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Research Article

Anti- Implantation Activity of the Hydroalcoholic Tuber Extract of *Gloriosa superba* Linn in Female Albino Rats

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ABSTRACT

The practice of traditional medicine for control of fertility in the country is based on the use of plant medicine for many years. The aim of the present study is to evaluate the effect of hydroalcoholic tubers extract of traditionally used *Gloriosa superba* Linn (Liliacaea) for anti-fertility activity using an anti-implantation model in female albino rats. After acute toxicity study, the extract at dose levels 30 and 60 mg/kg body weight were administered orally to female rats from day 1 to day 7 of pregnancy. At autopsy on day 10 post-coitum, number of implantation sites and the corpora lutea were recorded. The extract was found to possess significant dose-dependent anti-implantation activity and there was reduction in the number of litters born. The anti-fertility activity was reversible on withdrawal of the treatment of the extracts. Results indicated that the hydroalcohalic extract exhibited very promising anti-fertility activity in albino rats. The activity was attributed to the combined effect of alkaloids, tannins and phytosterols of the extract.

Keywords: *Gloriosa superba,* Anti-implantation, Anti-fertility.

INTRODUCTION

Ethanomedical literature contains thousands of references to the use of plants for variety of reproduction related purposes. The global search for an effective, safe and reversible anti-fertility agent is being envisaged to tackle the problem of population explosion that may lead to affect drastically the economic growth and health impact of family especially in developing countries like India. A large number of medicinal plants/herbal decoctions have been used by the women of rural natives, especially by the tribes, to prevent conception as recorded in ancient Indian literatures[1].Considering the marked serious side effects produced by continuous use of synthetic contraceptive agents[2], an approach was pursued to identify the new potent anti-fertility molecules from natural sources with minimal side effects and reversible contraceptive effect.

Gloriosa superba Linn. (Family-Liliaceae) is a semi-woody herbaceous climber on bushes distributed throughout the tropical forests of Western Ghats in India[3]. The plant is well documented traditionally in Ayurveda system of medicine and is used in inflammations, gout, rheumatoid arthritis, gonorrhea and relieving fever [4]. Extracts of the tuber are reported to cure ulcer, leprosy, bleeding piles, skin diseases and also have anti-dote property for snake bite. It is classified in Ayurvedic system as *Garbhapatani* (abotifacient) and used for promoting labour pains[5]. The leaf infusion is extensively used to overcome jaundice, parasitic helminthes infections and killing lice in hairs[6-7]. The anti-microbial and mutagenic properties are reported from the tubers of the plant[8]. Literature survey revealed that presence of colchicine, gloriosine and its related alkaloids in the tuber and seeds of the plant are highly valued in modern medicine[9]. Keeping in view of medicinal importance of *G. superba*, the present study has been focused to investigate the anti-fertility activity of the plant extract in experimental animal model.

MATERIAL AND METHODS

Collection and authentication of the plant material

The tubers of *G superba* were collected from Balehonnur**,** Chikkamagalur district, Karnataka during flowering in rainy season. The plant material was taxonomically identified with the help of available literature and authenticated by taxonomist. The voucher specimen (KCD/4657) of the plant has been deposited in K.C.D. Herbarium, Department of Botany, Karnataka University, Dharwad, Karnataka.

Preparation of extract

Freshly collected tubers were shade dried at room temperature and coarsely powdered (# 22). The powdered plant material (500 g) was extracted with hydroalcohol (70%) by continuous hot extraction method using Soxhlet apparatus for 18 h. The extract was concentrated in a rotary vacuum evaporator under reduced pressure and dried to obtain a dark brown semi-solid mass. The percentage yield of the extract was found to be 5.51% w/w with respect to air dried plant material.

Phytochemical screening

Identification of the chemical constituents was carried out on the plant extract in order to determine the presence of various phyto-constituents using specific reagents [10].

