

## Impact of feeding ethanolic extract of root bark of *Cananga odorata* (Lam) on reproductive functions in male rats

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The 50% ethanolic extract of the root bark of *C. odorata* administered orally at the dose of 1g/kg body weight /day for 60 days resulted in decreased epididymal sperm motility and sperm count in male albino rats. Morphological abnormalities were also observed in the sperms. The testicular glycogen, the activities of 3 $\beta$  hydroxy steroid dehydrogenase, glucose 6-phosphate dehydrogenase, malic enzyme, sorbitol dehydrogenase in seminal vesicle, fructose in seminal plasma and serum testosterone were significantly decreased in treated group. While testicular cholesterol level, the concentration of the fecal bile acids, urinary excretion of 17 ketosteroids, the activities of 17 $\beta$  hydroxy steroid dehydrogenase, epididymal lactate dehydrogenase and that of testicular HMG CoA reductase were increased in treated group when compared to control. The results suggest that the ethanolic extract of *C. odorata* possesses the spermatotoxic effects in male albino rats.

**Keywords:** Antifertility, *Cananga odorata*, Spermatotoxic, Testis, Testosterone

The use of plants as abortifacients and as contraceptives was well known to the ancient physicians of India. Various medicinal plant extracts have been tested for their antifertility activity both in male and female<sup>1</sup>. The studies on several plant extracts<sup>2-5</sup> have shown antifertility effect in male rats. But so far not a single herbal oral male contraceptive has been marketed. A number of plants were screened in and around our university campus for antifertility and among those *Cananga odorata* (Lam) was found to have spermicidal action in the *in vivo* and *in vitro* studies. Hence we selected this plant for detailed studies.

*Cananga odorata* is a medium sized tropical tree belonging to the family Annonaceae. Its oil has a soothing effect, therapeutically slowing excessive heart rate, calming and lowering blood pressure<sup>6</sup>. Traditionally, the plant is known to be effective against diarrhoea, malaria, boil and stomatic. Flowers of this plant are used in cephalgia, ophthalmia and gout<sup>7,8</sup>. Ethanolic extract (50%) of the root bark of this plant has been used in this study to highlight its role in reproductive functions in male albino rats.

### Materials and Methods

*Preparation of the extract*—Root bark of *C. odorata* was collected and dried at 60°C. Dried

material (10%) was refluxed in 50% ethanol at 65°C for 90 min and after that it was filtered and evaporated to dryness at 65°C. Dried root bark (100g) of *C. odorata* yielded 11g of the crude extract.

Male albino rats (Sprague Dawley strain, body weight 150  $\pm$  5g) bred in University animal house and maintained on a normal laboratory diet (Lipton India Ltd. Bangalore, India) were used. Water and food were given *ad libitum*. The rats were housed in polypropylene cages in a room at 25°C with 12:12 hr light and dark cycle.

### *In vitro* study

For *in vitro* studies semen was collected from cauda epididymis of healthy male rats and was diluted with normal saline and kept at 37°C. The motility of the sperm was noted under a light microscope. Extracted material (2mg) was added to 1ml of the diluted semen sample and incubated at 37°C. The motility of the sperm was observed at every 2min intervals.

### Dose dependent studies

Rats (24) were randomly divided into following 4 groups of 6 each:

Group I—Control

Group II—administered with crude extract (50mg/100g body weight/day)

Group III—administered with crude extract (70mg/100g body weight/day)

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Group IV—administered with crude extract (100mg/100g body weight/day)

The extract was suspended in distilled water and given orally by gastric intubation. Control animals were given only the vehicle. The duration of the experiment was 60 days. At the end of the experimental period the rats were fasted overnight and sacrificed and semen was collected from cauda epididymis. Sperm motility, sperm morphology (abnormalities in tail, head and shape of the body) and count were determined by the methods given in WHO manual<sup>9</sup>.

