolute ethanol for 24 h at room temperature with constant shaking using a flask shaker (Model, Denly A - 500). The extract was filtered with Whatman No. 1 filter paper and the resulting filtrate was evaporated to dryness using a rotatory evaporator at 40°C to give 5.74 g, the resultant concentrate was reconstituted in distilled water to give the required doses used in the study.

**Experimental Animals**

A total of twenty healthy sexually experienced Albino male rats (*Rattus norvergicus*), 2.5–3 months old, weighing between 190–230 g and thirty female rats between 2–2.5 months old and weighing between 150–180 g were obtained from the animal house unit of the Department of Biochemistry, University of Portharcourt, Rivers State. The animals were kept in a clean cage and housed in a well ventilated room at temperature 28–30°C under natural light and dark cycle with free access to grower’s mash and water.

**Experimental Design**

The twenty male rats were randomly divided into four groups (A–D), consisting of five rats each. Group A were orally administered once daily with 1 ml of distilled water (vehicle), groups B, C, and D were orally administered with 50, 100 and 150 mg/kg body weight of the extract in 1 ml of the vehicle for 21 days.

**Mating Behaviour Test Procedure**

The investigation was carried out on the 22nd day after the commencement of extract administration by adopting the methods described by (Gauthaman *et al.*, 2002; Yakubu and Akanji, 2011). The investigation was conducted between 19 and 22 h in the same laboratory and under a dim light. The female rats were artificially brought to oestrus by administering estradiol benzoate 10 µg/100 g body weight orally 48 h prior to mating and progesterone injected subcutaneously at a dose of 0.5 mg/100 g 6 h prior to the mating (Szectman *et al.*, 1991; Yakubu *et al.*, 2005). The artificially warmed female was introduced to the cage of the male. The male and female were observed from the cage side for proceptive, precopulatory and copulatory behaviours. The occurrence of events and phases of mating was monitored for 30 min observatory period and the following male sexual behavior indices recorded/calculated. Mount latency: the time interval between the introduction of the female to the first mount by the male; Mount frequency: the number of times the male assumed copulatory position but failed to achieve intromission; Intromission latency: the time interval from the introduction of the female until the first intromission of the female (vaginal penetration) by the male; Intromission frequency: the number of intromissions (vaginal penetration) made by the male from the first time of introduction of the female; Ejaculatory latency: the time interval between the first intromission and ejaculation, it is usually characterized by longer, deeper
pelvic thrusting and slow dismount, followed by a period of reduced activity; Ejaculation frequency: The number of times there was expulsion of semen by the males after vaginal penetration characterized by rhythmic contraction of the posterior abdomen; Post ejaculatory interval (PEI): the time interval from ejaculation to intromission of the next mating series; Copulatory efficiency: number(s) of intromission divide by number(s) of mount multiplied by 100 %. (Yakubu et al., 2005, and Yakubu and Akanji, 2011).

**Method of Collection and Handling of Serum**

At the end of the treatment period, the animals were anaesthetized in a chloroform chamber and blood samples collected from the jugular vein into sample bottles, the blood samples were allowed to clot for 10 min at room temperature and subsequently centrifuged to obtain serum for hormonal assay.

**Assay Kits**

Testosterone, follicle stimulating hormone (FSH), luteinizing hormone, prolactin and progesterone radioimmunoassay test kits are products of BYK-Sangtic Diagnostica, GmbH and Co. KG, while estradiol benzoate and progesterone are products of Sigma Chemicals, St. Louis, USA and Shalina Laboratories, Mumbai, India, respectively.

**Hormonal Assay**

Serum samples were assayed for the following hormones testosterone, follicle stimulating hormone, luteinizing hormone, prolactin and progesterone by using the procedure described by BYK-Sangtic Diagnostica. This was based on the principle of radioimmunoassay of competitive binding between the sample serum and the standards for a constant amount of the antisera. (Tietz, 1995).

**Statistical Analysis**

The results were expressed as the mean of five replicates ± standard deviation (S.D), means were analysed using one way analysis of variance (ANOVA) followed by Posthoc (Turkey). $P < 0.05$ was regarded as significant. The Statistical Package for Social Sciences (SPSS) Computer software version 16 was used for data analysis.