Experimental animals

Anti-fertility experiment was performed on-bread adult, cyclic virgin female albino rats (2-months-old and weighing 150-180 g body weight). Albino mice weighing 25-30 g were used for the acute toxicity study. The animals were fed with standard diet pellets and water *ad libitum.* They were housed in polypropylene cages and maintained under standard laboratory conditions (12:12 h light and dark cycles; temperature 25±2^{0}C and relative humidity 55±10%). Experiments were performed in accordance with the current guidelines of CPCSEA norms[11] after obtaining approval from the Institutional Animal Ethics Committee.

Acute toxicity (LD_{50}) studies

Acute toxicity study of the extract was performed in overnight fasted albino mice by following fixed dose method as per OECD guidelines No.420 (anx-2d) [12]. Mortality & toxic symptoms in the treated animals were observed continuously for the first 3 h after dosing, periodically during the first 24 h and then daily observation for a total period of 14 days.

Evaluation of anti-fertility activity by anti-implantation model

Rats found in proestrous phase of the cycle, were caged with males of proven fertility in the ratio of 2:1 and examined the fallowing morning for evidence of copulation. Animals exhibited the copulation plug or thick clumps of spermatozoa in their vaginal smears were separated and that day of mating was designated as day 1 of pregnancy [13].

The pregnant rats were randomly divided into four groups containing six in each group. Group I served as control, Group II served as standard group (Estradiol, 0.45 mg/kg). Groups III and IV were treated with hydroalcoholic extract at 30 mg/kg and 60 mg/kg body weight respectively. The extract was administered orally after making a suspension in the vehicle of 1% Tween-80 in distilled water from 1 to 7 day of pregnancy. All the test animals were laparotomized under light anesthesia and semi sterile conditions on day 10 of pregnancy. The uteri were examined to count the number of implantation sites and the corpora lutea in both ovaries. The rats were allowed to recover and deliver after full term. The number of litters born was counted after the completion of one gestion period in both the control and test groups. The litters were allowed to grow in order to check for postnatal growth and any congenital abnormalities.

The reversibility of the activity effect of the extract was also studied. After 21 days of drug-free period, the animals were allowed to mate with males of proven fertility in the ratio of 1 male to 2 females. After the completion of one gestation period (21 days), the number of titters was determined.

The anti-implantation percentage was calculated by using formula:

(Ic-It) x 100/ Ic

Where **Ic** – number of implantation sites in control**;** **It -** number of implantation sites in test.

Statistical analysis

The result are expressed as mean ± SEM (n=6). Statistical difference between control and experimental values were analyzed by one-way analysis of variance (ANOVA), followed by Dunnett's test. Probability level less than 0.01 were considered as significant.

RESULTS

Phytoconstituents

Preliminary phytochemical analysis of the hydroalcoholic extract of *G superba* revealed the presence of alkaloids, tannins and traces of phytosterols.

Acute toxicity studies of the hydroalcoholic tuber extract of *G superba*

Acute toxicity studies were carried out to evaluate toxicity and to determine the minimum lethal dose of the drug extract using *Swiss* albino mice. Fixed dose method of OECD Guideline No.420 given by CPCSEA was adopted for toxicity studies. It was found that no mortality and changes in the behavior

were observed up to dose 300 mg/kg body wt. Therefore, 1/5th and 1/10th of the maximal and sub-maximal tolerated safe HAGS doses (60 and 30 mg/kg body wt) were selected for screening of anti-implantation activity.

Effect of hydroalcoholic tuber extract of *G superba* on implantation

An anti-fertility effect of *G superba* was carried out with an anti-implantation model in female rats. Anti-implantation activity is expressed as the percentage inhibition of implantation sites in the uteri when laparotomized on day 10 of pregnancy. The hydroalcoholic extract from the tubers of *G superba* at doses of 30 and 60 mg/kg body wt was administered on the days 1-7 post-coital in female albino rats and result obtained as shown in Table-1 and Graphs 2, 3 and 4.

