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Effect of *Asteracantha longifolia* seeds on the sexual behaviour of male rats

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The aim of the present study was to study the effect of seeds of *Asteracantha longifolia* on the sexual behaviour of male albino rats. The ethanolic extract of seeds of *A. longifolia* was administered to groups of rats in 100, 150 and 200 mg kg\(^{-1}\) doses for a period of 28 days, and the action compared with control rats. The changes in body and organ weight, sexual behaviour, histo-architecture and fructose levels of seminal vesicles were observed. The sexual behaviour was assessed by determining parameters such as mount frequency (MF), intromission latency, mount latency (ML) and post-ejaculatory latency. The ethanolic extract exhibited pronounced anabolic effects in treated animals, as evidenced by gains in the body and reproductive organ weights. Increased spermatogenesis due to treatment with extracts was also witnessed in transverse section. The treatment further markedly affected sexual behaviour of the animals, as reflected by the reduction of ML, increase in MF and enhanced attractability towards females. A significant increase in the sperm count as well as fructose levels of seminal vesicles was noted.

**Keywords:** *Asteracantha longifolia*; fructose; sperm count; Vajikaran; spermatogenesis, Rasayana

1. Introduction

The role of the male in the sexual process and reproduction is two-fold: (1) to undertake successful intercourse with the purpose of deriving pleasure for himself and simultaneously satisfying the female partner; and (2) to contribute the sperm for successful fertilization of the ovum towards procreation. The problem of male infertility and impotency is increasing day by day. The global incidence of couple infertility is estimated at 10–15% (Krausz, 2000). The contribution of the male factor alone to couple infertility is believed to be about 20% (World Health Organization, 1987). The remedies for male sexual dysfunction in modern medicine are limited. Allopathic drugs such as sildenafil have been developed for the management of erectile dysfunction, but produce side effects which limit their utility (Vitezic & Pelcic, 2002). Ayurveda realised the problem of male sexual dysfunction thousands of years ago and developed a separate field of therapeutics known as ‘Vajikaran’, meaning potentiation in sexual performance. In recent years, many plants categorised as Vajikaran have been tested for their aphrodisiac activity viz – *Mucuna pruriens* (Saksena & Dixit, 1987), *Caesalpinia benthamiana* (Zamble et al., 2008),

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Chlorophytum borivilianum (Thakur & Dixit, 2006), Curculigo orchioides (Chauhan, Rao, & Dixit, 2007), Montanoa tomentosa (Carro-Juarez, Cervantes, Cervantes-Mendez, & Rodriguez-Manzo, 2004), Dactylorhiza hatagirea (Thakur & Dixit, 2007), Orchis latifolia (Thakur & Dixit, 2008) and Syzygium aromaticum (Tajuddin, Latif, & Qasmi, 2004) have shown to improve sexual manifestation.

Working on the same line, Asteracantha longifolia Nees (Syn) Hygrophila auriculata (Schum) Heine, family Acanthaceae, a popular herb in traditional Indian medicine, has been evaluated for its purported aphrodisiac usage. A. longifolia is a wild herb commonly found in moist places on the banks of tanks, ditches, paddy fields, etc. throughout India and Sri Lanka. It is a source of the ayurvedic drug ‘Kokilaaksha’ and the Unani drug ‘Talimakhana’. The seeds are acrid, bitter, aphrodisiac, tonic, and sedative (Kirtikar & Basu, 1987). The plant is known to possess antitumour (Mazumdar, Gupta, Maiti, & Mukhejee, 1997), hypoglycaemic (Fernando, Wickramasinghe, Thabrew, & Singhai, 2006a, 2006b). The aerial parts of A. longifolia are reported to contain lupeol, stigmasterol and butelin (Misra, Singh, Pandey, Singh, & Pandey, 2001). The reported seed constituents are fatty acids (Quasim & Dutta, 1967), sterols (Parashar & Singh, 1965) and alkaloids asteracanthine and asteracanthinine (Basu & Rakhit, 1957a, 1957b). There are no reports available in the literature which may validate the traditional use of plant as an aphrodisiac. The present studies were thus undertaken to investigate the effect of the seeds of A. longifolia on the sexual behaviour of male rats.