**RESULTS**

**Effects of the Composite Mixture of *Z. leprieurii* and *P. guineense* on Mating Behaviour**

Upon the introduction of the female to the male cage, the male responded with immediate advances towards the female and displayed pre-copulatory behaviours such as chasing, anogenital sniffing which eventually culminated into mounting, the female also displayed receptivity that allowed mating to occur. The ethanol extract of the diherbal mixture of *Z. leprieurii* and *P. guineense* after 21 days of treatment at a dosage of 50, 100 and 150 mg/kg body weight was able to influence significantly ($P < 0.05$) on the mount latency, intromission latency, mount frequency, intromission frequency, ejaculatory latency, ejaculatory frequency and post ejaculatory interval, however, only the 150 mg/kg body weight of the extract had significant effect ($P < 0.05$) on the copulatory efficiency (Table 1.0).

**Effects of the Composite Mixture of *Z. leprieurii* and *P. guineense* on Hormonal Levels.**

The ethanol extract of the composite mixture of *Z. leprieurii* and *P. guineense* after 21 days of treatment at 50, 100 and 150 mg/kg body weight dose was able to significantly increase ($P < 0.05$) the levels of testosterone, luteinizing hormone and follicle stimulating hormone, however, only 100 and 150 mg/kg body weight was able to significantly ($P < 0.05$) increase progesterone. All doses of the extract studied were also able to significantly decrease ($P < 0.05$) the level of prolactin (Table 2.0).
### Table 1.0 Effect of 50, 100 and 150 mg/kg Body Weight of Ethanol Extract of Composite of *Zanthoxylum leprieurii* and *Piper guineense* on Mating Behaviour Parameters.

<table>
<thead>
<tr>
<th>S/N</th>
<th>MATING PARAMETERS</th>
<th>A CONTROL</th>
<th>B 50 mg/kg B.W</th>
<th>C 100 mg/kg B.W</th>
<th>D 150 mg/kg B.W</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Mount Latency (Seconds)</td>
<td>813.00 ± 4.95&lt;sup&gt;a&lt;/sup&gt;</td>
<td>746.00 ± 6.67&lt;sup&gt;b&lt;/sup&gt;</td>
<td>610.00 ± 7.21&lt;sup&gt;c&lt;/sup&gt;</td>
<td>493.00 ± 9.76&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>2.</td>
<td>Intromission Latency (Seconds)</td>
<td>820.00 ± 7.91&lt;sup&gt;a&lt;/sup&gt;</td>
<td>750.00 ± 6.63&lt;sup&gt;b&lt;/sup&gt;</td>
<td>618.00 ± 5.70&lt;sup&gt;c&lt;/sup&gt;</td>
<td>499.00 ± 8.63&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>3.</td>
<td>Mount Frequency</td>
<td>5.20 ± 0.84&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10.40 ± 2.07&lt;sup&gt;b&lt;/sup&gt;</td>
<td>14.20 ± 1.92&lt;sup&gt;c&lt;/sup&gt;</td>
<td>18.80 ± 1.48&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>4.</td>
<td>Intromission Frequency</td>
<td>3.00 ± 0.71&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.80 ± 1.64&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9.60 ± 1.52&lt;sup&gt;c&lt;/sup&gt;</td>
<td>13.00 ± 1.58&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>5.</td>
<td>Ejaculatory Latency (Seconds)</td>
<td>234.00 ± 6.52&lt;sup&gt;a&lt;/sup&gt;</td>
<td>274.00 ± 9.19&lt;sup&gt;b&lt;/sup&gt;</td>
<td>310.00 ± 11.81&lt;sup&gt;c&lt;/sup&gt;</td>
<td>409.00 ± 8.54&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>6.</td>
<td>Ejaculatory Frequency</td>
<td>1.00 ± 0.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.00 ± 0.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.20 ± 0.45&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.20 ± 0.84&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>7.</td>
<td>Post Ejaculatory Interval (Seconds)</td>
<td>400.00 ± 7.91&lt;sup&gt;a&lt;/sup&gt;</td>
<td>353.40 ± 8.44&lt;sup&gt;b&lt;/sup&gt;</td>
<td>308.60 ± 6.84&lt;sup&gt;c&lt;/sup&gt;</td>
<td>241.20 ± 7.01&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>8.</td>
<td>Copulatory Efficiency (%)</td>
<td>57.33 ± 7.23&lt;sup&gt;a&lt;/sup&gt;</td>
<td>65.01 ± 3.70&lt;sup&gt;a&lt;/sup&gt;</td>
<td>67.49 ± 2.46&lt;sup&gt;a&lt;/sup&gt;</td>
<td>69.00 ± 360&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are means of five replicates ± standard deviation, values with different superscript letters b, c and d are significantly different (P < 0.05) from the control ‘a’. BW: body weight.

