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Validation of traditional use of *Gloriosa superba* L., tubers as an early-term abortifacient agent

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Abstract

Gloriosa superba L. (Glory lily), mentioned as *Garbhaghatini* in ayurvedic texts, is a highly valued medicinal herb traditionally used for a wide range of therapeutic purposes. This study is aimed to investigate the influence of ethanol extract of *G. superba* tuber (GSTE) on female reproductive function via its effects on implantation and post-implantation embryo survival in Wistar rats (*Rattus norvegicus)*. Pregnant rats were subjected to intragastric administration of GSTE suspended in 1% Tween 80 at doses of 20 and 40 mg/kg body weight for seven days either from gestation day (GD) 1 to GD 7 or from GD 9 to GD 15, following which they were euthanized and the uterine contents examined. GSTE interfered with normal reproductive physiology during pregnancy. Though no significant anti-implantation activity was observed, the extract caused a significant dose-dependent reduction in post-implantation embryo survival and the higher dose of GSTE demonstrated 100% abortifacient activity in rats. The extract exerted these effects through its action on the corpus luteum, subsequently inhibiting progesterone secretion; serum progesterone levels were significantly reduced in treated females on GD 21 compared to the control females. In contrast to a few previous reports, no significant anti-implantation activity was attributable to GSTE. It is concluded that, *G. superba* tubers possess a strong abortifacient character and may serve as a source for the development of a commercial drug for medical termination of pregnancy.

Keywords: Birth control, Folk medicine, Glory lily, Progesterone levels

1. Introduction

Throughout recorded history, fertility control has been promoted through several methods. Herbal resources are important reservoirs of a huge variety of therapeutic substances, and plants and plant-based preparations have long been used as oral contraceptives and early-term abortifacients in different cultures throughout the world. The literary sources give a clear distinction between contraception and abortion, avoiding conception is contraception whereas getting rid of what has been conceived is termed as abortion. Several Greek, Roman, and medieval physicians documented *Ferula communis* L., *F. tingitana* L., *F. persica* Willd. and *F. galbaniflua* Boiss. & Buhse for the regulation of female fertility. A Greek physician and writer of gynaecology, Soranus of Ephesus (A.D. 98–138) described *silphium* and *opopanax* possible species of genus *Ferula* Tourn. ex L. (family Apiaceae), which were valued both as contraceptives and early-term abortifacients (Riddle, 1991).

In India, Ayurveda and other traditional texts document several herbal preparations for both male and female, as remedies for problems related to reproductive health and function (Jadhav and Bhutani, 2005). Many modern medicines have been developed using the clues obtained from the ancient knowledge of our ancestors and tribal and rural populations still largely depend on natural agents for therapeutic purposes (Yadav *et al*., 2006). Modern research plays a key role in establishing the acceptability and efficacy of such folk practices (Dabire and David, 2021).

Gloriosa superba L. (family Colchicaceae), is a herbaceous climber (Fig. 1) found up to an altitude of 6000 ft., above mean sea level in tropical Asia and Africa (Jana and Shekhawat, 2011). The geographical range of the plant practically extends all over India and the plant is listed in the checklist of plants of India on 'eFlora of India'. *G. superba* is identifiable by its beautifully shaped red-yellow flowers that blossom once a year during the monsoon (Behera *et al*., 2008). It is known as 'Malabar glory lily' and 'Flame lily' in English, '*Kalihari*' in Hindi and '*Gourihoovu*/ *Gourigadde*' in Kannada. More than 20 Sanskrit names, including *Agnishikha*, *Agnimukhi*, *Kalikari*, *Langaliki*, *Vahnishikha*, *Garbhaghatini*, *Trisha* and *Vishalya* are recorded for this herb in various sources (Warrier *et al*., 1993; Pullaiah, 2002). The high medicinal value of *G. superba* may be attributable to the alkaloids found in all its parts, mainly tubers and seeds. The plant is an important commercial source of colchicine, an amino alkaloid derived from tyrosine and phenylalanine (Sivakumar *et al*., 2004).

