Available online at www.ijpsdr.com
International Journal of Pharmaceutical Sciences and Drug Research 2012; 4(1): 84-87

Research Article ISSN 0975-248X

Screening of Isolated Fractions of *Dendrophthoe falcata* Methanol Stem Extract for Its Effects on Reproductive Functions of Male Rats

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ABSTRACT
In our previous study methanol crude extract of *Dendrophthoe falcata* Ettingsh (family-Loranthaceae), stem showed contraceptive like activities in male albino rats. In the present study proven fertile male rats were treated with different isolated fractions (Chloroform: Methanol) of *D. falcata* stem at 50 mg/rat/day for 60 days. On day 61th the animals were autopsied, testes and accessory sex organs i.e. epididymides, seminal vesicle, ventral prostate were dissected out and weighed. Sperm dynamics and serum testosterone level were assessed; also testicular histological studies were done. The sperm motility and density were significantly reduced in all the treatment groups. Testosterone levels were declined significantly. Evaluations of testicular cell dynamics and histological studies suggest disrupt or inhibited spermatogenesis.

Keywords: *Dendrophthoe falcata,* testosterone, testicular cell dynamics, spermatogenesis.

INTRODUCTION

Since ancient times, plants have been an exemplary source of medicine. The use of traditional medicine is widespread and plants still present a large source of structurally novel compounds that serves as leads for novel drugs. These medicinal plants are free from any toxic side effects.

D. falcata Ettingsh belonging to family Loranthaceae is a branched hemiparasite. *D. falcata* is a plant used in folk remedies as an antifertility agent. [1] Our previous experimental study also support that it has contraceptive like activities in male rats. [2] *D. falcata* treats kidney stones [3], having diuretic and antilithiatic activity. [4] It is also used as antibiotics. [5] Phytochemical studies showing the presence of some flavonoids like Quercetin, Kaempferol, Quercitrin, Rutin, Quercetagetin, Meratin and Hyperoside. [6]

In the present study, various isolated fractions of *D. falcata* were investigated that their contraceptive efficacy persist or not and also which of the fraction is most effective.

MATERIALS AND METHOD

Plant Extraction and Fractionation

Fresh stems of *Dendrophthoe falcata* were collected from Nahargarh Biological Garden, Amber, Jaipur and submitted to Deptt. of Botany, University of Rajasthan, Jaipur to get a voucher number (RUBL-19905). Shade dried stems were powdered and extracted with 100% methanol. This extract was subjected to column chromatography with Fr. I Chloroform: Methanol (3:1); Fr. II Chloroform: Methanol (1:1) and Fr. III Chloroform: Methanol (1:3).

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Animals

Male albino rats of Wistar strain were used for the study. Rats were housed in standard environmental conditions with free access of water and feed. Animals were divided in four groups of 10 animals in each. Animals of Group-I was treated with distilled water, Group II, III and IV were treated with Fr. I, Fr. II and Fr. III respectively at the dose level of 50 mg/rat/day for 60 days.

Autopsy schedule

After 55 days of treatment, male rats were cohabited with proestrus females (in the ratio of 1:3) for fertility test. On day 61st rats were autopsied by using light ether anesthesia. Body weight and reproductive organ weight were measured. Various sperm parameters [7], serum testosterone levels [8], testicular cell dynamics and tesicular histological studies were conduct to assess the reproductive function.

Statistical analysis

Data were expressed in mean ± SEM and are analyzed by using the Student's "t"-test followed by ANOVA.

RESULTS

Results of various fractions treatment revealed the non-significant changes in body weight, however a highly significant ($P \leq 0.001$) changes in the weight of reproductive organs i.e. testes, epididymides, seminal vesicles and ventral

prostate were found (Table-1). Significant reduction in sperm density as well as in sperm motility was found after fractions treatment (Table-2). Highest reduction (61.23%) in serum testosterone levels was seen in Fr. III (Table-2).

