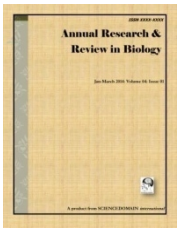

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Two-generation Reproductive Toxicity Study of Barrisal (Herbal Drug) on the Sprague Dawley Rats

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Authors' contributions

This work was carried out in collaboration between both authors. Author MA designed the study and wrote the protocol. Both authors MA and AA wrote the manuscript, analyze the data, performed the statistical analysis together. Both authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aim: Barrisal is a herbal medication used in Pakistan for hepatic and spleen ailments. It is composed of Aloevera. The present study was aimed to evaluate the teratogenic effects of barrisal in two generations of rats.
Study Design: Two generation teratogenic activity
Place and Duration of Study: Dr. Hafiz Muhammad Ilyas Institute of Pharmacology and Herbal Sciences (Dr. HMIIPHS) Hamdard University, Karachi, Pakistan, between June 2002 and July 2004.
Methodology: In first phase two groups of female rats (10 rats each group) were used. One group received normal saline (as control group).Group II were treated with a single oral dose of 10ml/kg barrisal (10 female rats) during the whole period of gestation till the delivery of pups named as F1 generation. For second phase study 10 females were selected from F1 generation control and barrisal treated group and administered the saline and barrisal (10ml/kg) from day first of gestation until they delivered pups (F2 generation).
Results: There were not any aborted or absorbed fetuses in normal saline group and barrisal treated group. Total number of collected pups after barrisal treatment in F1

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generation and F2 generation are comparable to control rats. Control and barrisal treated rats had no resorption sites, malformation and any congenital defects.
Conclusion: In conclusion, Barrisal (10ml/kg) during pregnancy not led to fetal growth retardation or fetal death at this dosage any abortion, fetal death, fetal growth retardation and teratogenic effect was not occurred and it is safe in rats.

Keywords: Barrisal; teratogenic activity; two-generation reproductive toxicity, F1 generation; F2 generation; Aleo vera.

1. INTRODUCTION

Barrisal syrup is a herbal preparation that is prepared by Hamdard Laboratories (Waqf) Pakistan. Barrisal is composed of only one medicinal plant *Aloe vera* syn. *Aloe barbadensis* Mill. (*A. barbadensis* Linn. family; Liliaceae). It is widely prescribed in Pakistani alternative medicine during hepatic and spleen disorders with raised levels of LFT (Liver function test). Author [1] has recently reported the hepatoprotective effect of barrisal on CCl_4 induced hepatic damage in rats. Furthermore, in alternative medicine *A. vera* has been recognized as a good medicine for the treatment of many disorder like asthma, gastrointestinal ulcer, constipation, wounds, herpes simplex virus, diabetes and inflammation [2]. The use of Barrisal in alternative medicine in Pakistan for the treatment of hepatic disorder is appealing because it is readily available and cheap. Hakims (Herbalists) have been practicing and describing barrisal for ages. Author [1] has already suggested that up to the dose of 10ml/kg barrisal treatment did not cause any mortality and growth arrest. Autopsy and biochemical analysis also showed that Barrisal treatment did not produce any side effect in liver kidney and heart [1]. Furthermore, the complications that might be associated with their use during pregnancy have not been investigated. Previously, several drugs and herbs were often used in pregnancy despite of their reported toxicities and side effect in this regard the case of thalidomide was a big disaster in the 1960's [3]. Author [4] also reported the teratogenicity of *Asparagus racemosus* (a herbal drug) and concluded that Asparagus racemosus caused higher rate of resorption in pups. *Apilia Africana* used in ethanomedical practice in Africa also showed neonatal liver distortion [5].

Now a day the drug prescription and administration of drug in pregnant female is done with care. The objective of this research is to observe the teratogenic effect of barrisal and safety of this drug during pregnancy.

2. MATERIALS AND METHODS

2.1 Barrisal

Barrisal (herbal syrup) used in the teratogenic activities was obtained from Hamdard Laboratories (Waqf), Karachi, Pakistan.

2.2 Animals

Normal Virgin female rats (*Sprague dawley*) of about 200-225 grams were selected from the Animal House Dr. Hafiz Muhammad Ilyas Institute of Pharmacology and Herbal Sciences (Dr. HMIIPHS). Animals fed with standard rat diet and water *ad libitum,* and were housed

and looked after according to the Animal House Committee regulations on “the Care of Experimental Animals”.

2.3 Grouping or Design of study

Reproductive toxicological study in two-generation reproductive were performed according to guideline approved by FDA reproduction studies [6,7].

The present study was conducted in two phases:

Phase I: 20 virgin female rats + 4 male rats were randomly selected from animal house and used for teratogenic activity
Group 1: 10 Control females were treated with saline from day first until they delivered pups
Group 2: 10 females were treated with Barrisal (10ml/kg) from day first until they delivered pups

Virgin females were kept into the cages into the group of 5. One male rat was placed in one cage with 5 female rats for mating. Saline was administered in Control group while the treated female received the barrisal 10ml/kg orally daily till the end of pregnancy/ delivery.