Fig. 1. *Gloriosa superba* L., located at Karnatak University Campus, Dharwad, India

Indigenous populations across India have been known to make use of different parts of *G. superba* for the management of various conditions related to female reproductive function and fertility. It tuber is mentioned in various traditional medicinal texts for a variety of therapeutic purposes and has been described as acrid, purgative, alexiteric (*prativisha*), heating, anthelmintic and abortifacient in nature (Ravishankar and Shukla, 2007; Badwaik *et al*., 2011). The tuber of *G. superba* is used topically for purposes such as labor induction and easing childbirth (Swarnkar and Katewa, 2008), relieving pain during parturition (Mohan *et al*., 2008; Augustine *et al*., 2010) and expelling retained placenta post-parturition (Prakash *et al*., 2008). It is used orally as an abortive agent during the early stages of pregnancy by various local and tribal populations of Satpura hills, Maharashtra (Mali *et al*., 2006; Kosalge and Fursule, 2009), Adilabad district, Andhra Pradesh (Madhu and Suvartha, 2009), Vellore district, Tamil Nadu (Thirumalai *et al*., 2009), Western Ghats, Kerala (Pushpangadan and Atal, 1984), southern Rajasthan (Jain *et al*., 2004) and Chhattisgarh (Husain *et al*., 2017). The locals of Dindigul district, Tamil Nadu use tubers as a uterine stimulant (Balayogan *et al*., 2014). Aboriginal communities in the African nations of Botswana, Zimbabwe, Namibia, Zambia, Rwanda, South Africa and Nigeria also use the tuber as a traditional abortifacient (Toyin *et al*., 2014). However, there are few scientific studies to demonstrate the safety and percent efficacy of this herb during various stages of pregnancy. The present study aimed to determine the effect of oral administration of *G. superba* tuber extract on implantation and post-implantation pregnancy survival in Wistar rats.

2. Materials and methods

2.1. Experimental plant

Fresh tubers of *G. superba* were procured from Sri Murugan Impex, Tiruppur, Tamil Nadu and authenticated by the experts at the Department of Botany, Karnatak Science College, Dharwad, Karnataka. A voucher specimen (accession no. KUD/Zoo/2020-21/3) was collected from Banavasi, Uttara Kannada, Karnataka (14.5341°N, 75.0177°E) and deposited in the Karnatak University Herbarium, Dharwad, Karnataka. No rules concerning biodiversity rights were violated in procuring the plant material for the present study.

2.2. Preparation of plant extract

The tubers were checked for microbial infestation, washed under tap water, sliced, fully shade-dried and pulverized using an electronic grinder. The powder was sieved, weighed and stored in an airtight container until extraction. 40 g of tuber powder was extracted with 300 ml of ethanol in the Soxhlet apparatus. The obtained extract was filtered using Whatman Grade 1 filter paper, evaporated in a rotary evaporator and dried overnight in a laboratory oven at 30°C. Dried extract (GSTE) was stored at 4°C until further use. The yield was 5.6%.

2.3. Preliminary phytochemical analysis

GSTE was subjected to preliminary phytochemical tests for the identification of major phytoconstituent groups (e.g., alkaloids, phenols, flavonoids, tannins, cardiac glycosides, and phytosteroids) according to the standard protocols previously described (Edeoga *et al*., 2005; Kodangala *et al*., 2010; Mir *et al*., 2013; Rufai *et al*., 2016; Gul *et al*., 2017).

2.4. Experimental animals

Sexually mature healthy Wistar rats (females weighing 200-250 g, males weighing 250–300 g) were obtained from the animal facility of the Department of Zoology, Karnatak University, Dharwad, Karnataka, and housed in polypropylene cages containing paddy husk. The animals were kept under natural light/dark cycle and temperature of 29 ± 2°C and fed with commercial food pellets (VRK Nutritional Solutions, Sangli, India) and tap water *ad libitum*. The research was conducted following the internationally accepted principles for laboratory animal use and care per the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi guidelines.