All control rats treated with Tween-80 (1%) have shown normal number of implantation sites when laparotomized on day 10, exhibiting 10.50 ± 0.61 mean number of implants and the mean number of corpora lutea were 13.33 ± 0.84. The number of litters delivered was 9.50 ± 0.56. Hydroalcoholic extract at low dose level (30 mg/kg) is effective as all the experimental animal exhibit implantation sites with average of 3.16 ± 0.16 as shown in figure-1.

The same extract with maximum dose level (60 mg/kg) has cause the failure of implantation. The average number of implants is reduced to 2.00 ± 0.25. It observed that HAGS strongly inhibit the implantation sites with both dose levels.

The hydroalcohol extract found to have significant anti-fertility activity as indicated by the significant reduction in the number of implants after day 10 and reduced the number of litters born. The hydroalcohol extract at dose levels of 30 and 60 mg/kg body wt reduced the implantation up to 69.90 % and 80.95 % (**P<0.01) respectively. Estradiol (0.45 mg/kg body wt) as a reference standard was found to 95.07 % inhibition of implantation sites as shown in the Graph-3.

The HAGS exhibited significant anti-implantation activity in a dose-dependent manner. None of the treatments altered the number of corpora lutea, which was similar to that of the control. Resorption sites on day 10 were present in treated groups and no teratogenic effect was observed at the various doses used.

The reversibility of the anti-fertility effect of the extract after 21 days of drug-free period, the animals was produced 8.0 ± 0.26 litters on the average. This showed that there was no statistically significant change from the control group. This resulted in pregnancy and deliver of normal litters, indicating that the action of the extract was reversible.

DISCUSSION

The use of plant preparations for anti-fertility activities, especially for prevention/ interruption of pregnancy has been practiced since ancient time in India[14]. Findings of the present study establish the anti-fertility activity of *G.superba* in female reproductive system.

Oral administration of hydroalcoholic extract of *G superba* at two different doses (30 and 60 mg/kg body wt) showed most significant dose dependent anti-fertility activity. The treated animals showed anti-implantation activity in postcoital study (administered from days 1 to 7). After parturition, the number of litters born was significantly less than that of control. This study clearly reveals that the extract is effective before and after the implantation occurred. Hence, the drug indicated the highest anti-fertility activity. The loss of implantation may be due to their anti-zygotic, blastocytotoxic, anti-implantation or by early abortifacient activity[15].

It is well know that for implantation exact equilibrium of estrogen and progesterone is essential, and any disturbance in the balance of these hormones may cause infertility[16].The compound of hormonal values usually disturbs the hormonal milieu in the uterus and provokes infertility effect in experimental animals[17].

The anti-fertility effect may be due to uterine failure to form deciduoma in the endometrium, which is essential for blastocyst implantation. It indicated that the anti-implantation activity may be due to estrogenic activity causing the expulsion of ova from the tube or disrupting the luteotropic activity of the blastocyst[18].

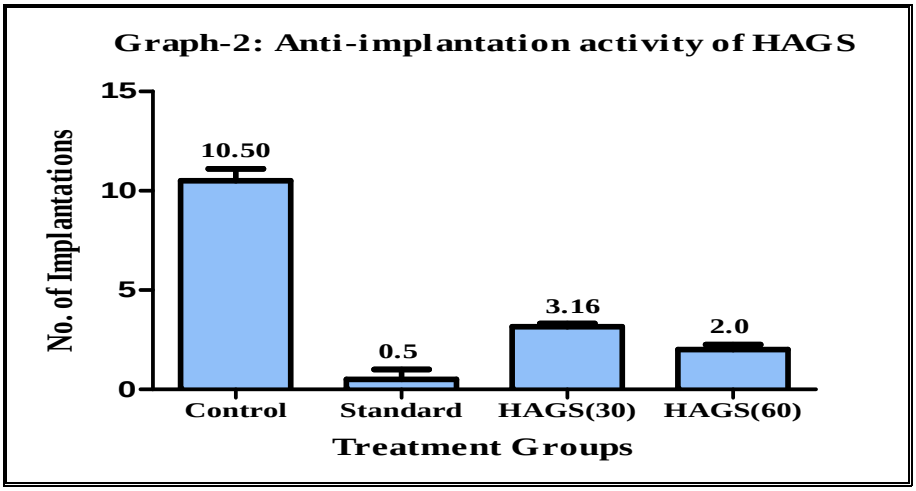
Phytochemical studies, there are reports of thirty alkaloids from tuber, seeds and flowers of *G superba* Linn. The plant is rich in colchicines and its derivatives are reported to be used for a variety of medicinal purpose[19].Several alkaloids are known to exhibit anti-fertility properties[20]. Therefore, the anti-fertility effect of hydroalcoholic extract of *G superba* Linn. may be attributed to the presence of such compounds.