2.4 Confirmation of Pregnancy

5 female rats were kept placed in one cage with a virile male. Presence of spermatozoa in vagina confirmed the mating in rats. This presence of sperm (sperm positive date) would be considered as day zero. After that day females were constantly monitored and were weighed daily. On the 10^{th} day of gestation significant increase in weight gain was confirm the pregnancy.

Delivered pups were considered as control and barrisal treated F1 generation. The objective of this study was to count the quantity of delivered pups that was delivered by control (F1generation for control) and experimental groups (F1 generation for barrisal) for statistics. Female rats were sacrificed if weight did not gain significantly to check resorption sites. Pups with malformed structure were also noted. Normal Pups were individually marked when weaned. These F1 rats were nurtured for 8 weeks, after 8 weeks mated again to obtain F2 generation in phase two study [8] as shown in Fig. 1.

Phase II: 20 Female rats + 4 male rats from F1 generation (delivered pups when they are matured) were used for second phase teratogenic activity

Group 1: 10 Control females from F1 generation were selected randomly and were treated with saline from day first until they delivered pups
Group 2: 10 females were F1 generation were selected randomly and treated with Barrisal (10ml/kg) from day first until they delivered pups

All experimental procedures were same as done in phase 1 study.

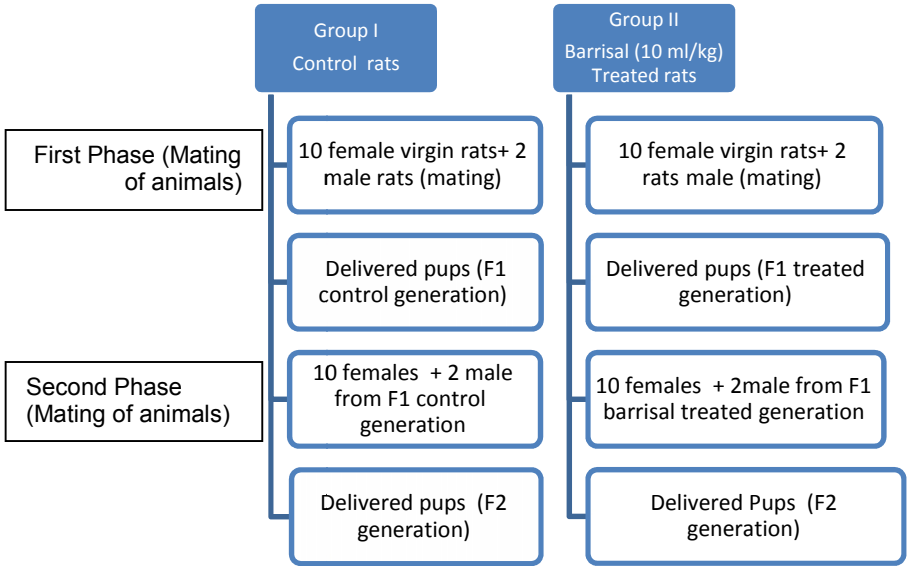

Fig. 1. Study design

3. RESULTS AND DISCUSSION

There were not any aborted or absorbed fetuses from normal saline group and barrisal treated group. Total number of collected pups from control and barrisal treated group (F1 generation) were 62 and 64 respectively. While in F2 generation the number of pups from control and barrisal treated groups were 63 and 77 respectively as presented in Table 1. These results indicate that the obtained generation (F1 and F2) from the barrisal treated groups did not has any congenital malformations, even control groups did not show any such malformations (Table 1). The result further showed that resorption sites were not observed in both the control and barrisal treated rats.

Table 1. Teratogenic activity of barrisal (herbal drug) in rats

	F1 generation		F2 generation	
	Control	**Barrisal treated (10ml/kg)**	**Control**	**Barrisal treated (10ml/kg)**
Dams	10	10	10	10
aborted or absorbed fetuses	0	0	0	0
Total number of pups	62	64	63	77
Males	26	32	23	39
Females	36	32	40	38
Gross congenital malformation	0	0	0	0

Barrisal is composed of plant *Aloe vera* syn. *Aloe barbadensis* Mill. The present study suggests that barrisal (10ml/kg) did not produce any harmful teratogenic effects in rats. Previously it is [9] reported that Aloe vera (*Aloe buettneri)* have property to increase ovarian and uterus weight and also raising serum and ovarian estradiol levels. In another study done [10], it was suggested that extract of *Aloe vera* might contain estrogen content and it might

be responsible to increase ovarian steroidogenesis and significantly increase production of estradiol and progesterone. In present study after barrisal treatment (10ml/kg) all female rats become pregnant might be due to an increase ovarian steroidogenesis because of presence of *Aloe vera*. There were not any aborted or absorbed fetuses as it is reported [11] that *Aloe vera* extracts are not fetotoxic and have no abortus effect.

4. CONCLUSION

Two-generation reproductive toxicity study concludes that Barrisal (10ml/kg) having medicinal plant *Aloe barbadensis* during pregnancy not led to fetal growth retardation or fetal death. At this dosage no abortion, fetal death, fetal growth retardation and teratogenic effects were occurred. In conclusion, our study revealed that Barrisal did not show any degree of teratogenic effects and it is safe in rats. It is recommended that further studies be carried out to examine these findings in other species.

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ETHICAL APPROVAL

Authors hereby declared that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the departmental ethics committee".

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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