2.5. Experimental design

Females in the proestrus stage of the reproductive cycle were co-housed overnight with sexually mature males (2F:1M) and mating was confirmed the next morning by the presence of spermatozoa in the vaginal lavage. This day was designated as day one of gestation (GD 1) (Srikanth *et al*., 2013). Mated females were isolated, weighed and randomly divided into two groups; one group (A) was used for the determination of anti-implantation activity and the other (B) was used for the abortifacient study. Groups A and B were subdivided into three groups of six animals each (n = 6). The doses were selected based on the results of a 14 days repeated dose oral toxicity study performed following the procedure of Yun *et al*. (2018). No observed adverse effect level (NOAEL) of GSTE in Wistar rats was 80 mg/kg body weight, and 1/4 and 1/2 of this concentration were selected for our study. Females of groups A1 and A2 received 20 mg/kg and 40 mg/kg body weight GSTE in 1% Tween-80, respectively from GD 1 to GD 7 (Salhab *et al*., 1999). Group A3 served as the control and received only vehicle (10 ml/kg) from GD 1 to GD 7. Groups B1 and B2 received 20 mg/kg and 40 mg/kg GSTE, respectively from GD 9 to GD 15 (Choudhary *et al*., 2017). Group B3 served as the control and received only vehicle from GD 9 to GD 15. Doses were administered once daily by gavage. Females of groups A1-A3 were weighed on GD 8 and euthanized by sodium pentobarbital (Sigma-Aldrich, US) overdose. Females of groups B1-B3 were allowed to complete the term and euthanized on GD 21. The experimental procedures were approved by the Institutional Animal Ethics Committee of Karnatak University (No. 639/GO/Re/S/02/CPCSEA).

2.6. Calculation of implantation and pregnancy related indices

Ovaries and uteri were excised from all the experimental females post-euthanasia. Ovaries were examined for the number of corpora lutea present, and uteri were examined for the implantation sites/number of foetuses. The number of corpora lutea in both the ovaries of a pregnant female rat is a measure of the actual number of ova released during the ovulatory phase. Any difference between the number of corpora lutea and the number of implantation sites suggests a loss of implantation. The uteri that appeared non-gravid were stained with approximately 5 ml of 10% (v/v) ammonium sulfide for one hour to detect early resorption sites (Narotsky *et al*., 1997). Implantation-related indices were calculated for groups A1-A3 as follows (Lilaram and Ahmed, 2013):

Implantation index = number of implantation sites/ number of corpora lutea × 100

Pre-implantation loss = (number of corpora lutea in both the ovaries – number of implantation sites)/ number of corpora lutea × 100

Vaginal bleeding, if any, was recorded for the females of groups B1-B3. Litter size, the number of live/dead fetuses, maternal weight and fetal weight were recorded. Fetuses were observed for gross morphological abnormalities. The following were calculated (Nishimura *et al*., 2012; Lilaram and Raichur, 2014):

Resorption index = total number of resorption sites/total number of implantation sites × 100

Post-implantations loss = (number of implantations – number of live fetuses)/number of implantations × 100

Total pregnancy loss per dam = (No. of corpora lutea – No. of live embryos)/No. of corpora lutea × 100

Survival ratio = number of live fetuses/total litter size × 100

2.7. Chemiluminescence immunoassay for serum levels of female sex hormones

About 5 ml of blood was collected from all experimental animals immediately after euthanasia by puncturing the right cardiac ventricle. Samples were allowed to clot for about 30 minutes at room temperature and centrifuged at 3000 rpm for three minutes. Serum progesterone and estradiol (E2) levels were estimated using chemiluminescence immunoassay (CLIA) kits (Diagnostic Automation/Cortez Diagnostics Inc., CA, US) based on the competitive immunoassay technique involving progesterone-HRP conjugate or E2-HRP conjugate and the respective hormone antibodies. The assays were performed according to the manufacturer's instructions.

2.8. Histology of the ovary

Ovaries were excised from each animal, separated from adherent tissue, and fixed in 10% neutral buffered formalin for 48 hours. The organs were processed for

histological preparation according to the procedures described by Kiernan (2000). In brief, 5 µm thick serial paraffin sections were sectioned on a fully automated rotary microtome (RM2255, Leica Biosystems, Germany) and stained with hematoxylin and eosin. Stained tissue sections were photographed using a Jenoptik camera and imaging software (ProgRes® C3 CapturePro 2.10.0.1) attached to an Olympus BX51 microscope.