#### Detailed *in vivo* studies

Rats (12) were randomly divided into 2 groups of 6 each, control and experimental. The extract was suspended in distilled water and given orally by gastric intubation. Control animals were given only the vehicle. The duration of the experiment was 60 days and the dose was 1g/kg body weight/day. On day 60, 24 hr urine was collected for estimation of 17 ketosterols. At the end of the experimental period the rats were fasted overnight and sacrificed, liver, testis, epididymis and seminal vesicle were removed to ice cold containers for various analyses.

Body weights and weight of reproductive organs were recorded. Epididymal sperm motility, morphology and count were determined as per the methods given in WHO manual<sup>9</sup>.

**Biochemical analysis**—The testicular lipids were extracted according to the procedure of Folch *et al*<sup>10</sup> and cholesterol was estimated according to the method of Abell *et al*<sup>11</sup>. Testicular HMG CoA reductase activity was assayed as described by Rao and Ramakrishnan by determining the ratio of HMG CoA/mevalonic acid<sup>12</sup>. Activities of 3 $\beta$ -hydroxysteroid dehydrogenase<sup>13</sup>, 17 $\beta$ -hydroxysteroid dehydrogenase<sup>14</sup>, glucose 6- phosphate dehydrogenase<sup>15</sup>, malic enzyme<sup>16</sup>, sorbitol dehydrogenase<sup>17</sup> and lactate dehydrogenase<sup>18</sup> were assayed. 17 ketosteroids<sup>19</sup> and fecal bile acids<sup>20,21</sup> were extracted and estimated. Testicular glycogen<sup>22</sup> and fructose in seminal plasma<sup>23</sup> were determined.

Serum testosterone level was estimated by RIA method using the kit supplied by BRIT India. The protein content was also estimated after precipitation with TCA by the method of Lowry *et al*<sup>24</sup>.

**Statistical analysis**—The results were analyzed using a statistical programme SPSS/PC+, Version 5.0 (SPSS Inc., Chicago, IL, USA). A one-way ANOVA was employed for comparison among the six groups. Duncan's post-hoc multiple comparison tests of significant differences among groups were determined.  $P \leq 0.05$  was considered to be significant.

#### Results

***In vitro* studies**—Result showed that 50% ethanolic extract of the root bark of *C. odorata* caused the complete immobilization of sperms within seconds. So dose dependent studies were conducted with this extract.

**Dose dependent studies (Table 1)**—Results of the dose dependent studies showed that group III and IV had similar results for sperm motility and morphology. But sperm count decreased significantly only in group IV when compared to all other groups. So 100mg/100g body weight/day [1g/kg body weight/day] dose was selected for detailed *in vivo* studies.

#### Detailed *in vivo* studies

**Body and organ weights (Table 2)**—The root bark extract of *C. odorata* did not cause any significant

Table 1—Effect of different doses of root bark extract of *C. odorata* on sperm motility, count and morphology  
[Values are mean  $\pm$  SD of 6 animals in each group]

Groups (mg crude extract /100g body wt/day)	Sperm motility [time taken for the complete immobilization of sperm]	Sperm count (millions/ml)	Sperm morphology
I (control)	30 $\pm$ 1.98	48 $\pm$ 1.10	96% normal
II (50)	5 $\pm$ 0.38*	45 $\pm$ 0.91	65% abnormal
III (70)	5 seconds*	44 $\pm$ 0.91	94% abnormal
IV (100)	5seconds*	26 $\pm$ 0.35*	94% abnormal

\* $P < 0.05$  between control and treated groups

Table 2—Effects of *C. odorata* root bark extract on body and organ weights  
[Values are mean  $\pm$  SD of 6 animals in each group]

Groups	Body weight (g)		Relative organ weights (mg/100g bodyweight)			
	Initial	Final	Testis	Seminal Vesicle	Epididymis	Prostate gland
Control	153 $\pm$ 0.01	246 $\pm$ 0.02	13.23 $\pm$ 1.23	4.67 $\pm$ 0.61	0.93 $\pm$ 0.08	2.55 $\pm$ 0.23
Experimental (100mg/100g body wt/day)	150 $\pm$ 0.01	251 $\pm$ 0.02	12.38 $\pm$ 1.15	6.95 $\pm$ 0.64*	0.95 $\pm$ 0.08	2.26 $\pm$ 0.21

\* $P < 0.05$  between control and treated groups

change in the body weight, weight of testis, epididymis and prostate gland of treated rats. But the weight of seminal vesicle increased significantly in experimental group when compared to control.

