The reproductive toxicity of saponins isolated from *Cortex Albiziae* in female mice

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[ABSTRACT] Saponin from *Cortex Albiziae* (SCA) are extensively used in the clinical treatment of tumor and depression. However, SCA may cause several adverse effects, including reproductive toxicity. The present study was designed to assess the mechanism by which SCA cause reproductive toxicity in female mice. The general reproductive toxicity testing was accomplished in female Kunming mice. The animals were divided into four groups: three groups that were treated by oral gavage with 135, 270, and 540 mg·kg⁻¹·d⁻¹ of SCA prepared in physiological saline, respectively, and one vehicle control group that was treated with physiological saline only. The gestational toxicity tests were conducted at 540 mg·kg⁻¹·d⁻¹. The general reproductive toxicity results showed that the pregnancy rate of the SCA-treated group decreased with the pregnancy rate being decreased by 70% at 540 mg·kg⁻¹·d⁻¹. SCA elicited maternal toxicity in the ovary and the uterus, but no fetal toxicity or teratogenicity was observed. The rates of implantation in the early, middle, and late pregnancy were all decreased, with stillbirths and maternal deaths being observed. Histopathological changes showed that SCA adversely affected the ovary and the uterus. In conclusion, SCA-induced reproductive toxicity in female mice is most likely caused by its damage to the ovary and the uterus.

[KEY WORDS] Saponin; *Cortex Albiziae*; Female mice; Reproductive toxicity


Introduction

Plants are important sources of drugs, and modern pharmaceutical industries largely gain benefits from research on new natural medicines. There are rapid advancements in natural medicines often derived from seeds or fruits of plants by extraction [¹]. For example, saponins from *Cortex Albiziae* (SCA), known as a traditional natural medicine in Southeast Asia, which is the dried bark of *Albizia julibrissin* Durazz, one of the leguminous deciduous plants. SCA contain various chemical constituents, such as triterpenoids, flavones, lignans, alkaloids, tannins, and polysaccharides [²]. Saponins are a particularly important class of component derived from *Cortex Albiziae* [³-⁴] and exhibit various bioactivities, including antitumor, anti-depression, and sedation effects [³-⁵]. However, SCA may cause several adverse effects, such as reproductive toxicity. Gupta *et al* have reported toxic effects of SCA on spermatogenesis [⁶-⁷].

Reproductive toxicity has been recognized as an adverse condition in general toxicology [⁸-¹⁰]. Approximately 1% of infertility cases in women aged < 40 years are attributed to premature ovarian failure (POF). POF is almost spontaneous and may be related to the use of exogenous drugs. Exogenous drugs with reproductive toxicity act against the female repro-
Productive system and may disrupt ovarian cycle or cause infertility. This condition possibly leads to an increased rate of spontaneous abortion, offspring dysplasia, and fertility decline, among others. Therefore, the reproductive toxicity and mechanism of drugs in the female reproductive system should be determined.

Previous studies have shown that SCA elicit toxic effects on the reproductive organs of male mice by influencing spermatogenesis in the testis and destroying the spermatogenic tissue [11]. In the present study, toxicological tests were conducted to evaluate the reproductive effects of SCA in female mice and to assess the mechanism by which SCA induce reproductive toxicity in female mice. This study could provide a reference for further clinical application of SCA.

Materials and Methods

Test material

SCA extracted from the dried bark of *Albizia julibrissin* Durazz with 20% water were supplied by Shaanxi Ciyuan Biotech Co., Ltd. (Batch Number: CY120512). All of the chemicals used in the present study were of analytical grade.

Plant material

Cortex Albiziae was collected in 2011 from Shaanxi, China. And the plant material was authenticated by Prof. YIN Zhong-Qiong, College of Veterinary Medicine, Sichuan Agricultural University. And the Saponins from Cortex Albiziae were characterized by HPLC analysis [11].

Animals

The animal experiment was conducted in accordance with the Guidelines of the International Committee on Laboratory Animals implemented by the College of Veterinary Medicine, Sichuan Agricultural University, Chengdu, China.

Sexually mature female and male Kunming mice (a closely related strain coming from Kunming, Yunnan Province, China), weighing 25–27 g, were purchased from Chengdu Dossy Experimental Animals Co., Ltd., (License Number: SCXX (Sichuan) 2008-24). The mice were kept at room temperature of 22 ± 2 °C, relative humidity of 40% to 65%, and a 12 h/12 h light/dark cycle. They were fed with a standard diet from Nuvital Nutrients and provided free access to tap water. The mice were acclimated to laboratory conditions for one week before experiment.