Testicular cell dynamical studies shows that number of Sertoli cells was unchanged, whereas other spermatogenic elements i.e. spermatogonia, preleptotene, pachytene, secondary spermatocyte and round spermatids were declined in all the fractions (Fig. 1). The number of mature Leydig cells was decreased, where as the degenerating Leydig cells was increased significantly (P≤0.001), however the fibroblast like cells were found non-significant (Fig. 2). After testicular histological study increased number of abnormal seminiferous tubules were seen, the percentage of normal tubule was reduced by 47.93%, 57.38% and 67.45% with 75:25 ($CHCl_3$:CH_3OH), 50:50 ($CHCl_3$:CH_3OH) and 25:75 ($CHCl_3$:CH_3OH) respectively whereas, the abnormal tubules raised significantly (Fig. 3).

DISCUSSION

Treatment of rats with different fractions of *D. falcata* stem extract i.e. 75:25 ($CHCl_3$: CH_3OH), 50:50 ($CHCl_3$: CH_3OH), 25:75 ($CHCl_3$: CH_3OH) did not affect the final body weight in any of the treatment group. The same results were also obtained after treatment with hydromethanolic extract of *Austroplenckia populnea*. [9]

However, statistically significant reductions were observed in the weight of testes and accessory reproductive organs (epididymides, seminal vesicle and ventral prostate) of treated male rats. In the testes, a large component of the weight of tissue is associated with spermatogenic function. [10] Tubules and germinal elements of a testis account for approximately 98% of the testicular mass. [11] The reduction in the number of spermatogonia, spermatocytes and spermatids found in our treatment resulted in decreased weight of testis. These findings are also supported by D'souza and Narayana [12] and Vijay Kumar *et al.* [13]

Table 1: Effect of various fractions of *D. falcata* on body and reproductive organ weights

Treatment	Body Weight (g)	Testes	Epididymides	Seminal Vesicle	Ventral Prostate
			mg/100 g. b.wt.		
Group-I [Control or Vehicle treated]	184.22 ± 6.98	1198.36 ± 34.89	435.52 ± 15.61	601.33 ± 17.92	381.92 ± 14.58
Group-II [*D. falcata* stem fraction 75:25 ($CHCl_3$:CH_3OH) 50 mg/rat/day]	180.92 ± 6.77 ^{ns}	997.30 ± 23.51*	351.15 ± 9.18*	510.16 ± 14.49*	343.61 ± 10.97^{ns}
Group-III [*D. falcata* stem fraction 50:50 ($CHCl_3$:CH_3OH) 50 mg/rat/day]	173.81 ± 6.93 ^{ns}	897.16 ± 20.86**^{a}	315.92 ± 3.45**^{a}	487.01 ± 15.86*	324.53 ± 11.22*
Group-IV [*D. falcata* stem fraction 25:75 ($CHCl_3$:CH_3OH) 50 mg/rat/day]	179.92 ± 7.01 ^{ns}	752.40 ±19.39**$^{a+b+}$	287.79 ± 4.23**$^{a+b+}$	450.43 ± 14.41**^{a}	310.01 ± 10.27*

Values are mean ± SEM (n=10); Levels of significance - ns = non significant

* P≤0.01; ** P≤0.001 compared with Group I (Control); ^{a} P≤0.01; $^{a+}$ P≤0.001 compared with Group II; $^{b+}$ P≤0.001 compared with Group III

Table 2: Effect of various fractions of *D. falcata* on sperm dynamics and testosterone levels

Treatment	Sperm Motility (%) (Cauda Epididymides)	Sperm Density (million/ml) Testes	Sperm Density (million/ml) Cauda Epididymides	Testosterone (ng/dl)
Group-I [Control or Vehicle treated]	74.81 ± 4.54	5.57 ± 0.27	51.89 ± 2.47	5.03 ± 0.11
Group-II [*D. falcata* stem fraction 75:25 ($CHCl_3$:CH_3OH) 50 mg/rat/day]	33.24 ± 1.13**	3.25 ± 0.23**	25.19 ± 1.20**	2.58 ± 0.09**
Group-III [*D. falcata* stem fraction 50:50 ($CHCl_3$:CH_3OH) 50 mg/rat/day]	27.64 ± 1.13**^{a}	2.22 ± 0.15**^{a}	18.55 ± 1.17**^{a}	2.20 ± 0.05**^{a}
Group-IV [*D. falcata* stem fraction 25:75 ($CHCl_3$:CH_3OH) 50 mg/rat/day]	21.76 ± 0.98**$^{a+b}$	1.60 ± 0.09**$^{a+b}$	13.43 ± 0.96**$^{a+b}$	1.95 ± 0.06**$^{a+b+}$