**Spermatotoxicity (Tables 3, 4)**—Results of *in vivo* experiments showed that sperms of control group had 100% rapid progressive movement at zero time. In experimental group only 50% of the total sperms had slight progressive movement at zero time and after 5min they were completely immotile. The treated group had a significant decrease in sperm count and morphological changes were also seen in experimental group when compared to control.

**Biochemical findings**—The activities of testicular HMG CoA reductase, 17 $\beta$  hydroxy steroid dehydrogenase, epididymal lactate dehydrogenase and testicular cholesterol level were increased significantly in experimental group (Table 5). HMG CoA reductase activity is measured as ratio of HMG CoA to mevalonic acid. Hence lower the ratio higher

the activity. But the activity of testicular 3 $\beta$ -hydroxysteroid dehydrogenase, glucose 6-phosphate dehydrogenase, malic enzyme and sorbitol dehydrogenase in seminal vesicle were decreased significantly in experimental group. Concentration of testicular glycogen, fructose in seminal plasma, and serum testosterone were decreased significantly in experimental group. The fecal bile acid and urinary excretion of 17 ketosterols were increased in experimental group.

Table 3—Effects of *C. odorata* root bark extract on sperm motility

Groups	Motility of sperm at 0 min and mode of movement	Motility (%) after			
		5 min	10 min	20 min	30 min
Control	Rapid progressive 100% motility	80	70	10	0
Experimental (100mg/100g bodywt/day)	Slight progressive 50% motility	0	-	-	-

Table 4—Effects of *C. odorata* root bark extract on sperm counts and sperm morphology [Values are mean  $\pm$  SD of 6 animals in each group]

Groups	Sperm count (millions/ml)	%					
		Tail less	Head less	Body twisted	Tail round	Head round	Total
Control	62.5 $\pm$ 5.84	-	-	2	2	-	4
Experimental (100mg/100g bodywt/day)	19.0 $\pm$ 1.77*	45	2	5	40	2	94

\* $P < 0.05$  between control and treated groups

Table 5—Effects of *C. odorata* root bark extract on biochemical parameters [Values are mean  $\pm$  SD of 6 animals in each group]

Groups	Testicular parameters						
	3 $\beta$ -hydroxy steroid dehydrogenase (absorbance <sup>a</sup> /mg protein)	17 $\beta$ -hydroxy steroid dehydrogenase (absorbance <sup>a</sup> /mg protein)	HMG CoA Reductase (HMG/mevalonate)	Glucose 6-phosphate dehydrogenase (Units <sup>b</sup> /mg protein)	Malic enzyme (Units <sup>c</sup> /mg protein)	Cholesterol (mg/100g tissue)	Glycogen (mg/100g wt tissue)
Control	7.74 $\pm$ .72	3.94 $\pm$ .36	2.39 $\pm$ .22	40.38 $\pm$ 3.85	20.49 $\pm$ 2.02	206.88 $\pm$ 19.30	0.483 $\pm$ .04
Experimental (100mg/100g bw/day)	5.66 $\pm$ .53	6.83 $\pm$ .63	1.53 $\pm$ .14	32.18 $\pm$ 3.01	10.76 $\pm$ 1.0	244.12 $\pm$ 22.85	0.243 $\pm$ .02
Groups	Epididymal lactate dehydrogenase (IU <sup>d</sup> /mg protein)	Sorbitol dehydrogenase (IU <sup>e</sup> /mg protein)	Fructose in seminal plasma (mg/ml)	Serum testosterone (ng/ml)	Fecal bile acid (mg/g fecal wt)	Fecal neutral sterols (mg/g fecal wt)	Urinary 17-ketosterols (mg/24 hr in sample)
Control	500.15 $\pm$ 46.78	2.68 $\pm$ .25	0.15 $\pm$ .01	65.08 $\pm$ .14	18.50 $\pm$ 1.73	12.24 $\pm$ 1.14	121.25 $\pm$ 11.34
Experimental (100mg/100g body wt)	694.77 $\pm$ 64.27	1.48 $\pm$ .13	0.08 $\pm$ .01	10 $\pm$ .03	22.3 $\pm$ 2.09	14.68 $\pm$ 1.42	220.51 $\pm$ 20.62