General reproductive toxicity test

Eighty female Kunming mice were randomly divided into four groups (20 mice/group): The mice in vehicle control group (group I) were treated with physiological saline only and The mice in the three treatment groups were treated by oral gavage with 135 (group II), 270 (group III), and 540 (group IV) mg·kg⁻¹·d⁻¹ SCA prepared in physiological saline for 19 days respectively [12-13]. All of the mice were marked with trinitrophenol. After the mice were treated with SCA or control for one week, the females were co-habited with mature males at a ratio of 2 : 1 (F : M). The presence of sperm and vaginal plug in the vaginal smear the next morning were indicated positive mating. The mated and unmated females were then separated [13-15]. The females continued to receive the drug or physiological saline. During the treatment, body weight was measured once a week, and behavior was observed daily. Blood samples were collected from the female mice by cardiac puncture 30 min after the last treatment to evaluate the toxic effects of SCA on the liver and kidneys. All of the animals were euthanized. A detailed gross necropsy examination was carefully conducted, and the pregnancy rate was calculated [16]. The uterus and ovaries were removed. After the removal of fat and connective tissues, the tissues were fixed in 4% paraformaldehyde solution for 24 h, dehydrated in alcohol, and embedded in paraffin. The paraffin blocks were sectioned with a thickness of 5 µm and stained with hematoxylin-eosin solution for histopathological observations.

Gestational toxicity tests

Implantation toxicity test

Female mice were co-habited with mature male mice at a ratio of 2 : 1 to induce mating. After sperm and vaginal plug were observed in the vaginal smear, the females were separated from their male partners. The day that the spermatozoa were detected in the vaginal smear was recorded as 0 d of gestation. The separated pregnant females were randomly divided into two groups(20 mice/group). The experimental group was treated saline by oral gavage with 540 mg·kg⁻¹·d⁻¹ SCA in physiological saline from Day 1 to Day 5 d of gestation. The vehicle control group received physiological saline During the experiment, vaginal bleeding was regarded as a symbol of threatened abortion. All of the animals were euthanized at 4 d after the 5-day treatment. The number of implanted embryos was observed under anatomical lens and recorded [17]. The implantation rate was then calculated. Necrosis, absorption, and observed traces in the embryo indicated the toxicity of SCA to embryo implantation.

Early pregnancy toxicity test

Female mice were treated at first day of gestation by using the same method as the aforementioned implantation toxicity test. The pregnant mice were randomly divided into two groups (20 mice/group). The experimental group was treated by oral gavage with 540 mg·kg⁻¹·d⁻¹ SCA in physiological saline from Day 6 to Day 9 of gestation; the vehicle control group received physiological saline All of the mice were euthanized at 4 d after the 4-day treatment, and an autopsy was carefully conducted. During the experiment, the toxicity of SCA to early pregnancy was characterized by flaking of the embryo from the uterus wall or shrinking of the embryo.

Middle pregnancy toxicity test

Similarly, the pregnant mice were randomly divided into two groups (20 mice/group). The experimental group received a daily oral dose of 540 mg·kg⁻¹ SCA from Day 10 to Day 14 of gestation. The vehicle control group received physiological saline. At 4 d after the 5-day treatment, all of the animals were euthanized, and the number of live fetuses was counted.
During the experiment, abortion was observed and recorded. 

**Late pregnancy toxicity test**

The pregnant mice were randomly divided into two groups \((n = 20\text{ each})\). The experimental group received a daily oral dose of 540 mg·kg\(^{-1}\)·d\(^{-1}\) SCA from Day 15 to Day 19 of gestation; the vehicle control group received physiological saline. In this part of the test, abortion was observed and recorded. After parturition, all of the animals were euthanized, the number of live fetuses was counted, and fetal malformations or stillbirths were observed and recorded.

### Statistical analysis

The data were expressed as mean ± standard deviation (SD) and analyzed using SPSS 17.0 for Windows. The significance of differences among groups was determined using one-way analysis of variance (ANOVA), followed by the Student-Newman-Keuls test, and one-way analysis of variance (ANOVA), followed by the

**Effects of SCA on blood biochemical parameters**

Blood biochemical parameters were determined in the general reproductive toxicity test (Table 2) SCA did not cause any significant changes in the levels of Alanine Aminotransferase, Total Cholesterol, Blood Urea, and Serum Creatinine \((P > 0.05)\); the levels of Total Protein and Aspartate Aminotransferase in groups II and III did not exhibit any significant changes \((P > 0.05)\). By contrast, a significant increase in these parameters was observed in group IV \((P < 0.05)\). Significant changes were also observed in the levels of Globulin, Albumin/Globulin, and Triglyceride \((P < 0.01)\). All of the data were compared with those of group I (vehicle control group).