2.9. Statistical analysis

Arithmetic means were calculated for individual data groups and the results were expressed as mean ± standard deviation (SD). Results were subjected to a one-way analysis of variance (ANOVA) using SPSS statistics version 21.0 (IBM Corporation, New York, US). *p* value less than 0.05 ($p < 0.05$) was considered to be statistically significant for all comparisons.

3. Results and discussion

3.1. Phytochemical analysis

Preliminary qualitative phytochemical analysis of the extract indicated the presence of alkaloids, phenols, phytosteroids, flavonoids and saponins as the major constituents of GSTE. Moderate presence of flavonoids, glycosides, carbohydrates and proteins was detected as well while tannins were present in trace amount.

3.2. Anti-implantation activity

The mean numbers of implantation sites on GD 8 in the uteri of GSTE-treated females of both groups were not significantly different from that in the control females. Similarly, the implantation index and the pre-implantation loss, which is a measure of the number of successful implantations against the number of eggs actually released from the ovaries, were not significantly different between the control and GSTE-treated groups. These results have been illustrated in Table 1 and Plate 1 a & b.

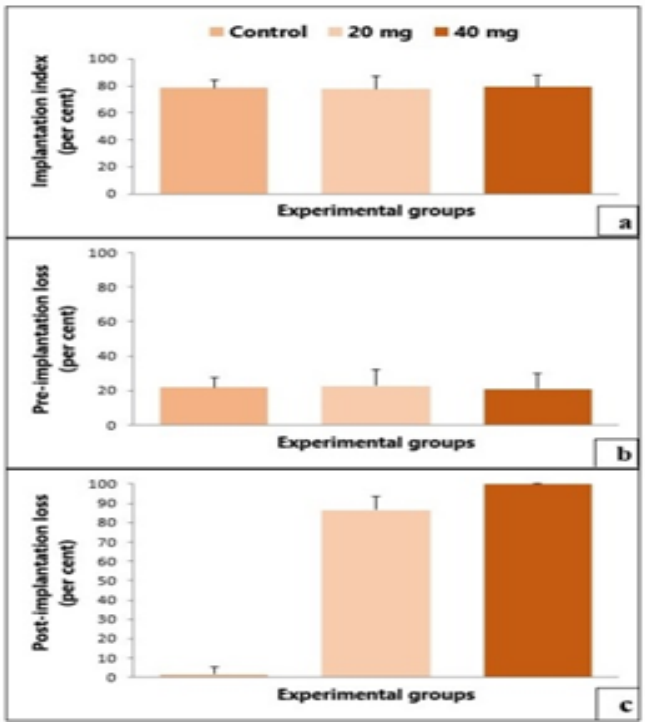

Plate 1. a. Implantation index in control and GSTE-treated female rats on gestation day (GD) 8; b. Pre-implantation loss (%) in control and GSTE-treated female rats on GD 8; c. Post-implantation loss (%) in control and GSTE-treated female rats on GD 21.

3.3. Abortifacient activity

GSTE exhibited a dose-dependent adverse effect on *in utero* embryo survival. On GD 21, the mean number of fully developed and live fetuses in group B1 was significantly lower than that in the control group. Though the mean body weight of surviving fetuses of group B1 was lower than those of the control group, no morphological abnormalities were recorded. Group B2 females exhibited 100% reproductive failure and no fully developed fetuses were present in this group on GD 21. No vaginal discharge/ bleeding was observed in any group during the treatment. Mean litter size, number of live pups, resorption index and pregnancy loss data have been summarized in Table 2. Mean fetal

Table 1. Implantation-related indices in control and GSTE-treated female rats on gestation day 8

Sl. No.	Implantation-related indices	Control	A1	A2
1	Number of corpora lutea	14.50 ± 1.52*	11.67 ± 1.63*	13.00 ± 0.89*
2	No. of implantation sites	11.33 ± 1.37*	9.00 ± 1.41*	10.33 ± 1.63*
3	Implantation index (%)	78.21 ± 5.81*	77.45 ± 9.47*	79.20 ± 8.88*
4	Pre-implantation loss (%)	21.79 ± 5.81*	22.55 ± 9.47*	20.80 ± 8.88*

Values have been expressed as mean ± SD (n=6). Groups marked with the same number of asterisks in a row are not significantly different from each other; (p ≥ 0.05).

