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**Contraceptive efficacy of *Plumbago zeylanica* root extract (50% etoh) in male albino rats with special emphasis on testicular cell population dynamics**

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#### **Abstract**

The administration of *Plumbago zeylanica* root (50% EtOH) extract to intact rats at the dose of 150 mg/kg body weight for 60 days caused arrest of spermatogenesis. The diameter of seminiferous tubules and Leydig cell nuclei were reduced. The production of spermatocytes (primary and secondary) and spermatids were significantly reduced (P£ 0.001; 83.57%, 89.69% and 69.47% respectively). The total number of immature and mature Leydig cells was significantly decreased (P£ 0.001; 68.62% and 71.14%), where as degenerating cells were significantly increased (58.26%). Decreased testicular cell population reflects contraceptive or antispermatogenic nature of *Plumbago zeylanica* extract and may be of vital use in fertility control.

#### **Introduction**

In recent years emphasis is being laid on male antifertility regulating agents rather than female. A number of synthetic compounds have been reported to arrest spermatogenesis<sup>1-2</sup> but they are unsuitable for human use due to toxic manifestation. Attention has been focused on plant products<sup>3-4</sup>.

*Plumbago zeylanica* is an evergreen perinneal herb growing wild or cultivated in the gardens. The root of *Plumbago zeylanica* has been reported to yield plumbagin<sup>5</sup>. The root juice of this plant was found to be the most potent in stimulating the uterus<sup>6</sup>. The present study is aimed to assess the contraceptive efficacy of *Plumbago zeylanica* (50% EtOH) root extract in male rats with a view to develop a contraceptive of plant origin for human male.

## Material and Method

20 mature male Swiss albino rats weighing 180-200 gm maintained on a standard diet (Hindustan Lever Ltd., Mumbai) and water *ad libitum* were distributed into two groups. In each group 10 rats were used.

**Gr. A:** Animals of this group served as control and second group

**Gr. B:** Animals of this group received *Plumbago zeylanica* root extract (50% EtOH) orally for 60 days at the dose of 150 mg/kg body weight.

The roots of *Plumbago zeylanica* were commercially obtained from the market. The authentic samples were powdered and later subjected to soxhalation in 50% ethanol for 18 hours. The ethanol was distilled off under reduced pressure. The solid dark brown mass so obtained was administered.

The animals were screened for fertility test from day 55th - 60th days with female (1: 3) and sacrificed on 61<sup>st</sup> day by using light ether anaesthesia. The sperm motility and density were assessed in the cauda epididymidis and sperm density was assessed in testis<sup>7</sup>. Testis was fixed in Bouins fluid. Paraffin sections were prepared and examined for testicular population dynamics.

The evaluation of germinal cell population dynamics is based on the calculations made for each type per cross-tubular section. All raw counts were transformed to nuclear points<sup>8</sup>.

Interstitial (Leydig cell) cell types such as fibroblast, immature, mature and degenerating Leydig cells were estimated applying a different count over 200 cells of this cell population statistically verified by the binomial distribution<sup>9</sup>. Seminiferous tubule and Leydig cell nuclei diameter were traced with camera lucida at x80 and x800 respectively and the means were calculated.

## Result and Discussion

*Plumbago zeylanica* (50% EtOH) fruits extract administration did not alter the body weight but sperm dynamics showed that motility as well as density was significantly reduced in the treatment groups. Similarly the diameter of seminiferous tubule and Leydig cell nuclei were reduced (P£ 0.001). The fertility showed 90% negative (Table- I).

The plant material reduced the germinal cell population to highly significant level (P£ 0.001). Similarly the number of immature and mature Leydig cells was also reduced (P£ 0.001, 68.62% and 71.14%). However the number of degenerating cells was significantly increased (P£ 0.001, 58.26%) in treated animals when compared with control. (Table- II).

The process of spermatogenesis is androgen dependent<sup>10-11</sup>. Decreased androgen production reflects in reduced number of Leydig cells. In the present study the number of degenerative Leydig cells were significantly increased thereby reflecting the probable depletion of

androgen level. It was further supported by shrunken seminiferous tubule diameter and decreased number of germinal cells, as these stages are completely androgen dependent<sup>12-13</sup>.

Significant reduction in the sperm motility of cauda epididymides was observed in *Plumbago zeylanica* treatment. This may be due to

inhibitory effect of *Plumbago zeylanica* on the enzyme of oxidative phosphorylation<sup>14-15</sup>.

In conclusion *Plumbago zeylanica* root extract showed its contraceptive effect via affecting Leydig cell function. Further comprehensive chemical and pharmacological investigations are in progress.

**Table I**  
**Sperm dynamics and histometry of control and *Plumbago zeylanica* treated rats**  
**(Mean± SEM of 10 animals)**

Treatment groups	Sperm Density million/ml		Sperm Motility %	Fertility test %	Seminiferous tubule diameter -----µm-----	Leydig cell nuclear diameter -----µm-----
	Testes	Cauda				
<b>Intact Control (Gr.A)</b>	4.8 ±0.3	52.4 ±2.8	69.60 ±1.89	90%(+)	296.7 ±0.370	6.89 ±0.31
<b><i>Plumbago zeylanica</i> 150 mg/kg body weight orally for 60 days (Gr. B)</b>	0.70 <sup>c</sup> ±2.34	11.67 <sup>c</sup> ±3.32	10.12 <sup>c</sup> ±10.12	90% (-)	175.80 <sup>c</sup> ±0.09	3.09 <sup>c</sup> ±0.35

P≤0.001 = c , Gr.B compared with Gr.A (Highly significant)Mean ± SEM of 10 animals

**Table II**  
**Testicular cell population dynamics of control and *Plumbago zeylanica***  
**treated rats (Mean ± SEM of 10 animals)**

Treatment Groups	Germinal Cell Types				Interstitial Cell Types			
	Spermatogonia	Primary spermatocyte	Secondary spermatocyte	Spermataids	Fibroblast	Immature Leydig cell	Mature Leydig cell	Degenerative cell
Intact (Control) (Gr.-A)	11.30 ± 3.04	58.68 ± 2.60	86.63 ± 2.61	69.30 ± 2.67	110.3 ± 9.01	37.32 ± 6.96	39.41 ± 4.42	12.15 ± 2.57
<i>Plumbago zeylanica</i> extract 150 mg/kg.b Wt./oral for 60 days (Gr-B)	6.80 ± 0.07 <sup>c</sup>	9.63 ± 1.6 <sup>c</sup>	8.90 ± 0.15 <sup>c</sup>	2.45 ± 0.06 <sup>c</sup>	101.26 ± 5.92 <sup>d</sup>	7.96 ± 0.15 <sup>c</sup>	8.69 ± 0.2 <sup>c</sup>	82.09 ± 0.63 <sup>c</sup>
Percent Deviation	39.82	83.57	89.69	96.47	8.15	68.62	71.14	58.26

<sup>c</sup> P ≤ 0.001 (Highly significant) <sup>d</sup> P ≤ N.S. (Non significant), Gr.-B was compared with Gr.-A

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