

**EUPHORBIA NERRIFOLIA LINN: HERBAL TOOL FOR BIRTH CONTROL**

Maskare Rina*, Mishra Manisha, Turaskar Ashish

Manoharbhay Patel Institute of Pharmacy (B-Pharmacy), Kudwa road, Gondia, Maharashtra, India

*Corresponding Author Email: rinamaskare@yahoo.com

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ABSTRACT

In the present study, antifertility activity of hydroalcoholic extract of *Euphorbia nerifolia* Linn was evaluated. The ethanolic extract of *Euphorbia nerifolia* showed significant antifertility activity. Pre treatment with ethanolic extract showed significant inhibition of number of implant site at a dose of 400 mg/kg. There was no change in ovulation, hence the antifertility activity observed in the present study with *Euphorbia nerifolia* can be attributed largely to its anti implantation activity.

Keywords: *Euphorbia nerifolia*, anti fertility activity, anti implantation activity.

INTRODUCTION

The medicinal plants are of great interest to human health. Plant based medicines have been a part of traditional healthcare in most parts of the world for thousands of years^{1,2}. Plants contain numerous biologically active compounds, many of these have been shown to exhibit antifertility properties and therefore they were in use as antifertility drugs in traditional medicines. Plants used in traditional medicine contain a vast array of substances that can be used to treat chronic and even infectious diseases. According to a report of World Health Organization, more than 80 % of world's populations depend on traditional medicine for their primary health care needs. Knowledge of the phytochemicals is desirable not only for the discovery of healthcare products, but also in disclosing new sources of economic materials like alkaloids, tannins, oils, gums etc³. The systematic screening of plant extracts or plant derived substances still remains an interesting strategy to find new lead compounds in many plant species. *Euphorbia nerifolia* leaves are used as aphrodisiac, diuretic and also used in the treatment of bronchitis, bleeding piles and in ano-rectal fistula⁴. The plant is useful in dominal troubles, bronchitis, tumors, leucoderma, piles, inflammation and enlargement of spleen, anemia, ulcers, fever and in chronic respiratory troubles⁵. *Euphorbia nerifolia* hydroalcoholic extract was found to contain sugar, tannins, flavonoids, alkaloids, triterpenoidal saponin on preliminary phytochemical analysis. Several triterpenoids like glut-5-en-3b-ol, glut-5(10)-en-1-one, taraxerol and b-amyrin has been isolated from powdered plant, stem and leaves of *Euphorbia. nerifolia*⁶. Neriifolione, atriterpene and a new tetracyclic triterpene named as nerifoliene along with euphol were isolated from the latex of *Euphorbia nerifolia*^{7,8}. Antiquorin have been isolated from ethanolic extract. Anti-inflammatory and analgesic effect of *Euphorbia nerifolia* is reported by⁹. There are reports on the mild CNS depressant, wound healing and immune modulatory activities of the hydroalcohol leaf extract¹⁰⁻¹². However, to the best of our knowledge, literature surveys are available on antifertility properties whatever constituent are responsible for antifertility activity that is present in *Euphorbia nerifolia*. Hence, in the present study an attempt has been made to evaluate the antifertility activity of hydroalcoholic extracts of plant stem isolates to ascertain the rationale for its use in traditional medicine.

MATERIALS AND METHODS**Chemicals and reagents**

All materials and chemicals used in the study were of analytical reagent grade and of highest quality available, and were purchased from reliable firms and institutes.

Plant material collection

Euphorbia nerifolia stems were collected from the local areas of Gondia District in Maharashtra, India, in the month of may 2012. The plant was identified with the help of available literature and authenticated by Botanist of S. M. Bhuskute, Bhavbhuti Mahavidyalay Amgaon, Gondia District, India.

Preparation of hydro-ethanolic crude extract

Freshly collected *Euphorbia nerifolia* stems were air dried in shade and coarse powder (500 g) was defatted in 1.5 L of ethanol (70 % v/v) using Soxhlet apparatus. The extracted mixture was evaporated at 40°C, using a hot air oven and kept in dissector for two days. The yield of the extract was 20 % w/w of the powdered plant material. Dried extract was collected and stored at 5°C in airtight container. Preliminary phytochemical studies showed the presence of flavonoids, glycoside, carbohydrates, tannins, amino acid and sterols etc. The residue was designated as hydro-alcoholic extract and used to assess antifertility activity.

Experimental animals

Wistar rats (150-200 g) of either sex were obtained from pharmacology laboratory, Manoharbhay Patel Institute of Pharmacy, (B-Pharm), Kudwa, Gondia, India for the study. They were housed under standard condition of temperature (24 ± 10 C), relative humidity (65 ± 10 %), light and dark cycle (14:10 h) and fed with standard pellet food. The initial body weight of each animal was recorded. The vaginal smear of the female rats was studied microscopically for estrus cycle every morning at 8-9 am. Only female rats with normal estrus cycle were selected for the antifertility activity evaluation. All experimental procedures were carried out in strict accordance with the guidelines prescribed by the Committee for the Purpose of Control and Supervision on Experimentation on Animals (CPCSEA Reg. No. 928/ab/06/cpcsea) and were approved by the Institutional Animal Ethics Committee.

Acute Toxicity Study

Acute toxicity study of hydro-alcoholic extract of *Euphorbia nerifolia* Linn were carried out in mice according to OECD guidelines. Extract at different doses up to 2000 mg/kg, p.o. was administered and animals were observed for behavioral changes, any toxicity and mortality up to 48 h. There was no toxic reaction or mortality, and found to safe. Based on acute toxicity result we have selected 150 mg/kg and 300 mg/kg for antifertility evaluation.