Table 2. Pregnancy-related indices in control and GSTE-treated female rats on gestation day 21

Sl. No.	Pregnancy-related indices	Control	B1	B2
1	No. of implantation sites	11.83 ± 1.17*	10.83 ± 1.47*	11.33 ± 1.21*
2	No. of resorption sites	0*	9.00 ± 1.41**	11.33 ± 1.21**
3	Resorption index (%)	0.00*	83.03 ± 6.30**	100**
4	Mean Litter size	11.83 ± 1.17*	1.83 ± 0.75**	0.00**
5	Mean live fetuses per dam	11.67 ± 1.37*	1.50 ± 0.84**	-**
6	Total pregnancy loss per dam (%)	19.51 ± 4.87*	87.58 ± 6.69**	100**
7	Litter survival ratio (%)	98.48 ± 3.71*	77.78 ± 4.37**	0.00**

*Values have been expressed as mean ± SD (n=6). Groups marked with the same number of asterisks in a row are not significantly different from each other; *groups marked with different numbers of asterisks indicate significant difference ($p < 0.05$)*

Table 3. Fetal and maternal body weights in control and GSTE-treated female rats on gestation day 21

Sl. No.	Fetal and maternal body weight indices	Control	B1	B2
1	Maternal body weight (g)	345.83 ± 10.21*	266.67 ± 17.79**	218.33 ± 10.33**
2	Average weight of unborn pups (g)	3.59 ± 0.17*	3.19 ± 0.18*	-
3	Mean placental weight	0.621 ± 0.03*	0.803 ± 0.04**	-

Values have been expressed as mean ± SD (n=6). Groups marked with the same number of asterisks in a row are not significantly different from each other; groups marked with different numbers of asterisks indicate significant difference ($p < 0.05$)

loss data have been summarized in Table 2. Mean fetal and maternal body weights in control and treated groups on GD 21 have been shown in Table 3.

3.4. Hormone assay

Compared to the control group, circulatory levels of female sex hormones were significantly altered in GSTE-treated pregnant rats. Serum progesterone in females of B1 and B2 groups was significantly lower than that in control females on GD 21 (Plate 2a). The decrease was 35.03% and 85.58% in groups B1 and B2, respectively against control. In contrast, serum estradiol levels increased significantly in GSTE-treated females (Plate 2b).

3.5. Histology of the ovary

Histological preparation of the ovaries showed signs of gradual luteal regression in GSTE-treated pregnant rats. On GD 21, the mean diameter of corpora lutea in control females was significantly greater than that in experimental females that received GSTE from GD 9 to 15. B2 females exhibited a greater extent of luteal regression than B1 females (Plate 3).

3.6. Discussion

Antifertility effects of plants on female reproductive health can be broadly classified as estrous cycle disruption and suppression of ovulation, fertilization inhibition, anti-implantation, and abortion. Implantation in rats generally begins on gestation day 5 and is usually accomplished by GD 7. Most of the blastocysts are held in position within the uterine