**Effects of SCA on the histopathological characteristics of uterus and ovaries**

Histopathological changes in the uterus and ovaries were observed. The ovaries of the control group showed normal characteristics. Ovarian follicle (OF) and follicular fluid (FF) were visible under a light microscope (Fig. 1A). The OF of the mice in the SCA-treated group also appeared normal in size and shape, but hyperemia, necrosis,
Degeneration, nuclear condensation, and nuclear fragmentation were observed in the OF (Figs. 1B and 1C). In the uterus of the control group, endometrium (En), myometrium (My), and perimetrium (Pe) showed normal characteristics under a light microscope (Fig. 1D). In the SCA-treated group, hyperemia was visible in My, and edema was found in uterus glands (Fig. 1E). Nuclear condensation and fragmentation were also visible in the uterus, which showed significant thickening of the luminal epithelium (Fig. 1F).

Fig. 1  Effect on the histopathological characteristics of the uterus and the ovary
(A): Ovary of the control female mice showing ovarian follicle (FC) and follicular fluid (FF), 200 ×. (B, C): Ovary of saponin from Cortex Albiziae-treated mice showing (B) hyperemia (↘) and degeneration (↙) in OF, 200 ×, and (C) necrosis (↓) in OF, dissolution and disappearance of the nucleus in OF (↘), 200 ×. (D) Uterus of the control mice showing normal characteristics, and endometrium (En), myometrium (My) and perimetrium (Pe) were visible, 400 ×. (E) Hyperemia (↓) was visible in My, and edema (↘) was found in uterus glands, 400×. (F) Nuclear condensation and fragmentation (↘) were visible in the uterus, and En showed a significant increase (↖) in height, 400 ×.

Effects of SCA on implantation
To determine the implantation toxicity of SCA, we observed the pathological damages in the reproductive organs (ovaries and uterus) of female mice. As shown in Table 3, SCA decreased the implantation rate. The implantation rates of the SCA-treated group and the control group were 10% and 100%, respectively. In the autopsy, the uterus of the control group showed a normal shape (Fig. 2A); by comparison, the uterus of the SCA-treated group was thinner (Fig. 2B). Hyperemia (Fig. 2C) and edema (Fig. 2D) of the uterus were also visible. SCA showed a significant anti-implantation effect by decreasing the number of implantation sites and absorption of the existing implants compared with the control group.
Table 3  Implantation toxicity effect of SCA

<table>
<thead>
<tr>
<th>Groups</th>
<th>Female number</th>
<th>Implantation number</th>
<th>Implantation rate/%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>20</td>
<td>20</td>
<td>100</td>
</tr>
<tr>
<td>SCA</td>
<td>20</td>
<td>2</td>
<td>10</td>
</tr>
</tbody>
</table>

Effects of SCA on early pregnancy

In this test, the effects of SCA on early pregnancy was observed. As shown in Table 4, the early pregnancy rates of the SCA-treated group and the control group were 30% and 100%, respectively. SCA showed significant anti-early pregnancy effect by decreasing the rate of early pregnancy compared with the control group.

Table 4  Effects of SCA on early pregnancy

<table>
<thead>
<tr>
<th>Groups</th>
<th>Female number</th>
<th>Early pregnancy number</th>
<th>Early pregnancy rate/%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>20</td>
<td>20</td>
<td>100</td>
</tr>
<tr>
<td>SCA</td>
<td>20</td>
<td>6</td>
<td>30</td>
</tr>
</tbody>
</table>

Effects of SCA on middle-term pregnancy

In this test, vaginal bleeding (Fig. 3A) and fetal absorption (Fig. 3B) were observed in the experimental group. As shown in Table 5, the average number of fetuses in the SCA-treated group was 2, and 20 females gave birth to 34 fetuses. The fertility rate of the SCA-treated group was significantly decreased, compared with that of the control group.

Table 5  Effect of SCA on middle-term pregnancy

<table>
<thead>
<tr>
<th>Groups</th>
<th>Female number</th>
<th>Fetus number</th>
<th>Average number of fetus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>20</td>
<td>20334</td>
<td>10</td>
</tr>
<tr>
<td>SCA</td>
<td>20</td>
<td>34</td>
<td>2</td>
</tr>
</tbody>
</table>

Effects of SCA on late-term pregnancy

As shown in Table 6, the average number of fetuses in the SCA-treated group was 6, and 15 females gave birth. Five female mice died before parturition. The fertility rate of the SCA-treated group was significantly decreased, compared with that of the control group.

Discussions

Reproductive toxicity is defined as the hazardous effects of exogenous substances on male and female reproductive systems in which biological processes, including ovulation and spermatogenesis (from germ-cell differentiation to whole-cell development), are impeded. These substances may disrupt the development of an embryonic cell; as a result, biochemical functions and structures are drastically changed. Furthermore, reproductive capacity and offspring are negatively affected. In our previous study, SCA were shown to elicit reproductive toxicity against male mice by destroying the spermatogenic function of the testis and the epididymis. In the current study, the reproductive toxicity, mechanism, and target organ of SCA on female mice were investigated. After the female Kunming mice were treated with SCA, no significant changes in body weight or other general toxicities were observed, indicating that the general metabolic condition...
of the mice remained normal under the current assay conditions.

