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Research Article

REVERSIBLE GERM CELL TOXICITY OF ETHANOLIC EXTRACT OF GLORIOSA SUPERBA IN MALE RATS

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ABSTRACT

The present study was designed to evaluate the antifertility potential of ethanolic extract of *Gloriosa superba* in male rats. The parameters related to fertility of males such as sperm count, sperm motility, percentage fertility and weight of sexual organs were observed. Group I received vehicle only p.o. daily and served as control. Groups II and III received ethanolic extract of *Gloriosa superba* (GS) at 100 and 200 mg/kg, respectively for a treatment period of 45 days. Group IV also received the ethanolic extract of GS at 200mg/kg for the full treatment period of 45 days but for evaluating the reversible effect of drug extract a washout period of 30 days was given in group IV. It was found that GS administration in male rats produced germ cell toxicity but the effect was reversible as evident from the recovery group.

INTRODUCTION

Rising human population throughout the world particularly in developing and underdeveloped countries has detrimental effects on the life supporting systems on earth. Fertility regulation comprising contraception and management of infertility forms an important component of the reproductive health. Though considerable progress has been made in the development of highly effective, acceptable and reversible methods of contraception among females, progress and possibilities on males are still slow and limited (Gupta *et al*., 2006), but over the past few years there has been considerable interest in the development of contraceptive agents for both sex (Hilton *et al*., 1983). According to a survey of WHO, 61 % of the males who were using contraceptives were motivated by the problems of the female partners, including 35%, who had experienced a contraceptive failure, or by the desire to share responsibility (Geofffrer, 2003). Now a days, various scientists round the world are continuously trying to develop a reversible, effective and patient compliant male contraception. The development of an agent which is spermicidal is an effective method of contraception in males but the effect must be reversible (Agrawal *et al*., 2009).

In men, the major causes of infertility are asthenospermia, oligospermia, azoospermia and teratozoospermia, which report for 20–25% of cases (Marmor *et al*., 1995).

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There are numerous risk factors for example Sexully Transmitted Diseases involving *C. trachomatis and N. gonorrhoeae*. These cause changes in semen quality and leads to a block of the seminal vesicles (Drife, 1982). In the male reproductive tract, the major target site for fertility regulation is testis, where the production and maturation of sperms occur (Shivabasavaiah *et al*., 2011). The researches related to male contraceptive is still very limited and mainly involves the various types of barrier methods. So we work ahead in the fields of male contraceptives of herbal origin.

A number of plants are available with male antifertility potential (Priya *et al*., 2012). *Gloriosa superba* is a medicinal plant belonging to the family *Liliaceae*. It is a semi-woody herbaceous branched climber reaching approximately 5 meters height, with brilliant wavy-edged yellow and red flowers (Rajak and Rai, 1990). In the traditional systems of medicine, the tubers are used as tonic, antiperiodic, antihelmenthic, and also against snake bites (Gupta *et al*., 2005). It is also used in the treatment of fever, wounds, skin related problems, inflammation, piles, blood disorders, uterine contractions, general body toner and poisoning (Haroon *et al*., 2008). Till date, no specific literature is available on the possible male contraceptive properties of *Gloriosa Superba* bark.

MATERIALS AND METHODS

Collection and authentication of crude drug

Bark of *Gloriosa Superba* were collected from local market of Bareilly, U.P. India in the month of September, 2016 and were identified taxonomically in Department of Botany, M.J.P.

Rohilkhand University, Bareilly, (U.P), India. The Bark have been cleaned in fresh running water and shade dried.

Preparation of ethanolic extract

The bark was shade dried and pulverized in an electric grinder; 1.0 kg of the powdered drug was extracted with 3.0 L of ethanol for 48 h with occasional shaking. The filtrate (500 ml) was concentrated under reduced pressure at 40°C to yield 50 g (5.0% w/w) of a brown soluble residue. The residue was further dried in an oven at 37°C to eliminate traces of ethanol solvent and stored in a sealed dark airtight plastic container at 4–8°C, until use. The crude residue suspended in (0.5% CMC w/v) served as the dosage form for experimentation.

