

Full Length Research Paper

Evaluation of the anti-fertility activity of stem bark of *Crataeva nurvala* buch-hum

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Accepted 22 May, 2009

The ethanol and aqueous extracts of the dried stem bark of the plant *Crataeva nurvala* Buch-Hum (Capparidaceae) have been found to possess significant anti-fertility effects in rats. Both ethanol and aqueous extracts exhibited partial and complete resorption of implants at 300 and 600 mg/kg b.wt dose levels, respectively. In estrogenic activity study, both the extracts increased uterine weight and caused opening and cornification of vagina in immature rats. The present work justifies its effectiveness in preventing pregnancy in all rats at dose levels

Key words: Anti-fertility, anti-implantation, aqueous and ethanol extracts, *Crataeva nurvala*.

INTRODUCTION

The genus *Crataeva* (Capparidaceae) is named in honor of the Greek botanist Crataevas. *Crataeva nurvala* is commonly known as *barna* and *varuna* (Bhattacharjee, 1998) and distributed, throughout India and tropical regions of the world wild or cultivated (Kirtikar and Basu, 1984). It is found along streams and also in dry, deep boulder formations in sub Himalayan tract (Agarwal, 1997). It is useful as a laxative, demulcent, stomachic, and blood diseases and is reported to cure disorders of urinary organs (Drury, 1978). It is also very useful as anti-inflammatory drug and act as a good contraceptive for women. This plant is known to possess immense pharmacological activity-nephrotoxicity (Shirwaikar et al., 2004) arthritis (Geetha et al., 1998), lipidperoxidation in adjuvant induced arthritis (Geetha and Varalakshmi, 1999), urolithiasis (Varalakshmi et al., 1990), urinary disorders (Deshpanda et al., 1982) and antilithic properties. The major component isolated from this plant is lupeol, which is used to treat hypercrystalluria, hyperoxaluria and hypercalciuria (Anand et al., 1994). Triterpenoids and related compounds were isolated from bark of this plant (Gangandee et al., 2006). The tribes of

Shevaroyan hills use the bark of this plant to induce abortion. An extensive survey of literature available from all scientific sources revealed no information about the pharmacological validation of the anti-fertility activity of the plant. The present work was therefore undertaken to substantiate the folklore claims in a scientific manner using animal models.

MATERIALS AND METHODS

Plant material

The bark of *C. nurvala* was collected from Shevaroyan Hills Tamil Nadu, India, in the month of September, 2006 and the plant material was authenticated by a Botanist. A voucher specimen (CCB-94) has been kept in our college museum for future reference.

Preparation of the extract

The powdered material of *C. nurvala* was extracted separately by continuous hot extraction process using soxhlet apparatus with ethanol and aqueous extract by cold maceration (Kokate, 1994). After extraction, the extracts were concentrated under reduced pressure. The dried ethanol and aqueous extracts were subjected to various chemical tests to detect the presence of different phytochemical constituents like flavonoids, alkaloids and traces of carbohydrates.

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Table 1. Anti-fertility activity of stem bark *C. nurvala* Buch-ham in female Wister rats

Treatment (mg/kg)	No. of corpora lutea	No. of implantation sites	No. of resorped implantation	% anti implantation activity	% early abortifacient activity
Normal control 2 ml	17.33 ± 0.33	14.67 ± 0.21	0.00 ± 0.00	5.86 ± 2.22	0.00 ± 0.00
Ethanol extract 300	12.00 ± 1.03	5.17 ± 0.30*	4.26 ± 0.24	18.48 ± 0.44	56.33 ± 0.52
Ethanol extract 600	9.17 ± 0.47	3.00 ± 0.36*	6.44 ± 0.22	24.20 ± 0.36	65.42 ± 0.33
Aqueous extract 300	13.50 ± 0.56	6.83 ± 0.30*	5.43 ± 0.89	17.68 ± 1.30	38.02 ± 0.26
Aqueous extract 600	11.67 ± 0.66	4.00 ± 0.36*	6.86 ± 0.22	18.00 ± 0.79	40.02 ± 1.20

Values are expressed as Mean ± SEM of 6 rats in each group.

*P < 0.001, **P < 0.01 compared with control group.

The data was also analyzed by one way ANOVA followed by Dunnett Multiple Comparison Test.

Test animals

Anti-fertility testing was performed on adult female Wister rats weighing between 180-200 g which was obtained from M/S Venkateshwara enterprises (P) Ltd Bangalore. They were housed in polypropylene cages and fed with standard chow diet and water *ad libitum*. The animals were exposed to alternate cycle of 12 h of darkness and light each. Before each test, the animals were fasted for at least 12 h. The experimental protocols were subjected to the scrutinization of the Institutional Animal Ethics Committee and were cleared by the same (IAEC No: P. Col - 15/2006).

