ABSTRACT

Objective: Somina (herbal medicine) is used in Pakistan as Unani anxiolytics. It is composed of five medicinal plants. The current work was designed to evaluate the general reproductive and teratogenic effects of somina in two consecutive generations of rats according to the OECD guideline.

Methods: Fertility study (a two-phase study) was done in Sprague-Dawley rats. 1st part: three groups' female rats (10 rats each group) received different doses orally. First group: The control group (saline), a single oral human dose of somina (285 mg/kg/day) and the high dose of somina (3rd group: 1g/kg/day) during the whole period of gestation till the delivery of pups named as F1 Breed. For the second part of study ten females were selected from each F1 breed (control, somina 285 mg/kg/d, somina 1g/kg/day) and administered the same treatment from day first of mating than the entire period of gestation until F1 breed delivered pups (F2 breed). For F1 and F2 breed the fertility index and litter size were determined. Some of the female rats (F1 and F2) were anesthetized and autopsied. The blood sample was subjected to biochemical analysis and serum liver function test: bilirubin, gamma-glutamyl transferase (yGT), alanine aminotransferase (ALT: SGPT), aspartate aminotransferase (AST: SGOT) and alkaline phosphatase (ALP) were measured spectrophotometrically. The uterine growth index, fertility index, and litter size were also measured to evaluate the teratogenic effects of somina treated rats.

Results: The data showed that any significant different (P>0.05) was not found during the maternal examination (uterine growth index, fertility index) and reproductive parameters (litter size, the quantity of fetus, aborted or absorbed fetus) in somina treated rats as compare to control rats (P>0.05). Control and treated Pups did not show any significant (P>0.05) malformation and any congenital defects. Non-significant (P>0.05) pathological findings in the liver, kidneys, and uterus.

Conclusion: The oral administration of somina during the gestational period of pregnant female rats was not teratogenic/fetotoxic. Any adverse or deleterious effects were not observed at the dose of 285 mg/kg [human dose] or 1g/kg [3times greater than the human dose] during pregnancy, and it is safe in rats.

Keyword: Somina, Teratogenic activity, Two-generation reproductive toxicity, F1 breed, F2 breed, Uterine growth index, Fertility index

INTRODUCTION

Sagina is herbal medicine prepared by Hamdard Laboratories (waqf) in Pakistan. It is available in powder form and consists of five medicinal plants that are Sesamum indicum (14%), Lagernia vulgaris (12%), Prunus amygdalus (12%), Papaver somniferum (10%) and Lactuca scarioli (5%). It is used as curative unani anxiolytics in Pakistan [1, 2] also to strengthen all processes involved in memory and learned behaviour [3]. The role of somina in alternative medicine as curative unani anxiolytic is appealing because it is easily available and cheap. Natural herbs have vital medical importance [4, 5] and are being used in alternative medicine. Hakims (herbalists) have been practicing and describing these natural herbs or herb based formulation for ages. These herbal medicines may contain either one or mixture of different herbs or extracts for health benefits [6, 7].

However, these alternative herbal medicines have a risk of toxicity for human [8, 9]. There is some evidence of harm of traditional herbal medicines [10] either used singly or as poly-herbal. Regarding the safety of herbal medicine, information is contradictory. Therefore, In different countries, to make the use of herbal medicines safer and legislation for these products has undergone changes in recent years, and pharmaceutical companies are instructed to submit data on the efficacy and safety of herbal medicines [11]. Previously, according to Hodge and Sterner toxicity scale, the obtained value of the lethal dose (LD50) >10,000 mg/kg classified the somina as practically non-toxic herbal medicine [12]. However, the drawbacks that might be affiliated with somina during pregnancy have not been investigated. Reproductive performance and teratogenicity of some medicinal plants expose the fact that some herbs or plants did not have any side effect. That’s why, it is time to conduct safety studies and teratogenic study of somina in its standardized form and provides authenticated proved literature to guide the population about the use of somina, and clarify the side effects of somina, especially in pregnancy. The objective of this research was to observe the teratogenic effect of somina and safety of this drug during pregnancy in rats.