2. Results

2.1. Sexual organ weight and histopathological studies

The effect of the ethanolic extract of the seeds of A. longifolia on body weight, as well as weight of sexual organs, is given in Table 1. A general improvement in body weight of treated animals was noted. There was significant change in weights of sexual organs, and an increase in testes, prostate, seminal vesicles and epididymis was observed. The increase in weight was influenced by the dose of the extracts, the highest values being obtained with 200 mg kg⁻¹ dose. Testosterone treatment also caused increase in weight of sexual organs, which was comparable with extract treatments.

<table>
<thead>
<tr>
<th>Group</th>
<th>Body weight of animals (gm)</th>
<th>Weight of testis (mg 100 g⁻¹)</th>
<th>Weight of prostate (mg 100 g⁻¹)</th>
<th>Weight of seminal vesicles (mg 100 g⁻¹)</th>
<th>Weight of epididymis (mg 100 g⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>133.83 ± 3.25</td>
<td>955.5 ± 5.4</td>
<td>102.6 ± 2.4</td>
<td>623.1 ± 2.1</td>
<td>455.4 ± 3.8</td>
</tr>
<tr>
<td>Testosterone treated</td>
<td>139.33 ± 2.06</td>
<td>1112.2 ± 4.6*</td>
<td>112.6 ± 3.6**</td>
<td>635.2 ± 2.8**</td>
<td>473.6 ± 4.8**</td>
</tr>
<tr>
<td>Extract 100 mg kg⁻¹</td>
<td>142.66 ± 3.74</td>
<td>1128.2 ± 3.4*</td>
<td>105.6 ± 2.6</td>
<td>645.5 ± 4.2**</td>
<td>468.2 ± 6.8**</td>
</tr>
<tr>
<td>Extract 150 mg kg⁻¹</td>
<td>149.16 ± 2.04</td>
<td>1222.4 ± 4.8**</td>
<td>115.4 ± 1.8**</td>
<td>696.2 ± 3.28**</td>
<td>487.4 ± 2.2**</td>
</tr>
<tr>
<td>Extract 200 mg kg⁻¹</td>
<td>156.16 ± 5.52</td>
<td>1297.2 ± 3.2**</td>
<td>125.8 ± 3.2**</td>
<td>737.5 ± 4.2**</td>
<td>503.4 ± 4.2**</td>
</tr>
</tbody>
</table>

Note: Results are expressed as mean ± standard error (SE). *p < 0.05 considered significant, **p < 0.01 considered extremely significant.
Transverse sections of the testes of control group animals showed normal histoarchitecture. Sertoli and leydigs cells of normal size were present. The seminiferous tubules were normal in number with bundles of spermatozoa (Figure 1). In the extract-treated group, increased spermatogenesis was seen. In almost all of the extract-treated groups there was an increase in diameter of the seminiferous tubules. Instead of the normal round shape, many tubules became oblong in shape to accommodate the growth. Most of the nuclei were larger, and condensation of chromatin material was clearly marked. The blood vessels of testis were slightly dilated and connective tissues were compressed. Transverse sections of animals given treatment with 200 mg kg\(^{-1}\) of ethanolic extract show seminiferous tubules completely filled with sperm bundles, when compared to the other treated group. In the testosterone treated group, similar histoarchitecture was also present, and the increase in spermatogenesis was quite marked (Figure 1).

### 2.2. Sexual performance

Sexual behaviour of animals was highly influenced by treatment with ethanolic extract. The mount latency (ML) and post-ejaculation latency time was reduced significantly with ethanolic extract treatment in a dose dependent manner. A five-fold increase in mount frequency (MF) was recorded with a 200 mg kg\(^{-1}\) dose of ethanolic extract. The animals of the treated group also exhibited a significant decrease in ML (Table 2).