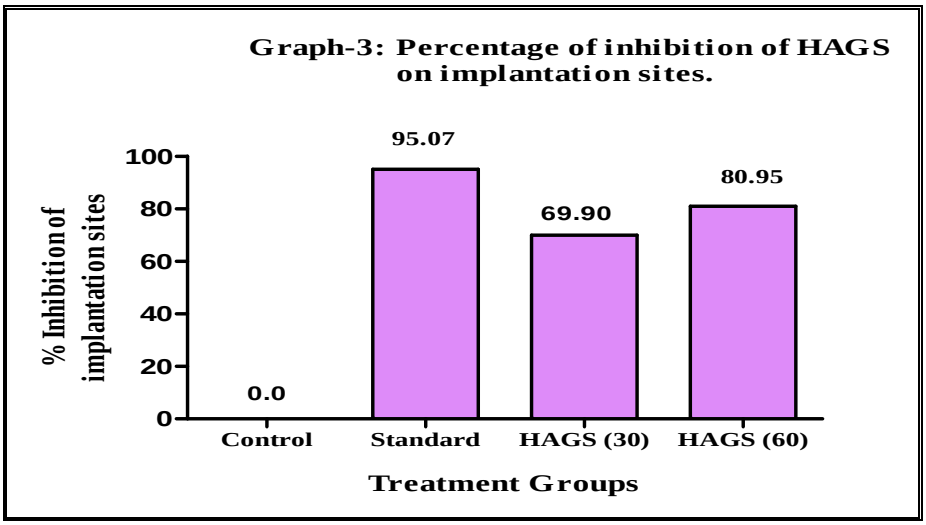
Table 1: Anti-implantation activity of hydroalcoholic tuber extract of *Gloriosa superba* (HAGS) when administered orally on day 1 to 7 post-coitum in female albino rats

Group	Treatment	Dose mg/kg p.o.	Laparotomized on day 10 Post-coitum			Litters born
			Corpora lutea	Implantation sites	% Anti-implantation	
I	Control	--	13.33 ± 0.84	10.50 ± 0.61	--	9.50 ± 0.56
II	Estradiol	0.45	0.00 ± 0.00	0.5 ± 0.50**	95.07	----
III	HAGS	30	12.66 ± 0.61	3.16 ± 0.16**	69.90	2.3 ± 0.21**
IV	HAGS	60	12.16 ± 0.75	2.00 ± 0.25**	80.95	1.16 ± 0.47**

N= 6 animals in each group. Values are expressed as mean ± SEM,
**P<0.01 Vs control (Dennett's t-test)