MATERIALS AND METHODS

Sagina

Sagina drug was received from Hamdard Laboratories (Waqf) Pakistan. Two doses of somina 285 mg/kg/d=human dose and 1g/kg/d=3 times greater than the human dose was calculated and prepared for administration.

Human dose of somina = two tablespoon twice a day = 20g/day

20g/day = 285 mg/kg/day

Chemicals and reagents

All biochemical tests were performed by using commercial assay kits. Kits used were: Mercobo test® for bilirubin, Ecoline® 25 for SGPT, Ecoline® S by Szasz method for yGT, Ecoline® 25 for ALP, Ecoline® 125 for ASAT (GOT).
**Animals**

Adult primiparae Sprague-Dawley rats (>3months: 150-200) were used. Animals fed with standard rodent diet and water *ad libitum*, and were housed and looked after according to the animal house committee regulations on "the care of experimental Animals." The study regarding general reproductive performance and teratology in rats was performed according to guidelines approved by food and drug administration (FDA) for reproduction studies [13, 14]. The Institutional ethic committee approved the study (Approval number: Reg.no.2005/04/S/HMIIPHS).

**Grouping or design of study**

The teratological study was performed according to established methods [15, 16] with slight modifications.

**Implantation and fertility study**

The study regarding general reproductive performance and teratology in rats was performed according to guidelines approved by FDA reproduction studies [13, 14].

The current work was conducted in two phases as described earlier [16]:

**Phase I:** 30 primiparous female rats +6 male rats were used for teratogenic activity

The rats were divided as follows,

- **Group 1:** 10 control females were treated with distilled water from day 0 until pups delivery
- **Group 2:** 10 control females were treated with *somina* (285 mg/kg/d=human dose) from day 0 until pups delivery
- **Group 3:** 10 control females were treated with *somina* (1g/kg/d=3 times greater than human dose) from day 0 until pups delivery

At day 0, male and female both received the first dose and then five primiparise females were kept in the cages with one male rat. Control females were kept overnight with control male and treated female rats were paired with treated male rats.

**Confirmation of pregnancy**

Mating was confirmed by the presence of sperm in the vagina the following morning (09H-10H) and this day was considered as day 1 of pregnancy. After that day, all female rats were regularly monitored, administered the dose and were weighed daily. On the 10th day of gestation, the significant increase in weight has confirmed the pregnancy. The doses were continued till the delivery [16].

Delivered pups were considered as control and *somina* treated F1 breed. After the completion of the work, the litter size was noted, and the fertility index calculated using the following formula [17]:

\[
\text{Fertility index} = \frac{\text{Number of Pregnant} \times 100}{\text{number of mated}}
\]

The goal of this study was to enumerate the quantity of delivered pups (F1 Breed for control: for *Somina 285 mg/kg* and *Somina 1g/kg*) for further statistics. Female rats were dissected if weight did not gain significantly to observe the resorption sites. For each delivered pups, a malformed structure was also noticed. Pups were individually marked when weaned. For eight weeks, the F1 breed was nurtured, and mortality was noted. After eight weeks received the first dose and mated again to obtain F2 breed in part two study [16].

**Phase II:** 30 Female rats +6 male rats from F1 generation (delivered pups when they matured) were used for second phase teratogenic activity

**Group 1:** 10 Control females from F1 breed were selected randomly and were treated with distilled from day 0 before mating until they delivered pups

**Group 2:** 10 females from F1 breed have been chosen randomly and treated with *Somina* (285 mg/kg/d) from day 0 before mating until they delivered pups

**Group 3:** 10 females from F1 breed were selected randomly and treated with *Somina* (1g/kg/d) from day 0 before mating until they delivered pups

All experimental procedure was same as done in phase 1 study. At the end of the study, the litter size was recorded, and the fertility index calculated using the following formula [17]:

\[
\text{Fertility index} = \frac{\text{Number of Pregnant} \times 100}{\text{number of mated}}
\]

After six weeks, six rats from each breed F1 and F2 were sacrificed, and their blood samples were was subjected to liver function test, and animals were autopsied [18].