<sup>a</sup>Change in absorbance of 0.001/min at 340nm, <sup>b</sup> $\mu$ moles of NADPH formed/min, <sup>c</sup>enzyme which causes increase in OD of 0.01/min)

<sup>d</sup> $\mu$  mole liberated/min, <sup>e</sup> $\mu$ g fructose liberated/min at 37°C.

( $P < 0.05$  for experimental values)

## Discussion

The weight of testis was not affected when root bark extract of *C. odorata* root bark extract was administered. But the weight of seminal vesicle increased significantly in experimental group. Seminal vesicle secretes the bulk of the fluid in which ejaculated sperms are suspended. The increase in weight of the seminal vesicle may be due to the accumulation of glandular secretions. This is in agreement with the studies of Watcho *et al*<sup>25</sup> which gave the same results when methylene chloride:methanol (1:1) extract of the dried root of *Mondia whitei* was given to male albino rats. In this case also only the weight of seminal vesicle was altered and the testicular weight was not changed.

The deficiency of testosterone causes a decline in the number of spermatozoa. In the present study reduction in the level of testosterone and also in the sperm count was observed. Cholesterol is an obligate precursor for the production of testosterone. Enhanced cholesterol level may be due to its increased production as evidenced by the elevated activity of testicular HMG CoA reductase. The increased cholesterol is not utilized for the production of testosterone since the activity of 3 $\beta$ -hydroxy steroid dehydrogenase was reduced. There was enhanced catabolism of cholesterol and testosterone as evidenced by the increased fecal bile acid content and enhanced excretion of 17 ketosterols. NADPH is essential for the conversion of cholesterol to testosterone. But the supply of this coenzyme was also reduced due to the decreased activities of glucose 6-phosphate dehydrogenase and malic enzyme in testis.

The decrease in the testicular glycogen and fructose in seminal plasma indicates the deficiency of nutrients for the development of sperms. The glycogen content in the cell represents the energy storage. Reduced glycogen content reflects decreased number of post-meiotic germ cells which are thought to be the sites of glucose metabolism<sup>26</sup>. Decreased fructose concentration in the seminal vesicle may be the result of a decreased secretory activity. Reduction in fructose content is due to the reduced activity of sorbitol dehydrogenase. Sorbitol dehydrogenase is an indicator of energy status of a cell. Moreover sorbitol dehydrogenase activity has been localized intensely in the elongating spermatids. Therefore lowering activity of sorbitol dehydrogenase showed low levels of energy production and destruction of spermatid

formation after treatment of *C. odorata* root bark extract. The increased activity of lactate dehydrogenase in the present study suggests a shift in the tissue respiration from anaerobic to aerobic type which would be adverse to the metabolism of stored spermatozoa in the epididymis. It is further substantiated by the reduced sperm count, motility and percentage of normal spermatozoa in the rats treated with *C. odorata* root bark extract.

The extract may be a combination of a number of hydrophilic and hydrophobic compounds. Hence it can be concluded that 50% alcoholic extract of the root bark of *C. odorata* suppresses the production of testosterone and increase its catabolism. Also it alters the micronutrients of the testis, causing a deficiency in the nutrients for the proper maturation and functioning of sperms.

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