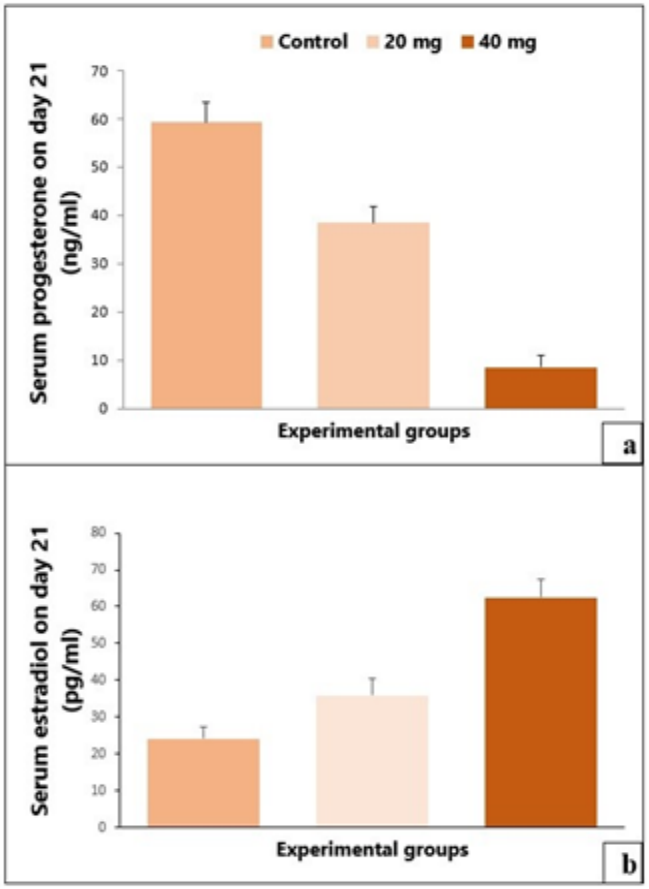

Plate 2. a. Serum progesterone levels in control and GSTE-treated female rats on gestation day 21; b. serum estradiol levels in control and GSTE-treated female rats on gestation day 21