Anti Ovulatory Activity

The animals used in this method were female rats divided into 3 groups (n = 6), fasted overnight and allowed free access to water *ad libitum*. Different groups of female rats were treated with test drug at 200 and 400 mg/kg, p.o. vaginal smear from each rat was examined daily for 15 days, and those rats exhibited three regular cycles (Hafez E.S.E.; 1970) were used. Drugs and vehicle were started in the estrous phase and administered orally, daily for 15 days. Group I received vehicle only (1 % gum acacia, p.o. daily) and served as control. Groups II and III received hydroalcoholic extract of *Euphorbia nerifolia* at 200 and 400 mg/kg, respectively. The 15-day treatment was to cover three regular estrous cycles. Vaginal smear from each animal was observed every morning between 9-10 A.M. On the 16th day, 24 hours after the last treatment, the rats from each group were anesthetized and sacrificed. Ovaries and uteri were dissected out, freed from extra deposition, and weighed on a sensitive balance. One ovary from each animal was processed for biochemical analysis of cholesterol¹³.

Anti Implantation Activity

Female rats of proestrus phase were kept with male rats of proven fertility in the ratio of 2:1. The female rats were examined in the following morning for evidence of copulation. The animal which showed thick clumps of spermatozoa in vaginal smear were separated from the male partner and divided into 3 groups (n = 6). Animals in the groups I given vehicle only and serve as control. Hydro-alcoholic extract of *Euphorbia nerifolia* at 200 mg/kg and 400 mg/kg were administered to group II and group III respectively from day 1 to 7 of pregnancy. The day when spermatozoa detected in vaginal smear was considered as day 1 of pregnancy. All the animals were sacrificed under light

ether anesthesia and laprotomy was performed to determine the number of implantation sites on the both uteri horn and the number of corpora lutea on the both ovaries. The fertility rate was calculated by the percentage of implantation per number of corpora lutea (representing number of eggs ovulated)¹⁴.

Statistical Analysis

The values are expressed as mean \pm SEM. ANOVA followed by Dunnett's was performed to determine the differences between means and $p < 0.05$ considered as statistically significant.

RESULTS

Anti Ovulatory Activity

Hydroalcoholic extract of *Euphorbia nerifolia* at 200 mg/kg and 400 mg/kg showed no change in the weight of ovaries and cholesterol level, when compared with vehicle treated group (Table 1).

Anti Implantation Activity

Both doses of extract showed significant inhibition of number of implant site Hydroalcoholic extract of *Euphorbia nerifolia* 400 mg/kg was showed 66.66 % inhibition of implants in uterine horns when compared with vehicle treated group (Table 2). Study using different animal species to establish its antifertility activity and also understand underlying cellular mechanism of action.

DISCUSSION

In the present study Hydroalcoholic extract of *Euphorbia nerifolia* was evaluated for its antifertility activity. In the anti ovulatory model, we determined the cholesterol level and ovarian weight. We found that, no change in cholesterol level and ovarian weight in extract treated group, since the cholesterol is the precursor for the steroidogenesis of ovarian endocrine tissues. It is well known that for implantation exact equilibrium of estrogen and progesterone is essential, any disturbance in level of these hormones causes infertility¹⁵. Presence of chemical constituents in *Euphorbia nerifolia* like saponins, triterpenoids flavonoids and alkaloids and these constituents might responsible for anti implantation activity. Loss of implantation caused by *Euphorbia nerifolia* may be due to anti zygotic or blastocytotoxic activity¹⁶.

Table 1: Effect of the Hydroalcoholic extract of *Euphorbia nerifolia* on ovarian weight and cholesterol level

Treatment and dose (mg/kg p.o.)	Ovarian weight in mg/100 g body weight	Cholesterol level in ovary (mg/50 mg)
Gum acacia (1 %, 1 ml/kg)	26.12 \pm 0.68	0.48 \pm 0.06
Hydroalcoholic extract of <i>Euphorbia nerifolia</i> (200)	30.11 \pm 0.83	0.41 \pm 0.10
Hydroalcoholic extract of <i>Euphorbia nerifolia</i> (400)	26.24 \pm 1.13	0.43 \pm 0.09

*P < 0.05, **P < 0.01 compared with vehicle treated control group

Table 2: Effect of Hydroalcoholic extract of *Euphorbia nerifolia* Linn on implantation site after oral administration for 7 days in female rats

Treatment and dose (mg/kg p.o.)	Days of administration	Number. Of rats without implant on day 10	Number of Implantation sites	Rats without Implantation sites (%)
Gum acacia (1%, 1 ml/kg)	1-7	0	8.4 \pm 0.51	0
Hydroalcoholic extract of <i>Euphorbia nerifolia</i> (200)	1-7	1	5.4 \pm 0.81*	16.66
Hydroalcoholic extract of <i>Euphorbia nerifolia</i> (400)	1-7	4	1.2 \pm 0.8**	66.66

*P < 0.05, **P < 0.01 compared with vehicle treated control group

The present experimental findings suggest that, the Hydroalcoholic extract of *Euphorbia neriifolia* has anti implantation activity rather than anti ovulatory activity. Hence, it's anti implantation action responsible for the anti fertility activity. Further detailed study using different animal species to establish its antifertility activity and also understand underlying cellular mechanism of action.

CONCLUSION

The present study indicates that the hydroalcoholic extract of *Euphorbia neriifolia* has antifertility effect. Further studies on mechanism of antifertility action and isolation of the active components responsible for antifertility effect are in progress.

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