Chemicals

Ethylenediamine-tetraacetic acid (EDTA) and Hank's balanced salt solution (HBSS) was obtained from HiMedia Laboratories Ltd, Mumbai.

Acute toxicity study

Acute toxicity study of ethanolic extract of *Gloriosa superba* were carried out in rats according to OECD guidelines. Extract at different doses up to 2000 mg/kg, p.o.were 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 safe. Based on acute toxicity result we have selected two doses 100 mg/kg and 200 mg/kg respectively for the evaluation of antifertility effect.

Experimental design

The animals used in this method were male rats divided into 4 groups (n=6), fasted overnight and allowed free access to water *ad libitum*. Group I received vehicle only (p.o. daily) and served as control. Groups II and III received ethanolic extract of *Gloriosa superba* (GS) at 100 and 200 mg/kg, respectively for a treatment period of 45 days. Group IV also received the ethanolic extract of GS at 200mg/kg for the full treatment period of 45 days but for evaluating the reversible effect of drug extract a washout period of 30 days was given. Spermatogenesis is a highly organized process and in rodents, sperms are produced from the progenitor spermatogonia after a series of meiotic and mitotic divisions, which takes approximately 42–56 days. This explains the basis of taking the sampling after 45 days of exposure.

Autopsy and organ weights

At the end of the treatment period, each rat was sacrificed by cervical dislocation. The testes, seminal vesicles and epididymis were dissected, freed from adherent tissue and weighed accurately up to milligram level.

Fertility test

Successful mating (male female ratio 1:2) was carried out with all the animals, five days prior to sacrifice period. The successful mating was confirmed in the forthcoming mornings by vaginal plug and spermatozoa in the vaginal smear. The inseminated females were separated and after gestation period the number of females delivered, number of litter born and fertility percentage were recorded (Shivabasavaiah *et al*., 2011).

Sperm motility and Count

After sacrificing the animal, a 1 mm incision was made in the caudal epididymis and drops of sperm fluid were squeezed onto the microscope slide and 2 drops of normal saline were added to mobilize the sperm cells. Epididymal sperm motility was then assessed by calculating motile spermatozoa per unit area and was expressed in percentage. Epididymal sperm counts were also done by homogenizing the epididymis in HBSS. Counting was then done using the counting chamber in the haemocytometer (Padmanabhan *et al*., 2008).

Histopathological studies

After sacrificing the rats, the testes were fixed in 10% formalin, dehydrated in increasing concentrations of ethanol and then embedded in paraffin. Tissue sections (5 µm) were mounted on glass slides which was already coated with Mayer's albumin and dried overnight. The sections were then de-paraffinized with xylene, rehydrated with alcohol and water. The rehydrated sections were stained, mounted with DP_X mounting media and examined under the microscope (Adeeko and Dada, 1998).

Statistical analysis

Results were shown as mean ± standard error of mean (S.E.M.) for each group. Statistical analysis was performed using Jandel Sigma Stat (Version 2.03) statistical software. Significance of difference between two groups was evaluated using Student's t-test. For multiple comparisons, One-way analysis of variance (ANOVA) was used. In case ANOVA showed significant differences, post hoc analysis was performed with Tukey's test.

RESULTS

Autopsy and organ weights- Ethanolic extract of *Gloriosa superba* at 100 mg/kg and 200 mg/kg show a significant change ($p \leq 0.01$) in the weight of seminal vesicle after the treatment period of 45 days when compared with vehicle treated group. However, no significant changes were observed after 30 days of withdrawing the treatment.

Table 1 Effects of drug treatments after 45 days on body, testicular, epididymal and seminal vesicle weights in wistar rats.

Treatment Gorup	Body weight (gm)		Testis (gm)	Epididymis (gm)	Seminal vesicle (gm)
	Initial	Final			
Group I (Control)	251±18.19	253±12.72	1.98±0.11	0.43±0.03	0.38±0.19
GroupII *Gloriosa superba* (100 mg/kg)	242±14.12	246±17.12	1.91±0.08	0.40±0.05	0.19±0.17*
GroupIII *Gloriosa superba* (200 mg/kg)	243±13.21	250±13.87	1.89±0.13	0.38±0.04	0.21±0.09*
Group IV (Recovery)	244±11.11	248±12.12	1.93±0.07	0.44±0.08	0.36±0.17

values are expressed as Mean ± SEM (n=6) * $p \leq 0.01$ when compared with control group

Fertility Test **-** There was a decrease in ratio between delivered and inseminated females in all the groups except for the recovery group when compared with vehicle treated group. However, all the delivered pups were normal and healthy.