![Figure 1. Photomicrographs of the testes of: (I) control rats, showing the normal stage of spermatogenesis; (II) testosterone treated rats, showing increase of spermatogenesis; (III) extract (100 mg kg\(^{-1}\)) treated rats, showing increases of spermatogenesis; (IV) extract (150 mg kg\(^{-1}\)) treated rats, showing increases of spermatogenesis; (V) extract (200 mg kg\(^{-1}\)) treated rats, showing increases of spermatogenesis (X 100).](image-url)
2.3. Fructose content
The treatment with ethanolic extract markedly increased the fructose concentration in the seminal vesicles. A 60% increase in fructose concentration was recorded with 200 mg kg$^{-1}$ treatment over the control group.

2.4. Sperm count
The in vivo sperm count in the control and extract test groups is presented in Table 3. There was a significant increase in the number of sperm in the extract-treated group, as compared to the control. The survival and motility of sperm was increased in the extract-treated group. Only a 27.16% reduction in sperm count was noted in the extract-treated group, whereas this was 47.05% in the control, showing a protective effect of the extract. The effect of extract on in vitro sperm preservation has been recorded in Table 4.

3. Discussion
The present investigation shows that the ethanolic extract of A. longifolia enhances sexual activity in male rats. Administration of the ethanolic extract of the plant to rats for 28 days caused an increase in the weight of the ventral prostate gland and seminal vesicles. The androgens affect the development of secondary sex organs in the male, as the growth of the ventral prostate and seminal vesicle is dependent on the presence of male sexual hormones (Bennassi-Benelli, Ferrari, & Pellegrini-Quarantotti, 1979; Islam, Tariq, Ageel, Al-said, & Al-Yhya, 1991). Increases in the body weight of the A. longifolia ethanolic extract $A$. longifolia $A$. longifolia $A$. longifolia.
extract-treated rats could be due to the androgenic properties of this plant, since androgens possess anabolic activity (Vogel, 2002).

Our results show that there is an increase in spermatogenesis and weight of sexual organs in the *A. longifolia* ethanolic extract-treated group compared to the control group. Administration of testosterone also causes stimulation in the growth of target tissue and spermatogenesis (De Krester, 1987). Our studies show a testosterone-like action of the ethanolic extract.

An increase in fructose content in seminal vesicles of animals of the treated group was observed, which is a suggestion of an increase in testosterone synthesis in the body. The seminal vesicles provide fructose substances to the seminal pathway, which is necessary to generate normal sperm motility. One important substance is fructose; it is synthesised and secreted by the vesicles. It has been demonstrated that fructose synthesis is regulated by androgens and it is correlated directly with testosterone levels (Gonzales, 1989). The increases in spermatogenesis depend upon the combined effect of the follicle stimulating hormone and testosterone (Chauhan & Dixit, 2008). The possibility of the same mechanism holds well in our case. The effect in the *A. longifolia* ethanolic extract-treated group is much more pronounced when compared to the administration of testosterone. Administration of *A. longifolia* ethanolic extract increases both the orientation as well as sexual behaviour towards female rats by male rats, conclusively suggesting better sexual performance.

The results thus suggest that the *A. longifolia* ethanolic extract improves sexual performance and spermatogenesis in rats. The findings also seem to support the traditional use of the plant as an aphrodisiac.

### 4. Material and methods

#### 4.1. Plant material

Seeds of *A. longifolia* were collected from the forest surrounding the university campus. The plant was authenticated in the Department of Botany, Dr H.S. Gour University, Sagar, and a herbarium sample deposited (Herbarium No. B/H/512).

#### 4.2. Preparation of the extract and characterisation

Powdered seeds of *A. longifolia* were first defatted with petroleum ether, and then extracted with ethanol (95%). The ethanol was evaporated under vacuum to yield

<table>
<thead>
<tr>
<th>Groups</th>
<th>0 min</th>
<th>30 min incubation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>119 ± 2.0</td>
<td>63 ± 1.26</td>
</tr>
<tr>
<td>Ethanolic extract</td>
<td>120 ± 1.2</td>
<td>87.4 ± 1.64*</td>
</tr>
</tbody>
</table>

Note: All values are expressed as mean ± SE; n = 6. *p < 0.01 considered significant as compared to control.
7% extract. Qualitative chemical examination confirmed the presence of alkaloids and steroids in the extract. The ethanolic extract gave the best resolution (nine spots) by mobile phase butanol : acetic acid : water (4 : 1 : 1), which was chromatographed on a thin layer of silica gel G.