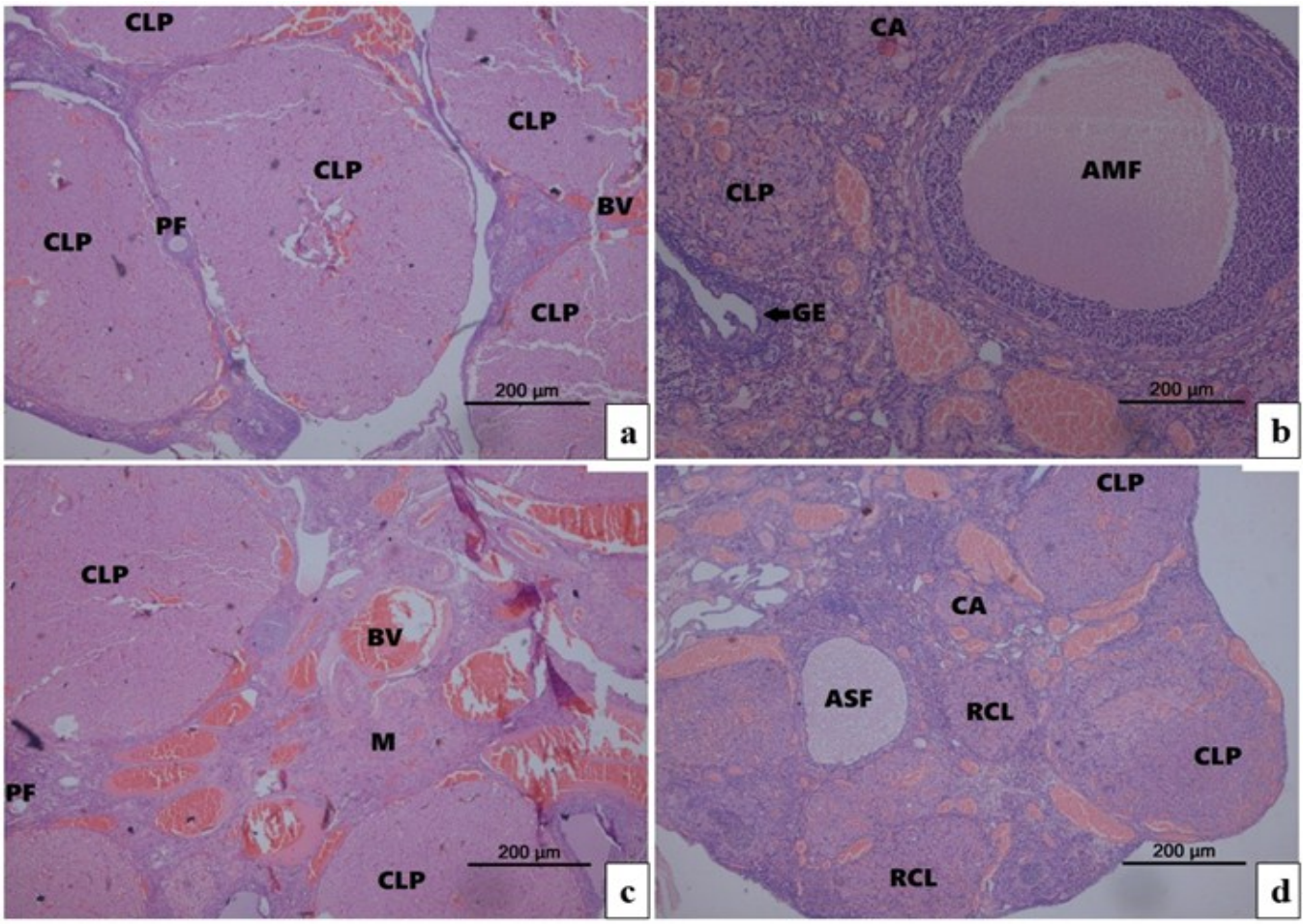

Plate 3. Cross-sections of the ovary showing the corpora lutea of pregnancy and luteal regression; Gradual luteal regression is seen in tissues from GSTE-treated experimental females: a-b. Control tissue; c. 20 mg/kg GSTE-treated; d. 40 mg/kg GSTE-treated

**CLP–corpus luteum of pregnancy, PF–primary follicle, BV–blood vessel, CA–corpus albicans, C–cortex, AMF–antrum of the mature follicle, GE–germinal epithelium, M–medulla, ASF–antrum of the secondary follicle, and RCL–regressing corpus luteum. Hematoxylin and Eosin. Magnification: 40×; Scale: 200 µm*

lumen by the end of GD 5, and an implantation chamber is formed on GD 6 (Enders and Schlafke, 1967). Any substance claimed as an anti-implantation agent must exert its action well within the implantation window by inhibiting the endometrial receptivity, destroying the fertilized ovum/free-lying blastocyst, altering zygote transport in the oviduct, or acting on the trophoblasts. The results of our study did not suggest any significant anti-implantation activity of GSTE in rats, the pre-implantation loss and the implantation indices being comparable between the control and treated groups. The negligible effect of GSTE administration on pre-implantation loss may be a reflection of its minimum interference with endometrial receptivity and implantation process during the early term which is mediated by cyclic changes in the levels of estrogen and progesterone. Some workers have used the term 'interceptive' to refer to such compounds that act after fertilization but before implantation (Maurya *et al*., 2004). Earlier or later administration of some of these agents may also render them contraceptives (anti-ovulatory or anti-fertilization) or abortifacients (Riddle, 1991). The compounds interfering with the secretion of proper amounts of sex steroids needed for successful implantation can also be referred to as interceptive.

In contrast to our findings, a few authors have previously reported that *G. superba* tubers possess significant anti-implantation activity in rats. Latha *et al*., (2013) orally administered hydroalcoholic tuber extract from GD 1 to 7 to post-coital female rats at two concentrations- 30 and 60 mg/kg. On GD 10, a statistically significant dose-dependent reduction in the number of implantations was observed in dosed

females when compared to the controls. The reduction was 69.90% and 80.95%, respectively against control. No teratogenic effect was observed. Experiments in which the test substance are administered to rats during the first week of pregnancy and which report only the number of implantation sites is not sufficient to determine the underlying interceptive mechanism, and detailed studies must be carried out to attest to the findings (Farnsworth *et al*., 1975). Indeed, the possibility of an early abortifacient action, occurring soon after implantation cannot be denied, which may lead to an error in interpreting and reporting the results. In another similar study, Malpani *et al*. (2011) reported that oral administration of aqueous tuber extract of *G. superba* at three different doses showed both abortifacient and anti-implantation activities in female rats, attributed to the oxytocic potential of the extract. It is important to note that the petroleum ether, alcohol, and aqueous extracts of *G. superba* tubers have been reported to be inactive as anti-implantation agents when given orally to pregnant rats from days 1 to 7 post-coitum (Saxena *et al*., 1970; Garg *et al*., 1978; Kamboj and Dhawan, 1982).

In our study, GSTE exhibited a dose-dependent adverse effect on post-implantation embryo survival *in utero*. The mean number of live fetuses in group B1 was significantly lower than that in the control group on GD 21 and group B2 females exhibited 100% pregnancy loss. No teratological malformations were observed in the surviving embryos of group B1. Shaikh *et al*., (2015) have reported a similar post-implantation anti-fertility action of the *kadamba* plant (*Anthocephalus cadamba* (Roxb.) Miq.) on mice. Oral administration of *kadamba* extract did not inhibit implantation, with implantation indices and pre-implantation loss in extract-treated mice being comparable to those in the control group. This suggested the inactivity of the extract as an interceptive. However, the extract caused significant post-implantation pregnancy loss and resulted in 100% abortion at a 1500 mg/kg dose.