Table 2 Effects of drug treatments on no. of females delivered/no. of inseminated females, total no. of pups, litter weight and mean percentage fertility (Male:female ratio, 1:2)

Treatment groups	No. of females delivered/no. of inseminated females	Total no. of pups	Litter weight (gm)	Mean percentage fertility (%)
Group I (Control)	12/12	76	9.4±0.93	100.0
Group II *Gloriosa superba* (100 mg/kg)	05/12	32	9.3±0.84	41.60
Group III *Gloriosa superba* (200 mg/kg)	06/12	37	8.7±0.71	50.00
Group IV (Recovery)	11/12	69	9.0±0.43	91.6

values are expressed as Mean ± SEM (n=6)

Effect on Sperm motility and sperm count- Decrease in sperm count was seen in all the groups except for the recovery group. The Ethanolic extract of *Gloriosa superba* at 100 mg/kg and 200 mg/kg show significant decrease in sperm count when compared with vehicle treated group but there is no effect on sperm motility.

Table 3 Effect of drug treatments after 45 days on sperm motility and sperm count in wistar rats.

Treatment Group	Sperm motility (%)	Sperm counts ($\times 10^6$/mL)
Group I (Control)	82.68±2.34	61.25±7.45
Group II *Gloriosa superba* (100 mg/kg)	83.62±1.31	42.17±5.62**
Group III *Gloriosa superba* (200 mg/kg)	84.98±3.21	49.88±4.61**
Group IV (Recovery)	80.22±2.12	61.88±6.53

values are expressed as Mean ± SEM (n=6) * p≤0.05, ** p≤0.01, ***p≤0.001 when compared with control group.

Histopathology of Testis

The histology of *Gloriosa Superba* treated group at dose of 100mg/kg and 200 mg/kg showed maturation distress of spermatozoa as compared to control group in which all the stages of spermatogenesis can be seen. The effect so produced by the drug treatment in Group III was scattered and the seminiferous tubules showed lack of spermatozoa along with the necrosis of the germ cells. But the histological slides of the recovery group showed all the stage of spermatogenesis.

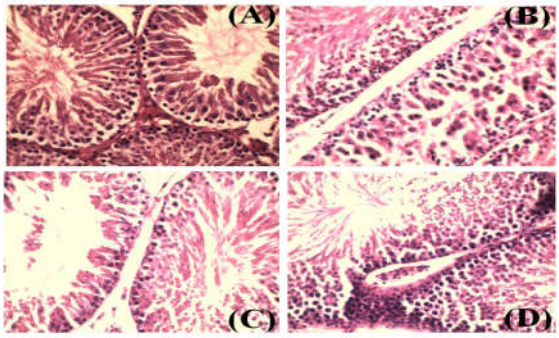

Figure 1 Photomicrographs of stained histological slides of the testis after 45 days of treatment; a) Group I (Control), b) Group II (Gloriosa Superba 100mg/kg), c) Group III (Gloriosa Superba 200mg/kg), d) Recovery

RESULT AND DISCUSSION

The results of this investigation reveals that the administration of GS interferes with the structure and function of major elements of male fertility as reflected from the marked reduction in percentage fertility, sperm count and decrease in weight of major sex organs but a significant decrease in motility of sperm was not observed. Thus, our results clearly demonstrate that GS administration in male rats produced germ cell toxicity in rats as evident from the decrease in sperm count, and decline in percentage fertility but the effect was reversible as evident from the recovery group. Furthermore, this was confirmed by histopathological analysis but the cause of this infertility at genetic level is not evaluated in this research paper. So, further researches are suggested to evaluate the exact chemical moiety which is responsible for this effect.

Conflict of interest

There is no conflict of interest.

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