Values are mean ± SEM (n=10); Levels of significance - ** P≤0.001 compared with Group I (Control)

^{a} P≤0.01; $^{a+}$ P≤0.001 compared with Group II; ^{b} P≤0.01; $^{b+}$ P≤0.001 compared with Group III

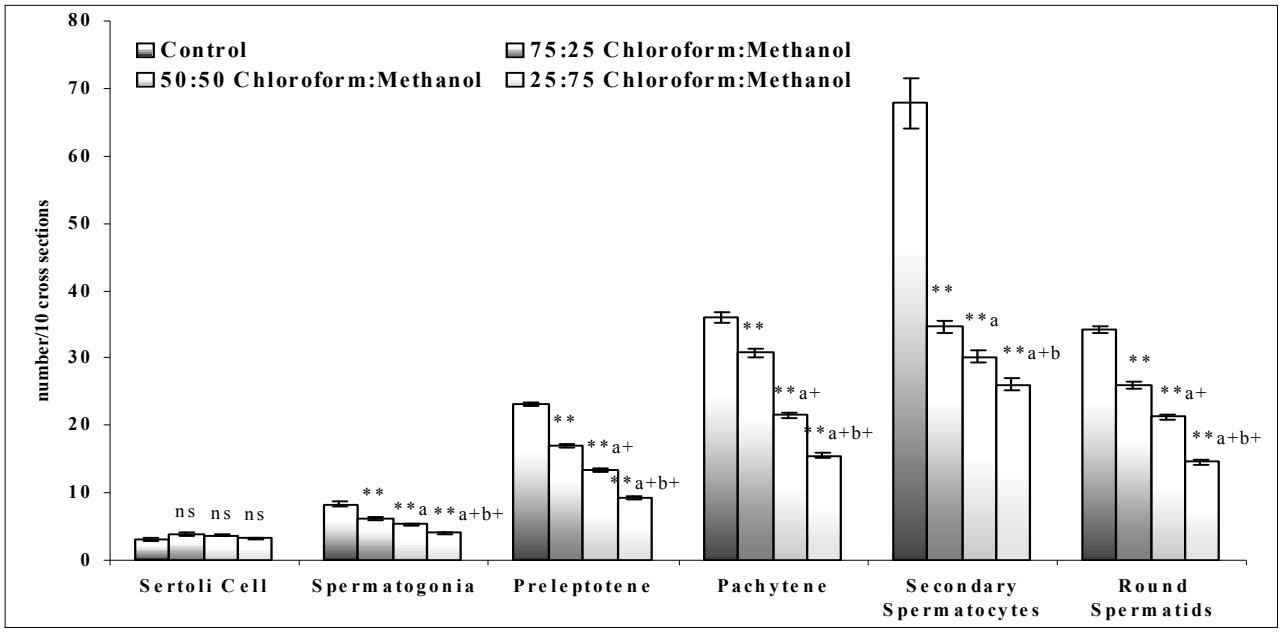

