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Antifertility Activity of Ethanolic Extract of *Allium cepa* Linn in Rats

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Abstract

In the present study, antifertility activity of ethanolic extract of *Allium cepa Linn* was evaluated. The ethanolic extract of *Allium cepa* showed significant antifertility activity Pretreatment with ethanolic extract showed significant inhibition of number of implant site at a dose of 300 mg/kg. There was no change in ovulation, hence the antifertility activity observed in the present study with *Allium cepa* can be attributed largely to its antiimplantation activity.

Key words: *Allium cepa,* antifertility activity, antiimplantation activity.

Introduction

India within, few years of time span will be the leading country as far as the population growth is concerned. Since the population rising tremendously, this may affect drastically the economic growth of India. Family planning has been promoted through several methods of contraception, but due to side effect produced by the use steroidal contraceptive[1] and use of abortifaciant drugs. There is a need of drug which is effective with lesser side effects.

The plant *Allium cepa* Linn. (Liliaceae) are proved to shown the antidiabetic[2], antioxidant[3], antihypertensive[4], antithrombotic[5,] hypoglycemic,[6]antihyperlipidemic[7]. Bulb extract shown to have ecobolic effect in rats[8]. The bulb of of *Allium cepa* contains Kampferol, β-sitosterol, ferulic acid, myritic acid, prostaglandins[9] .Traditionally plant containing these constituents used as abortifaciant, the bulb extract of Allium cepa had showed ecobolic effect in mice and rats[10]. The aim of present study was to evaluate antifertility activity of *Allium cepa* Linn in female rats.

Materials and methods

Plant material- Bulb of Allium cepa Linn was collected in month of January 2008 dried and sample was authenticated by Agharkar Research Institute, Pune, India. Extraction was done by maceration process for 72 h using ethanol. The percentage yield was found to be 4.8 % w/w. Preliminary phytochemical studies showed the presence of flavonoids, glycoside, carbohydrates, tannins, amino acid and sterols etc.

Animal-Wistar rats (150-200 g) of either sex were obtained from National Toxicological Centre, Pune, India for the study. They were housed under standard condition of temperature (24 $\pm 1^0$ C), relative humidity (65±10%), light and dark cycle (14:10 h) and fed with standard pellet food. (Amrut Laboratory animal feed diet. Pune, Maharashtra, India) and water *ad libitum.* 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. 198/99/CPCSEA) and were approved by the Institutional Animal Ethics Committee.

Acute toxicity study

Acute toxicity study of ethanolic extract of *Allium cepa* 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.

Antiovulatory activity [11]

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 150 and 300 mg/kg, p.o. Vaginal smear from each rat was examined daily for 15 days, and those rats exhibited three regular cycles[12] 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 ethanolic extract of *Allium cepa* at 150 and 300 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 16^{th} 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 cholesterol.

Antiimplantation activity [13]

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. Ethanolic extract of *Allium cepa* at 150 mg/kg and 300 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 leutea (representing number of eggs ovulated).

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

Results

Antiovulatory activity-Ethanolic extract of *Allium cepa* at 150 mg/kg and 300 mg/kg showed no change in the weight of ovaries and cholesterol level, when compared with vehicle treated group (Table 1.)

Table 1. Effect of the ethanolic extracts of *Allium cepa* Linn on ovarian weight and cholesterol level.

Treatment and dose (mg/kg p.o.)	Ovarian weight in mg/100g body weight	Cholesterol level in ovary (mg/50 mg)
Gum acacia (1 %, 1ml/kg)	38.46 ± 0.68	0.38 ± 0.06
Ethanolic extract of *Allium cepa* (150)	40.59 ± 0.83	0.43 ± 0.10
Ethanolic extract of *Allium cepa* (300)	37.24 ± 1.23	0.41 ± 0.09

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

Antiimplantation activity-Both doses of extract showed significant inhibition of number of implant site. Extract at the dose of 300 mg/kg was showed 66.66% inhibition of implants in uterine horns when compared with vehicle treated group (Table 2.)

Table 2. Effect of ethanolic extracts of *Allium cepa* 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 site (%)
Gum acacia (1 %, 1ml/kg)	1-7	0	8.4 ± 0.51	0
Ethanolic extract of *Allium cepa* (150)	1-7	1	5.4 ± 0.81*	16.66
Ethanolic extract of *Allium cepa* (300)	1-7	4	1.2 ± 0.8**	66.66

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

Discussion

In the present study ethanolic extract of *Allium cepa* was evaluated for its antifertility activity. In the antiovulatory 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 infertility[14].

Presence of chemical constituents in *Allium cepa* like Kampferol, β-sitosterol, ferulic acid, myritic acid, prostaglandins and these constituents might responsible for antiimplantation activity. Loss of implantation caused by *Allium cepa* may be due to antizygotic or blastocytotoxic activity.

The present experimental findings suggest that, the ethanolic extract of *Allium cepa* has antiimplantation activity rather than antiovulatory activity. Hence, its antiimplantation action responsible for the antifertility activity. Further detailed

study using different animal species to establish its antifertility activity and also understand underlying cellular mechanism of action.

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