4.3. Animals
Albino rats of either sex weighing 120–130 g were housed in standard environmental conditions, fed on a standard diet with water ad libitum at 24 ± 2°C and a day–night cycle 06:00–18:00 h. All the animal experimentations were carried out after permission from the Institutional Ethical Committee of Dr H.S. Gour University, Sagar (M.P.), India.

4.4. Treatment
The animals were divided into five groups of six rats each. Group I animals served as the control and received only vehicle. Group II was subcutaneously given 0.5 mg kg⁻¹ of testosterone suspended in arachis oil twice weekly, and served as the positive control for anabolic studies. Groups III, IV and V were given ethanolic extracts of 100, 150 and 200 mg kg⁻¹ respectively, for 28 days. Only male rats were used in forming a group.

4.5. Effect on sexual organ weight and histological studies
After 28 days of treatment, the body weights of animals were taken, after which all animals were killed by decapitation. Testis, seminal vesicles, epididymis and prostate glands were carefully removed and weighed. Testis of animals were cut into small pieces, fixed in Bovine’s fixative and dehydrated with varying percentages of ethanol for histological studies. Sections were cut (6 μ), stained with haemotoxylin and eosin and then analysed microscopically (Thakur & Dixit, 2006).

4.6. Effect on male sexual behaviour
After 28 days of treatment, the effect on the sexual behaviour of male rats was evaluated (Thakur & Dixit, 2006). In brief, male rats were placed in the glass observation chambers for 5 min to acclimatise to the cage environment. Sexually receptive female rats were then dropped silently in one side of the chamber as a stimulus. The observations for sexual behaviour: MF, intromission latency, post-ejaculatory latency and ML were recorded. ML was calculated as the time between the introduction of the female to the occurrence of first mount, MF was observed as the total number of mounts during the period of observation, intromission latency was considered as the time for first intromission after introduction of the female in the cage, and post-ejaculation latency was calculated as the lag time between the first intromission to the next, within 30 min.

4.7. Sperm count
4.7.1. In vivo sperm count
For counting the spermatozoa, the epididymides of rats of each group were homogenised and taken into 5 mL of 1% sodium citrate solution and squashed thoroughly with the help
of needle and forceps until a milky suspension was obtained. The solution was filtered through 80μ mesh and the volume was made up to 10ml with the same solution; the made-up volume was inclusive of the washings of the filter. The suspension was shaken thoroughly and the spermatozoa were counted in the five WBC counting chamber of the haemocytometer. The average number of sperm per chamber were reported (Saksena & Dixit, 1987).

4.7.2. In vitro sperm preservation

The epididymides of the rats were taken into 5mL of 1% sodium citrate solution and squashed thoroughly with the help of needle and forceps until a milky suspension was obtained. The solution was filtered through 80μ mesh and the volume was made up to 10mL, inclusive of washings of the filter. A 1 mg mL⁻¹ solution of extracts of *A. longifolia* (1 mg mL⁻¹) was prepared and added into the sperm specimens in the test sample in a ratio of 0.1 : 1 (100μL sperm solution : 1 mL extract solution). The sperms were counted at 0 and 30 min after incubation at room temperature. The spermatozoa were counted in a five WBC counting chamber. The average number of sperm per chamber were reported (Wiwanitkit, 2005).

4.8. Fructose content in seminal vesicles

The seminal vesicles were macerated with 3mL of distilled water and centrifuged at 4000 rpm for 12 min. To the supernatant fluid collected after centrifugation, 0.5mL of resorcinol and then 1.5 mL of HCl was added. The mixture was kept at 80°C for 12 min. The reaction with resorcinol developed a rosy colour, which was measured at 500 nm using spectrophotometer. A calibration curve was drawn using dilutions of fructose solution and measuring the colour which developed with resorcinol and HCl (Gonzales, 1989).

4.9. Statistical analysis

Results are reported as mean ± SE. The treated groups were compared to the control by ANOVA, following Dunnett’s test. Significance level was set at *p* < 0.5 and confidence level at 95%. Statistical analysis was carried out using Instat V. 2.1 software residing in a Pentium IV processor run on Windows XP.

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References