Fig. 1: Effect of various fractions of *D. falcata* on testicular cell dynamics

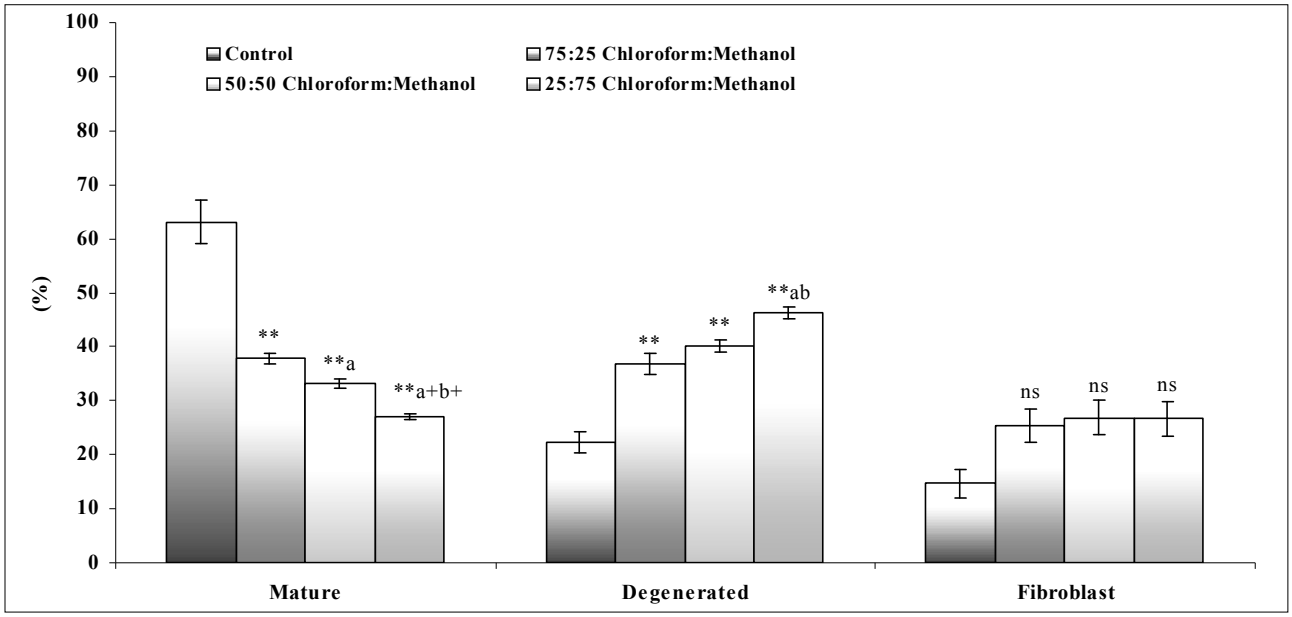

Fig. 2: Effect of various fractions of *D. falcata* on Leydig cells differential counts