**Liver function test**

After six weeks, six rats from each breed F1 and F2 were sacrificed, and the blood samples approximately (5 to 8 ml) were withdrawn from cardiac punctures from treated (F1 and F2) and control rats (F1 and F2). Blood samples were left at room temperature for 20 min, then incubated at 37 °C for 30 min and centrifuged separately in (BHG) Hermle Z230 (Germany) at the speed of 3,000 rpm for 20 min. Serum obtained (1 to 3 ml) was subjected for the study of the liver function test parameters: Bilirubin, gamma glutammyl transferase (γGT), alkaline phosphatase (ALP), aspartate aminotransferase (ASAT, SGOT) and alanine aminotransferase (ALT, SGPT). All tests were performed by using commercial assay kits. Kits used were: Mercko test® for bilirubin, Ecoline® 25 for SGPT, Ecoline® 5g/ml for γGT, Ecoline® 25 for ALP, Ecoline® 125 for SGOT (GOT). All these kits were purchased from diagnostic Merck (Germany), U-2000 spectrophotometer (Hitachi) was used to measure the absorbance of light.

**RESULTS**

**Effect of *somina* on implantation and fertility index**

Table 1 stated the results of the pre-coital administration of distilled water and two different doses of *somina* in rats (female). Treatment-induced pathological signs was not observed in any group. Compared to the control, *somina* (285 mg/kg or 1g/kg) did not show any significant implantation failure or inadequate uterine receptivity (P>0.05). All treated female rats become pregnant after successful mating. The fertility index was not affected and found to be 100% in all groups used in part I and F1 breeds used in part II (table 1). During full gestation periods, all females (control and *somina* treated) showed a significant increase in weight (P<0.05). The doses were continued till the delivery.

| Treatments | Implantation | Fertility |
|------------|--------------|-----------|
|            | Sperm positive female | Implanted females | Fertility Index |
| **Part I** |              |            |               |
| G1         | 10           | 10         | 100%          |
| G2         | 10           | 10         | 100%          |
| G3         | 10           | 10         | 100%          |
| **Part II (F1 breed)** | 10 | 10 | 100% |

Table 1: Effects of *somina* on implantation and fertility of adult female rats

Values are expressed as percentage. n = 10 female rats per group were randomized. G1-control, G2,*somina*285 mg/kg/d, G3-*somina*1g/kg/d
Reproductive parameters (embryo–fetal examination)

However, during the whole gestational period, any abortions were not noted in all groups. All cages were checked daily but did not find any aborted or absorbed fetuses from the control group and somina treated group. At the time of delivery, nonsignificant variations (P>0.05) were observed in quantity of fetus. A total number of collected pups from control and somina (285 mg/kg/d or 1g/kg/d) treated group (F1 generation) were 58, 56 and, 63 respectively. While in the F2 generation, the number of pups from control was 62. somina (285 mg/kg/d or 1g/kg/d) treated female delivered 66 and 72 pups respectively as presented in table 1.

Fetal malformations and variations

Delivered pups (F1 and F2 breeds) were monitored for any gross abnormality, and the result indicated that pups in all groups did not have any congenital malformations as showed in fig. 1 and table 2. External examination (fig. 1) did not reveal any abnormal findings concerning length, cranium, eyes, palate, limbs, tail, genitals, and sex.

Biochemical parameters

Pre-coital administration of distilled water and two different doses of somina in rats was continued till the delivery of pups. At the sixth week, randomly six rats from each delivered breed (F1 and F2 generation) were subjected to biochemical analysis (LFT). The results were presented in table 3. Liver function test (LFT) results showed that somina (285 mg/kg/d or 1g/kg/d) did not show any significant changes (P>0.05) in different liver marker enzymes (table 3). The levels were comparable to its control.

Table 2: Teratogenic activity of somina (herbal drug) in rats

|                  | F1 generation | F2 generation |
|------------------|---------------|---------------|
|                  | G1 | G2 | G3 | G1 | G2 | G3 |
| Aborted or absorbed fetuses | 0  | 0  | 0  | 0  | 0  | 0  |
| Total number of pups       | 58 | 56 | 63 | 62 | 66 | 72 |
| Gross congenital malformation | no | no | no | no | no | no |

Values are expressed as total. n = 10 female rats per group. G1-control, G2 somina-285 mg/kg/d, G3-somina1g/kg/d.