Acute toxicity studies

The animals were divided into six groups, and were treated orally with ethanol and aqueous extracts of *C. nurvala* at 200, 400 and 700 mg/kg, body weight doses, separately. The animals were continuously observed for 1 h, 4 h and intermittently for the next 6 h and then again at 24 and 48 h following drug administration. The parameters observed were grooming, hyperactivity, sedation, loss of righting reflex, respiratory rate and convulsion (Ghosh, 1994)

Anti-fertility activity

The anti-fertility study was performed as suggested by Thompson and Khanna methods (Thompson, 1990; Khanna and Chaudhury, 1968). The vaginal smears of such female rats of known fertility were examined daily and the rats in proestrous phase of the estrous cycle were left overnight with known fertile males. The female rats were examined the following morning for evidence of copulation. Those rats which showed thick clumps of spermatozoa in their vaginal smears were separated from the experiment and the day the spermatozoa were found was labeled as day one of pregnancy. Mated rats were randomly distributed into various groups of six animals in each. The ethanol and aqueous extracts to be tested were fed orally to these pregnant rats at the doses of 300 and 600 mg/kg b.wt, twice daily through an intragastric catheter, respectively. The control group received only vehicle (1%, w/v gum acacia) in a similar manner. Treatment was given for eight days and the animals were laparotomized under mild ether anesthesia on day 15. The 2 horns of the uterus were examined for the number of implants and prominent corpora lutea.

Estrogenic activity

Estrogenic activity of the extracts was assessed in immature female

rats as suggested by Zarrow et al. (1964). The selected rats were bilaterally ovariectomized under mild ether anesthesia through lateral incisions in the skin just below the last rib. The ovariectomized rats were divided into four groups of animals received one of the following through oral route vehicle (2 ml/kg), diethylstilbestrol (1.5 mg/kg), ethanol and aqueous extracts (300 and 600 mg/kg b.wt) once daily for a period of 4 d. After 24 h of last dose treatment, the animals were sacrificed and uteri were excised from adhering tissue and weighed. Vaginal opening and vaginal cornification were also recorded.

Statistical evaluation (Woodson, 1987)

All the data are presented as mean ± SEM. The differences between groups were evaluated by one-way analysis of variance (ANOVA) followed by the Dunnett multiple comparisons test. P < 0.01 was considered to be significant.

RESULTS

The results clearly showed that both extracts possess anti-fertility effect in a dose-dependent manner (Table 1). However, the ethanol extract is found to be more active than the aqueous extract. Resorption implants were observed with 600 mg/kg dose of both the extracts on laparotomy, as evidenced by scar marks of implantation sites in the uterine horns of animals. In animals treated with 300 mg/kg b.wt dose of both extracts, on laparotomy the uterine horns, showed reduced number of implantation sites compared to the control group animals.

Estrogenic activity

Oral administration of the ethanol and aqueous extracts at 300 and 600 mg/kg b.wt caused a significant increase in uterine weight in immature rats (versus control, P < 0.01). The uteri of these rats were inflated and full of fluid resembling the proestrous/estrous uterus. The epithelium of the endometrium consisted of spindle shaped cells with basal nuclei. The treated rats showed open vaginas. Examination of the vagina smears of treated rats

Table 2. Effect of extracts of stem bark *C. nurvala* Buch-ham on bilaterally ovariectomized immature rats.

Treatment (mg/kg)	Utrine weight (mg)	Vaginal opening (%)	Vaginal conification (%)
Control (Vehicle)	100.4 ± 6.8	0	0
Ethanol extract 300 mg/kg	204.8 ± 10.6	100	90
Ethanol extract 600 mg/kg	212.6 ± 8.6	100	100
Aqueous extract 300 mg/kg	198.2 ± 8.2	90	90
Aqueous extract 600 mg/kg	200.2 ± 10.6	100	100

Values are expressed as Mean ± SEM of 6 rats in each group.

*P < 0.001, **P < 0.01 compared with control group.

The data was also analyzed by one way ANOVA followed by Dunnett Multiple Comparison Test.

Table 3. Abortifacient activity of extracts of stem bark of *C. nurvala* Buch-ham.

Treatment (mg/kg)	No. of animals pregnant /no. of animals taken	Average no. of implantation sites	Average no. of litters delivered	Percentage abortifacient activity
Control	6/6	9.8 ± 0.75	9.8 ± 0.75	00
Ethanol extract 300	3/6	8.2 ± 0.98	4.9 ± 1.23	40
Ethanol extract 600	4/6	7.2 ± 0.32**	3.1 ± 1.65	57
Aqueous extract 300	3/6	8.6 ± 0.88*	5.2 ± 0.22	40
Aqueous extract 600	4/6	7.6 ± 0.44**	3.8 ± 0.42	58

Values are expressed as Mean ± SEM of 6 rats in each group.

*P < 0.001, **P < 0.01 compared with control group.

The data was also analyzed by one way ANOVA followed by Dunnett Multiple Comparison Test.

revealed predominantly cornified and nucleated epithelial cells (Tables 2 and 3)

DISCUSSION

In present study, the bark extracts of *C. nurvala* were tested for its anti-implantation and estrogenic properties. The loss of implantation caused by ethanol and aqueous extracts may be due to antizygotic, blastocytotoxicity or anti-implantation activity as described by Hafez (1970).

It is well known that for implantation exact equilibrium of estrogen and progesterone is essential and any disturbance in the level of these hormones may cause infertility (Psychoyos, 1966). Compounds with hormonal values usually disturbs the hormonal milieu in the uterus. Therefore, the anti-implantation activity may be due to estrogenic activity, causing the expulsion of ova from the tube, disrupting the luteotrophic activity of the blastocyst.

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