Antifertility effect of *Tinospora cordifolia* (Willd.) stem extract in male rats

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Oral administration of 70% methanolic extract of *T. cordifolia* stem to male rats at the dose level of 100 mg/rat/day for 60 days did not cause body weight loss but decreased the weight of testes, epididymis, seminal vesicle and ventral prostate in a significant manner. Sperm motility as well as sperm density were reduced significantly which resulted in reduction of male fertility by 100%. The stem extract brought about an interference with spermatogenesis. The round spermatids were decreased by 73.12%. However, the population of preleptotene and pachyphase spermatocytes were decreased by 47.60% and 52.85% respectively, followed by secondary spermatocytes (48.10%). Leydig cell nuclear area and mature Leydig cell numbers were significantly reduced when compared with controls. Serum testosterone levels showed significant reduction after *Tinospora* extract feeding. Seminiferous tubule diameter, Leydig cell nuclear area as well as cross sectional surface area of Sertoli cells were reduced significantly when compared to controls. Biochemical parameters i.e. protein, sialic acid, glycogen contents of testes decreased significantly. Seminal vesicular fructose also depleted whereas, testicular cholesterol was elevated significantly followed by a reduction in testosterone levels. These results suggested antifertility effects of the stem extract of *T. cordifolia* in male rats.

**Keywords**: Antifertility effect, Spermatogenesis, Testicular androgen, *Tinospora cordifolia*

The development of new fertility regulating drug from medicinal plants is an attractive proposition. *Tinospora cordifolia* (Willd.) (family: Menispermaceae) is a traditional plant commonly known as “Neem-giloy”. The roots of *T. cordifolia* are used for allohex diabetes. Pharmacological screening of crude methanolic extract of *T. cordifolia* stem showed immuno-modulatory and anti-potentiating activities. Some species have also been used for treatment of type-II diabetes. The present study was under taken to evaluate the antifertility effects of *T. cordifolia* stem extract.

**Material and Methods**

**Extract preparation**—*T. cordifolia* plants were collected from Jaipur region. Shade dried powdered stem was extracted with 70% methanol in a soxhlet apparatus to obtain a solid viscous brown mass, that is “crude extract”. On chemical screening of *T. cordifolia* stem two new compounds,—clerodane furano-diterpane (C_{20}H_{22}O_{13}) and clerodane derivative clerodane diterpenoid—have been obtained.

**Animals and treatment**—Male albino rats of Wistar-strain weighing 150 to 200 g were used for experimentation. Animals were housed in plastic cages with proper aeration and temperature. Maintained on standard rat feed (Hindustan Lever Ltd) and water ad libitum. The animals were divided in to two groups of 10 animals each.

**Group I** : Rats received vehicle (distilled water, 0.5 ml/rat/day) for 60 days.

**Group II** : Rats treated with crude extract (100mg/rat/day) for 60 days.

Crude extract was dissolved in 0.5 ml distilled water and administered orally. The mating exposure tests on control and treated groups were performed on day 55 using the method of WHO. The presence of sperms in the vaginal smear was the evidence of mating. On day 61 i.e. 24 hr after the last dose administration animals were sacrificed using ether anaesthesia. Blood was collected by cardiac puncture and serum was separated. Reproductive organs were dissected out.

**Histopathological preparation** — Tissue were fixed in Bouin’s fluid, paraffin sections were made and stained with hematoxylin and eosin.

**Sperm motility and sperm density** — Sperm motility and sperm density were assessed in cauda epididymis by the method of Prasad et al.

**Tissue biochemistry** — The parts of testes, epididymis, seminal vesicle and ventral prostate from each rat were kept at -20°C until assayed for protein, sialic acid, glycogen, fructose and cholesterol.

**Testicular cell dynamics** — The evaluation of cell population dynamics was based on the calculations made for each cell type per cross section of seminiferous tubule. Various cell components were
quantitatively analyzed using spherical tubules. Interstitial cell type such as fibroblast and mature degenerating Leydig cells were estimated applying a differential count and statistically varied by the binomial distribution.

Hormonal assay — Serum testosterone levels were assayed from frozen samples using radio immuno assay method (readily available). The sensitivity of the assay was 10 pg/ml and intra assay error was 4.5%.

Statistical analysis — The mean and standard error of mean (SEM) were calculated by using Student's t-test.

Result

Weight response — The orally tested T. cordifolia stem extract did not cause any significant change in the body weight of treated rats. However, the weight of epididymis, seminal vesicle and prostate gland were decreased significantly (P < 0.001). The weights of testes and epididymis were not significantly different from controls (Table 1).

Sperm dynamics and fertility test —The sperm motility in cauda epididymis was decreased and severe impairment of sperm density in testes and cauda epididymis were observed after treatment. The fertility test showed 100% negative fertility in T. cordifolia treated rats (Table 2).