Table 3: Effect of Somina on different liver enzymes

| Parameters      | F1 generation | F2 generation |
|-----------------|---------------|---------------|
|                 | G1 | G2 | G3 | G1 | G2 | G3 |
| Bilirubin       | 1.6±0.15     | 0.86±0.6     | 0.98±0.2     | 1.26±0.1     | 1.32±0.56     | 0.88±0.3     |
| γGT             | 4.26±0.95    | 6.48±1.2     | 6.2±2.95     | 5.95±1.26    | 6.48±1.2     | 6.2±2.95     |
| ALT: SGPT       | 18.85±0.5    | 19.58±3.6    | 13.68±1.0    | 19.85±2.4    | 22.58±5.5    | 23.68±5.2    |
| AST: SGOT       | 22.09±1.2    | 24.26±0.6    | 21.61±3.6    | 27.2±1.6     | 27.27±0.9    | 25.61±2.9    |
| ALP             | 15.9±2.5     | 14.13±2.2    | 15.62±2.9    | 20.05±1.9    | 10.73±2.8    | 21.62±3.4    |

Values are expressed as mean±SE. Non-significant difference against control group, n = 6 female rats per group were randomized. G1-control, G2 somina-285 mg/kg/d, G3-somina1g/kg/d. γGT-gamma glutamyl transferase, ALT: SGPT-alanine aminotransferase, AST: SGOT-aspartate aminotransferase, ALP-alkaline phosphatase
Autopsy

An autopsy stated that any abnormal lumps and lesions were not witnessed in any part of the body after examining the whole body, especially in the uterus. The texture of the uterus, liver, kidney and heart was comparable to normal. All vital organs were intact and did not show any sign of toxicity.

DISCUSSION

Many anxiolytic drugs are associated with many side effects especially teratogenic effects which decrease their clinical utility [19]. Therefore, the use of medicinal plants for neurological and psychiatric diseases has progressed significantly owing to their fewer side effects and better reliability [20]. The aim of the present study was to evaluate the implantation, fertility index and teratogenic properties of somina, a unani anxiolytic in rats. Two different doses of somina (285 mg/kg/d or 1g/kg/d) were used in this study. Previously, Ahmed [12] has reported that somina is practically nontoxic unani anxiolytic medicine, but the teratogenic effect of somina was not evaluated. The present study suggests that somina did not produce any harmful teratogenic effects in rats. In literature, individual constituents of somina were reported to have potential fertility property, Sesamum indicum in male rats [21, 22] and Prunus amygdalus [23]. The present study confirmed that somina as a whole did not cause any teratogenic effect. In the present study, pre-coital administration of somina (at both doses) in female rats was assessed because the precoital antifertility effects of neem oil have been confirmed [24].

However, in current study pre-coital administration did not provoke any failure of implantation (table 1). All female rats become pregnant, and fertility index was 100% (table 1). Fertility index and implantation in somina treated rats are in agreement with previous studies [21-23]. More important is the fact that somina is used during the widow period when the growing fetus is most sensitive to teratogens, week 3-8 [25]. None of the treated breed showed any malformation.

The results obtained suggested that somina did not produce any significant changes in gross behavior, autopsy [12] and liver function test (LFT) in rats (table 3) i.e. our results are in agreement of rats. Two studies suggest that different constituents of Somina Like sesame [26], Prunus amygdalus [27], Lagenaria vulgaris [28] and Lactuca scariola [29] individually, can be used without any adverse effect on liver function and protect the liver from oxidative damage [26]. In both generations, parameters regarding the LFTs are normal. The administration of somina (10g/kg) was safe as no mortality was observed [12]. In the present study, somina is also safe during pregnancy in rats.

CONCLUSION

Two-generation reproductive toxicity (F1 and F2 breed) study concludes that continuous administration of herbal drug somina during implantation and pregnancy did not show any maternal and fetal toxicity. It did not lead to fetal growth retardation or fetal death or any teratogenic effects. It is recommended that the oral dose of 1g/kg/d was found to be safe during the gestational period in pregnant rats.

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. (Approval number: Reg no.2005/04/3/HMPPHS)

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CONFICT OF INTERESTS

The authors have no conflict of interest.

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