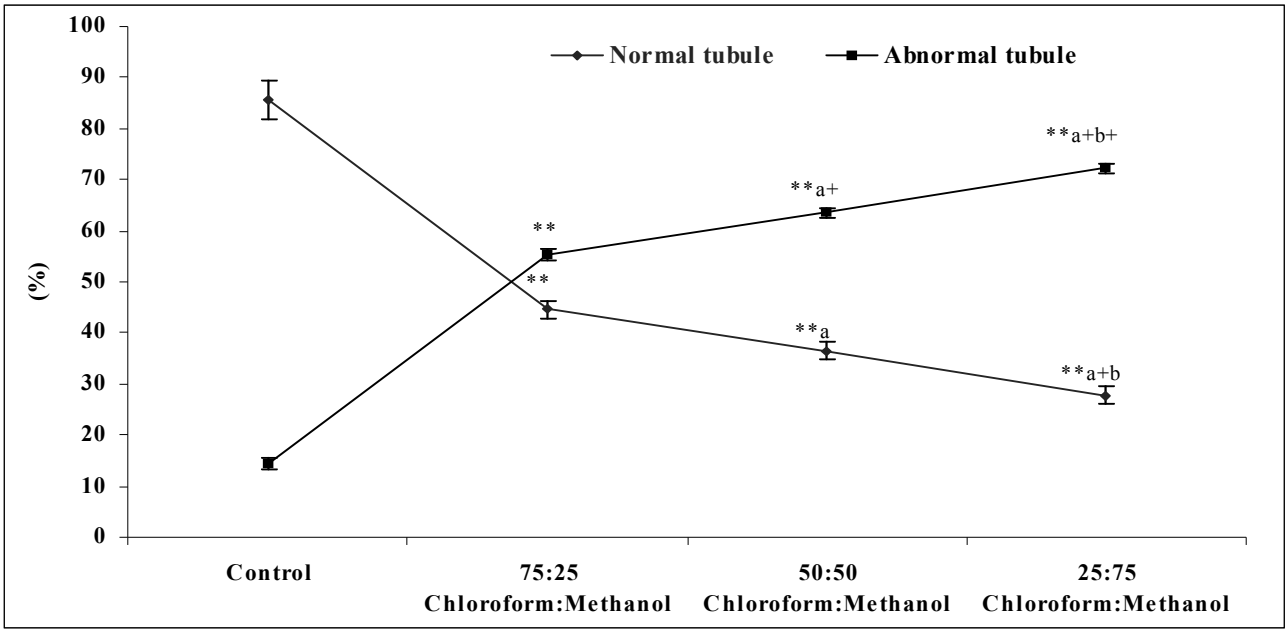

Fig. 3: Effect of various fractions of *D. falcata* on percent count of seminiferous tubules
Values are mean ± SEM (n=10); Levels of significance - ns = non significant
** P≤0.001 compared with Group I (Control); [a] P≤0.01; [a+] P≤0.001 compared with Group II; [b] P≤0.01; [b+] P≤0.001 compared with Group III

It is well established that androgens are the major regulators of the growth, structure and functions of accessory sex organs. [14] In accessory sex organs it is not testosterone, but rather the 5α-reduced metabolites, dihydrotestosterone and 3α, 17β-androstiendiol are the primary regulatory hormones controlling their structure and functions. [15] A decrease in such androgen metabolites might eventually result in decreased accessory sex organs weight.

In the present study, the inadequate sperm count and motility due to *D. falcata* fractions treatment could have resulted in an incompetence of the spermatozoa to reach the fallopian tubes and fertilize the egg, thus causing complete sterility. [16-17]

High levels of intratesticular testosterone are necessary for the proliferation and differentiation of spermatogenic cells and spermatogenesis. [18] While high circulating testosterone concentration is required for functional integrity of androgen-dependent accessory sex organs. [19]

Low levels of testosterone found in our study after *D. falcata* isolated fractions treatment confirmed the alterations found in the reproductive physiology of male rats.

Spermatogenesis is a complex process of male germ cell proliferation, regulated by endocrine and testicular paracrine/autocrine factors. [20] It starts with a differentiating division of the spermatogonial stem cells and continues with sequential cell divisions of spermatogonia and meiosis of spermatocytes to form round spermatids. In the present study spermatogenic elements were decreased in significant manner however, the most remarkable result in our study is that Sertoli cell counts were found within the normal range in

comparison to controls, which suggest the reversible nature of *D. falcata*.

Testosterone is synthesized and released by the Leydig cells in response to LH. [21] The Leydig cells are able to respond to changes in LH secretion within half an hour [22] and influence the seminiferous tubules by maintaining a high concentration of testosterone in the peritubular compartments of the testis. A significant decrease in the seminiferous tubular diameter, Leydig cell nuclear diameter and alterations in the Leydig cells differential counts probably correspond to decrease in testosterone production and or inhibin of pituitary gonadotropin secretion, hence, disruption of spermatogenesis occurred.

In conclusion the isolated fractions (Chloroform: Methanol) of *D. falcata* methanol stem extract at the dose level of 50 mg/rat/day persist the contraceptive like activities with inhibition of spermatogenesis and affects reproductive functions in male rats. Also after statistical analysis Fr. III was found the most effective fraction in all. Further investigations on isolated active principal/active compound of *D. falcata* are in progress.

ACKNOWLEDGEMENTS

Author Dr. Jai Bahadur Singh Kachhawa, acknowledges the financial assistance from UGC, New Delhi for Dr. D. S. Kothari Postdoc Research Fellowship.

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