Biochemical findings — A marked reduction in protein and sialic acid contents of testes and sex accessory organs were observed. However, testicular glycogen decreased significantly and fructose in seminal vesicle was also lowered. The testicular cholesterol was significantly elevated (P < 0.01) (Table 3).

Testicular histopathology and cell population dynamics — Administration of crude extract caused an effective inhibition of spermatogenesis in male

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Table 1 — Effect of T. cordifolia stem extract on body weight and organ weights. [Values are mean ± SE of six determinations]

<table>
<thead>
<tr>
<th>Organ weights</th>
<th>Body wt (g)</th>
<th>Testes</th>
<th>Epididymis</th>
<th>Seminal vesicle</th>
<th>Ventral prostate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Final</td>
<td>240 ± 1.40</td>
<td>1345 ± 4.70</td>
<td>529.5 ± 11.20</td>
<td>605.7 ± 9.50</td>
</tr>
<tr>
<td>T. Cordifolia stem extract 100 mg/rat/day oral for 60 days</td>
<td>172.5 ± 7.50</td>
<td>1155.15 ± 95.15</td>
<td>427.97 ± 42.97</td>
<td>302.49 ± 0.27</td>
<td>183.97 ± 9.53</td>
</tr>
</tbody>
</table>

*p < 0.01; **p < 0.001 vs Control (ns : Non significant)

Table 2 — Sperm dynamics and Testosterone level of T. cordifolia treated male rats. [Values are mean ± SE of six determinations]

<table>
<thead>
<tr>
<th>Sperm motility (%)</th>
<th>Sperm density (million/ml)</th>
<th>Fertility test</th>
<th>Testosterone (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>cauda epididymides</td>
<td>72.00 ± 0.81</td>
<td>4.62 ± 0.11</td>
<td>42.50 ± 0.92</td>
</tr>
<tr>
<td>T. Cordifolia stem extract 100 mg/rat/day oral for 60 days</td>
<td>32.92 ± 1.94**</td>
<td>2.45 ± 0.05**</td>
<td>11.13 ± 0.36**</td>
</tr>
</tbody>
</table>

**p < 0.001 vs Control

Table 3 — Tissue biochemistry of T. cordifolia treated male rats. [Values are mean ± SE of six determinations]

<table>
<thead>
<tr>
<th>Protein (mg/g)</th>
<th>Sialic acid (mg/g)</th>
<th>Glycogen (mg/g)</th>
<th>Fructose (mg/g)</th>
<th>Cholesterol (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Testes</td>
<td>Cauda epididymides</td>
<td>Ventral prostate</td>
<td>Seminal vesicle</td>
<td>Testes</td>
</tr>
<tr>
<td>Control</td>
<td>227.80 ± 4.56</td>
<td>± 3.6</td>
<td>± 0.18</td>
<td>± 3.40</td>
</tr>
<tr>
<td>T. Cordifolia stem extract 100 mg/rat/day oral for 60 days</td>
<td>194.70**</td>
<td>220.91**</td>
<td>144.19**</td>
<td>165.52**</td>
</tr>
</tbody>
</table>

* p < 0.01; ** p < 0.001 vs Control
rats. The production of round spermatids was decreased by 73.12%. The number of pre-leptotene, pachytene spermatocytes and secondary spermatocytes were decreased by 47.60%, 52.85% and 48.10% respectively. The mature Leydig cell number was significantly \((P < 0.001)\) depleted. The total number of Sertoli cells and seminiferous tubule diameter were also reduced as compared to control group (Table 4) (Figs 1 & 2).

**Hormonal assay**—Serum testosterone level of *Tinospora cordifolia* stem extract treated animals was decreased significantly in comparison to control group (Table 2).

**Discussion**

In the present study, administration of *T. cordifolia* stem extract to rats caused decrease in weight of testis, epididymides, seminal vesicle and ventral prostate which may be due to low plasma level of testosterone\(^{17}\), which is found to be decreased following *T. cordifolia* extract feeding to rats. Similar results were revealed\(^{16}\) after feeding leaf extract of *Stephania hermandifolia*. The decrease in the weight of accessory sex organs indicates the atrophy of glandular tissue and also reduction in secretory ability thus reflecting the decrease level of testosterone\(^{16}\). The reduced testicular weight and shrunk seminiferous tubular dimensions indicate wide spread damage\(^{20}\).