### Table 2.0 Effect of 50, 100 and 150 mg/kg Body Weight of Ethanol Extract of Composite of *Z. leprieurii* and *P. guineense* on Hormonal Levels.

<table>
<thead>
<tr>
<th>S/N</th>
<th>HORMONE</th>
<th>A CONTROL</th>
<th>B 50 mg/kg B.W</th>
<th>C 100 mg/kg B.W</th>
<th>D 150 mg/kg B.W</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Testosterone (nmol/L)</td>
<td>3.10 ± 0.16&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.22 ± 0.19&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.94 ± 0.21&lt;sup&gt;c&lt;/sup&gt;</td>
<td>10.62 ± 0.22&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>2.</td>
<td>Luteinizing hormone (IU/L)</td>
<td>2.86 ± 0.19&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.64 ± 0.38&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.98 ± 0.29&lt;sup&gt;c&lt;/sup&gt;</td>
<td>5.30 ± 0.31&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>3.</td>
<td>Follicle stimulating hormone (IU/L)</td>
<td>2.92 ± 0.23&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.12 ± 0.33&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.98 ± 0.15&lt;sup&gt;c&lt;/sup&gt;</td>
<td>6.08 ± 0.28&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>4.</td>
<td>Progesterone (nmol/L)</td>
<td>0.98 ± 0.24&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.34 ± 0.24&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.44 ± 0.18&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.64 ± 0.18&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>5.</td>
<td>Prolactin (ng/ml)</td>
<td>6.82 ± 0.37&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.14 ± 0.32&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.60 ± 0.46&lt;sup&gt;c&lt;/sup&gt;</td>
<td>5.04 ± 0.19&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are means of five replicates ± standard deviation, values with different superscript letters b, c and d are significantly different (P<0.05) from the control ‘a’. BW: body weight.
DISCUSSION

Aphrodisiacs can be defined as substances which are ingested, applied topically, smoked/snorted or otherwise delivered into the body to induce sexual arousal, heighten sexual experience and to improve sexual performance. History had it that many preparations from plants and animals have been used for this purpose e.g. Yohimbine, the mandrake plant, Ginseng, ground rhinoceros horn, sheep and bull testicle and the Spanish fly (Ang et al., 1997; Baljinder et al., 2010). This present study was therefore designed to investigate the aphrodisiac properties of the composite mixture of Zanthoxylum leprieurii and Piper guineense.

Mount latency and intromission latency are indices of sexual motivation and there is an inverse relationship between mount latency, intromission latency and sexual motivation (Yakubu and Akanji, 2011). The extract was able to significantly decrease ($P < 0.05$) the mount latency and intromission latency and this might imply stimulation of sexual appetite and arousal, thus lending credence to the sexual improving effect of the extract under study just like other aphrodisiac plants that have been studied e.g. Lepidium myenii (Cicero et al., 2001), Montanoa tomentosa (Carro-Juarez et al., 2004) and Microdemis kenyana (Zamble et al., 2008).