Misoprostol and mifepristone are among the most widely used medications for medical termination of pregnancy during early gestational stages, both known to obstruct progesterone and initiate pregnancy loss by blocking the essential actions of progesterone (Christin -Maitre *et al*., 2000). The abortifacient effectiveness of these medications has been previously reported. 300 µg/kg misoprostol causes 75% pregnancy loss in Sprague-Dawley rats (El-Ishaq *et al*., 2019), while 5.86 mg/kg mifepristone causes 100% pregnancy loss in pregnant mice and Wistar rats (Srikanth *et al*., 2013). Our study suggests a 100% abortifacient activity of GSTE at a dose of 40 mg/kg which is comparable to that of mifepristone; this indicates the prospect of utilizing GSTE as a herbal source for developing a commercial medical abortifacient.

In the present study, the circulatory levels of female sex hormones were significantly altered in treated rats when compared to the control. Serum progesterone decreased, whereas serum estradiol increased in the GSTE-treated groups in a dose-dependent fashion. Ovarian histology showed signs of gradual regression of corpus luteum (luteolysis) in GSTE-treated pregnant rats in a dose-dependent manner, and the mean diameter of corpora lutea in treated females was significantly reduced on GD 21. The corpus luteum is a collapsed cell mass mainly composed of granulosa cells of an ovarian follicle left behind after ovulation. If the oocyte is fertilized and the embryo is successfully implanted, the corpus luteum grows in size and is termed the corpus luteum of pregnancy (CLP) (Eroschenko, 2008). It secretes high levels of progesterone to maintain gestation. Human chorionic gonadotropin (hCG) secreted by embryonic trophoblasts continues to stimulate the CLP and prevent its regression. The influence of hCG resembles that of LH released from the hypophysis. As a result, the CLP persists for several weeks. In the absence of pregnancy, the corpus luteum regresses and eventually becomes non-functional scar tissue, the corpus albicans (Eroschenko, 2008). Functional luteolysis involves an initial reduction in progesterone secretion, whereas structural luteolysis leads to a change in the cytology of the corpus luteum and its subsequent involution into the ovary (McCracken *et al*., 1999).

Progesterone, which is secreted by the corpora lutea, is a key hormone essential for successful embryo implantation through its preparatory effects on the endometrium, and for the maintenance of pregnancy by decreasing the contractile activity of the myometrium (Noyola-Martínez *et al*., 2019). Levels lower than the threshold are bound to result in the physiological loss of these functions and thus, deficiency of progesterone has long been associated with infertility (Young, 2013). Estradiol is the major mammalian estrogen mainly secreted by the developing ovarian follicles and is essential for oocyte maturation within the follicle. It regulates the estrous cycle and also helps progesterone in the preparation of the uterus for implantation, but its role in pregnancy is not well understood. Some studies have suggested that estradiol too may have a role to play in pregnancy survival (Young, 2013). In a study by Hiremath *et al*. (1999), petroleum ether and ethanol

extracts of *Acalypha indica* L., exhibited potent estrogenic activity in pregnant rats. GSTE-induced luteolysis and a subsequent fall in the levels of progesterone seem an appropriate justification and the main cause of its observed post-implantation abortifacient activity.

4. Conclusion

G. superba is widely used to induce abortions by various tribal communities across India. Although the influence of *G. superba* tubers on female fertility has been studied in previous pieces of research, most of these studies, to our knowledge, have reported that the plant exhibits anti-implantation activity. We investigated the exact nature of the anti-fertility activity of the plant through its effects on implantation as well as post-implantation pregnancy survival and found that GSTE had strong abortifacient potential but no significant anti-implantation activity. Two concentrations (20 and 40 mg/kg) of the extract were tested, and both significantly lowered the chances of post-implantation pregnancy survival when given orally to pregnant rats. Hence, the plant has been rightly referred to as *garbhaghatini* in ayurvedic literature which translates to 'destroyer of the fetus' and indicates the post-implantation nature of its action on female reproductive function.

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