The reduction in sperm motility in cauda epididymis is of importance with regard to fertilization\(^{21}\). In many of the plant based contraceptives, inhibition of male fertility after administration of natural substance has been ascribed

![Fig. 1](image1.png) — Microphotograph of testes of control rat showing all the successive stages of spermatogenesis. Leydig cells are present in intertubular space. Lumen containing spermatozoa. X 200 HE

![Fig. 2](image2.png) — Microphotograph of testes of rats treated with *T. cordifolia* stem extract 100 mg/rat/day showing inhibition of spermatogenesis, note the absence of secondary spermatocytes and spermatids. Lumen is devoid of spermatozoa. X 200 HE

| Table 4 — Testicular cell dynamics of *T. cordifolia* treated male rats | Leydig cell differential count (%) | Seminiferous tubular diam. | Leydig cell nuclear diam. |
|———|———|———|———|———|
| Testicular cell counts/Cross section | Leydig cell count | Round spermatids | Fibroblast | Mature | Degenerated |
| Spermatogonium | Sertoli cell | Pre-leptotene | Pachytene | Secondary spermatocytes | | | |
| Control | 6.87 ± 0.02 | 2.81 ± 0.02 | 19.95 ± 0.19 | 29.29 ± 0.73 | 48.10 ± 0.60 | 35.10 ± 1.07 | ± 43.30 | ± 99.70 | ± 57.00 | ± 268.00 |
| *T. Cordifolia* stem extract 100 mg/rat/ day oral for 60 days | 2.36** | 1.65* | 10.45** | 13.80** | 12.34** | 9.43** | ± 63.94** | ± 74.33** | ± 62.61 | ± 192.90** | ± 4.93* |
| Percent deviation | -65.59 | -41.00 | -47.60 | -52.85 | -74.32 | -92.35 | ± 47.66 | ± 25.44 | ± 28.02 | ± 55.52 |

* \(P < 0.01\); ** \(P < 0.001\) vs Control
to decrease sperm motility and density\textsuperscript{22,23}. Decrease in sperm motility suggests alteration of sperm maturation in the epididymis\textsuperscript{24}. Administration of \textit{T. cordifolia} extract resulted in decrease sperm count which might be due to partial arrest of spermatogenesis as it was also confirmed by histopathological findings. A decrease in sperm reserve may be a reasonable cause for reduction in the weight of epididymis. The significant decrease in testosterone level in the treated animals supports this view\textsuperscript{25}, reduced androgen level may decrease sperm density in testes. The results indicate an inhibitory effect of the test substance on the testicular sperm production and epididymal sperm maturation which consequently resulted in the gradual decline in fertility rate, since decrease sperm count has positive correlation with infertility\textsuperscript{26,27}.

The reduction in number of spermatogenic cells may be due to non availability of testosterone, as spermatogenesis is activated by testosterone which is synthesized by Leydig cells and acts on sertoli and peritubular cells\textsuperscript{28}. Sertoli cells also facilitate the germ cell maturation but these cells are highly susceptible to extraneous damage\textsuperscript{29}. Low counts and structural changes in sertoli cells consequently diminished secretory function i.e. ABP (Androgen Binding Protein) resulted in depletion in number of spermatids along with secondary spermatocytes, since ABP is required for maintaining intratubular androgen concentration\textsuperscript{30}.

The impairment of Leydig cell function was evinced by its reduced nuclear area and lower number of mature Leydig cells. The number of mature Leydig cell has direct bearing on spermatogenesis. Deformation of Leydig cell further indicates the inefficiency of these cells to synthesize testosterone\textsuperscript{31}.

It is evident that decreased testicular and epididymal protein content could be correlated with absence of spermatozoa in the lumen\textsuperscript{32}. Structural integrity of acrosomal membrane is dependent upon sialic acid and due to alteration in its content the metabolism, motility and fertilizing ability of sperms also may be effected\textsuperscript{33}. It also acts as 'lubricant' to facilitate the downward movement of sperms and reduce friction among spermatozoa\textsuperscript{34}.

Glycogen acts as an energy producing source in tissues and constant supply of carbohydrate (glucose) is essential for gonadal maturation and for proper function of testes, as it maintains the tissue integrity ATP production and protein synthesis in the rat testes\textsuperscript{35}. Significant decrease in glycogen content after the treatment possibly could be due to inhibition of glycolysis during spermatogenesis\textsuperscript{36} as the doses of \textit{Tinospora cordifolia} stem extract inhibited glycolysis in spermatozoa which would ultimately influence fertilizing capacity of male rats.

The process of fructose formation in seminal vesicle is initiated and controlled by testicular androgens\textsuperscript{37}. Hormonal deficiency causes a decrease or even disappearance of seminal fructose and a compensatory treatment with androgens restores the ability of the accessory glands to produce this sugar\textsuperscript{38}.

The results thus suggests that oral administration of crude methanolic extract of \textit{T. cordifolia} stem can lead to a infertile state in male rats due to interference in the testicular androgen levels altering the process of spermatogenesis.

\textbf{References}


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