Histogram of the anti-implantation activity of HAGS





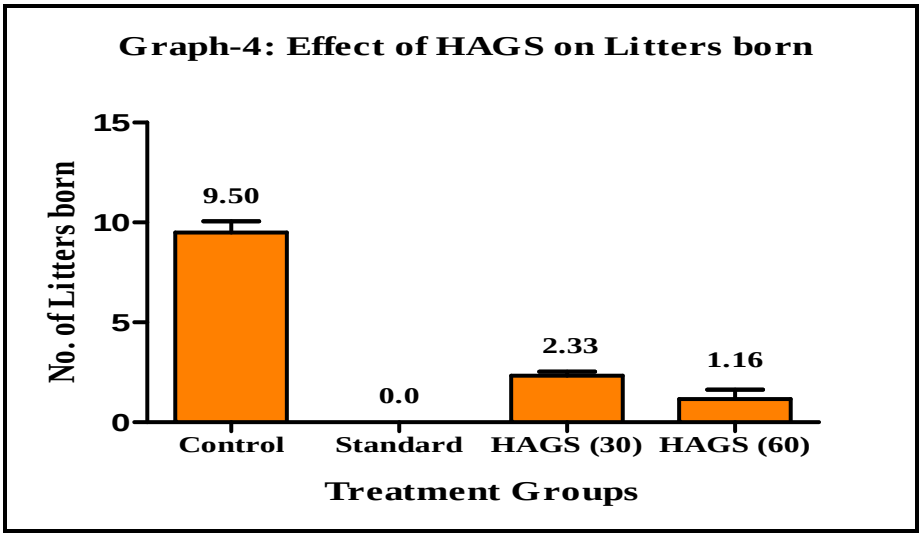


Fig. 1: Photograph of Anti-implantation activity of HAGS

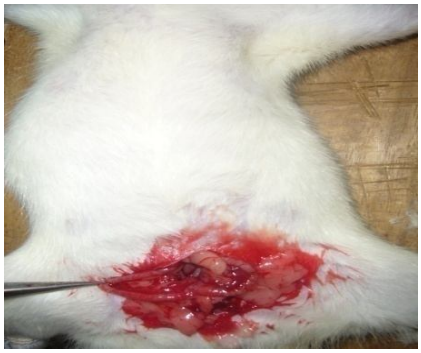


Fig. 1.1: Laparotomized on day 10 post-coitum

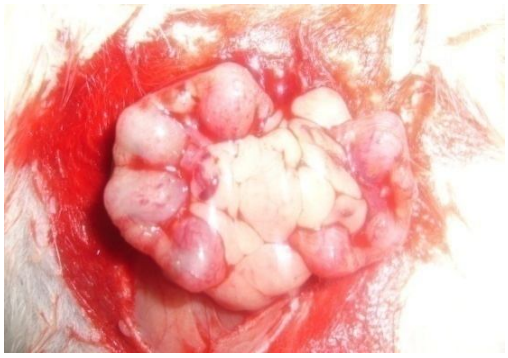


Fig. 1.2: Number of Implantation sites in control on day 10 post-coitum

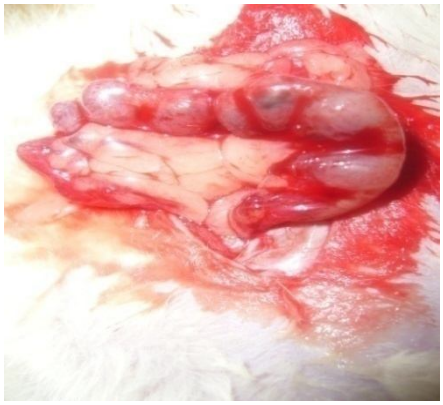


Fig. 1.3: Anti-implantation activity of HAGS on day 10 post-coitum

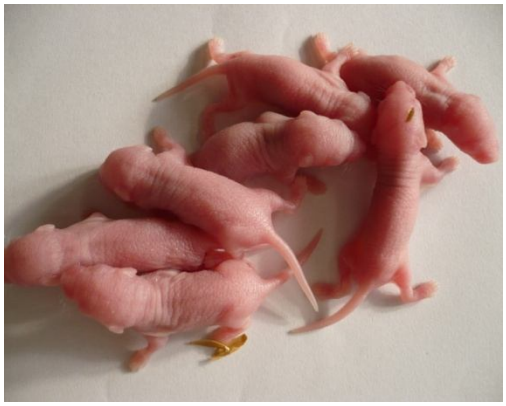


Fig. 1.4: Litters born after one gestation period (21-23 days) in control

CONCLUSION

The present investigation provides scientific basis for the traditional use of *G. superba* as potential anti-fertility plant. Further studies on mechanism of action and isolation of active components responsible for anti-fertility effect are in progress.

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