Mount and intromission frequencies are indicators of libido, sexual vigour, strength, power and energy (Yakubu et al., 2005). The extract was able to significantly increase ($P < 0.05$) the Mount and intromission frequencies, thus supporting the sexual improving effect of the extract as in the case of other aphrodisiac plants that have been studied e.g. Triholepis glaberrima (Padashetty and Mishra, 2007), Mucuna pruriens (Amin et al., 1994) and Terminalia catappa (Ratnasooriya and Dharmasiri, 2000). Increased intromission frequency is also a function of erection efficiency, penile orientation and the ease by which ejaculatory reflexes are activated (Agmo, 1997; Yakubu and Akanji, 2011).

Ejaculatory latency and ejaculatory frequency are pointers of enhanced copulatory performance, the extract was also able to significantly increase ejaculatory latency and ejaculatory frequency ($P < 0.05$). Ejaculatory latency also implies prolonged coitus duration which translates into increased staying power, strength and vigour thus validating its aphrodisiac properties just other aphrodisiac plants that have been investigated e.g. Vanda tsellata (Suresh et al., 2000), Dactylorhiza hatagirea (Thakur and Dixit, 2007) and Mondia whitel (Watcho et al., 2007).

Post ejaculatory interval is a positive marker of sexual potency, libido and a fast pace of recovery from exhaustion after the first series of mating, all doses of the extract significantly decreased the post ejaculatory interval ($P < 0.05$) and compared favourably with other aphrodisiac plants that have been studied e.g. Chlorophytum borivilianum (Thakar and Dixit, 2006) and Syzygium aromaticum (Tajuddin et al., 2004).

Copulatory efficiency is an indication that the copulatory action of the male was well enhanced with well coordinated pelvic thrusting and this further indicates sustained increase in interest, focus, agility and stamina in the sexual act. Only the 150 mg/kg body weight of the extract was able to increase the copulatory efficiency significantly ($P < 0.05$). Thus lending credence its aphrodisiac potency which compares to other aphrodisiac plants that have been studied e.g. Alpinia calcarata (Ratnasooriya and Tayakody, 2006) and Withania somnifera (Illayperuma et al., 2002).

The precopulatory and copulatory behaviours of the extract treated rats showed that the rats were extremely aroused and the effects of the extract on the mating behaviour were dose dependent with the 150 mg/kg body weight being more potent.

Sexual behaviour and erection are largely dependent on androgen which may act through central and peripheral mechanism (Mill et al.,
1996; Yakubu et al., 2011). All doses of the extract significantly increased \((P < 0.05)\) testosterone, luteinizing hormone and follicle stimulating hormone. Luteinizing hormone and follicle stimulating hormone are produced by the anterior pituitary lobe and are needed for maintaining testosterone levels, hence an increase in luteinizing hormone and follicle stimulating hormone automatically triggers an increase in testosterone levels (Yakubu et al., 2007). Studies have shown that testosterone supplementation helps to improve sexual function and libido (Aversa and Fabiri, 2001; Grahl et al., 2007), in addition to the intensity of orgasm and ejaculation (Morales, 1996). All doses of the extract except 50mg/kg body weight was able to increase the progesterone level significantly \((P < 0.05)\), optimal level of progesterone have associated with improved sexual function (Andersen and Tufik, 2006; Andersen et al., 2007). High levels of prolactin in men (hyperprolactinemia) have been associated with hypogonadism, decreased sperm count and motility, erectile dysfunction and decreased libido (Kruger et al., 2003; Paick et al., 2006), all doses of the extract was able to decrease significantly the levels prolactin in a manner that was dose dependent.

The observed improvement in mating behaviour seen between the control and the extract treated groups may be due to plant chemicals present in the extract (alkaloid, saponin, tannin, flavonoid, sterols), these plant chemicals are able to exert their effect through elevation of androgens and gonadotropins, vasodilation and generation of nitric oxide (Yakubu and Akanji, 2011), which are key factors in the initiation and sustenance of erection, libido and other sexual factors.

**CONCLUSION**

The systemic use of the ethanol extract of *Zanthoxylum leprieurii* and *Piper guineense* have marked enhancement on mating behaviour parameters and the sex hormones of male rats, thus corroborating the acclaimed use of this product as an aphrodisiac by the people of the Niger Delta region of Nigeria.

**REFERENCES**


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Conflict of Interest: None Declared