In female mammals, uterus and ovary are the main organs that maintain the normal reproductive capacity. Mature follicles ovulate as influenced by gonadotropin. After ovulation is completed, one egg cell moves to the fallopian tube, leaving granulosa cells in the ovary; granulose cells develop, forming the corpus luteum with the theca cell. Three complete estrous cycles are required to undergo development from the primary follicles to the final stage of the corpus luteum. In mice, one estrous cycle requires 4 to 5 days. Hence, female mice require three cycles to allow drugs to affect the fertilized eggs. In addition, the female mice should be continuously treated by SCA during mating and early pregnancy to evaluate test substances. Therefore, the estrous cycle in the general reproductive toxicity test lasted 19 days, and therefore Day 19 was set as the end point of our experiment. We evaluated the toxic effects, such as the ability to conceive, and adverse effects on the reproductive system and offsprings, when the mice were treated with SCA before and after mating. The results showed that the pregnancy rates of the experimental mice were lower than that of the controls. The pregnancy rate of group IV decreased by 70% compared with that of the control group. This result was related to our previous study, in which SCA elicits anti-fertility effects on male mice [10]. The results of the histopathologic observation also showed damages to the reproductive organs, particularly the uterus and ovaries. An evident increase in the height of the luminal epithelium was observed; loose and edematous stroma in uterus glands was also found. Under a light microscope, hyperemia was observed in all of the levels of follicular cavities, and parts of the follicular nucleus were broken; pyknosis occurred. Inflammatory cells were infiltrated. The results further indicated that the reproductive toxicity of SCA could be characterized by damages observed in the reproductive organs [18-20]. The results of blood biochemical parameters showed that the liver function (GLB, A/G, TG, AST, and TP) of the mice treated with high-dose SCA were significantly changed. By comparison, no significant changes were observed in the renal functions. This result indicated that SCA also elicited hepatotoxicity at high dosage. Zhao et al have indicated that SCA exhibits liver toxicity by shortening blood clotting time and decreasing antioxidant ability in major organs [13]. However, no pathological changes were observed in the liver of the SCA-treated mice in the present study. SCA may induce metabolic abnormalities in the liver, but the level of abnormality in the present study remained in a relatively safe range. The dosage of 540 mg·kg⁻¹·d⁻¹ used in our subsequent studies showed a certain degree of reliability (without general severe toxicity).

The results in gestational pregnancy toxicity tests showed that SCA elicited significant toxic effect on each stage of the gestation (embryo implantation and early, middle, and late pregnancy), indicating that SCA may prevent implantation, induce miscarriage in each stage of gestation, and cause fetal death before parturition.

Implantation is a process by which an embryo reaches En and undergoes development. This process is a unique reproductive activity in mammals. During implantation, embryo and En show a close relationship. They identify, accommodate, and interact with each other. After implantation, the embryo absorbs nutrition from the maternal blood for fetal development. Therefore, implantation is a process based on the cooperation between mother and fetus. Once exogenous factors interfere with implantation, drugs can be absorbed by the fetus in the early period and stillbirth may occur in the late period [8]. In the stage of implantation and early pregnancy, the rates of implantation and early pregnancy were significantly lower in the mice treated with 540 mg·kg⁻¹·d⁻¹ SCA, compared with the control mice (anti-implantation rate was 90% and anti-early pregnancy rate was 70%). SCA-induced pathological damages were also observed in the ovaries and uterus. SCA-treated mice showed thinner En, compared with the control mice. SCA also induced the atrophy of the embryo, which was not conducive to implantation. This result was
related to the anti-ovulation effect of SCA. The tension and contraction amplitudes of uterine muscle strips were significantly increased in the SCA-treated mice and the contraction frequency significantly decreased. The effects of SCA were possibly similar to oxytocin [2]. In particular, SCA could decrease the number of pregnant animals and normal embryos in mice [21]. These results indicated that SCA elicited reproductive toxicity in the early stages of pregnancy. Further studies will be conducted to investigate the specific causes of this toxicity.

Vaginal bleeding (threatened abortion), stillbirth, and dystocia were observed in females during the mid- and late-term pregnancy periods (middle and late pregnancy) in which fetuses showed better development. The number of fetuses was also significantly decreased in the SCA treated group. Furthermore, these results may occur possibly because SCA exhibited a higher affinity than progesterone. As such, SCA competed with progesterone for the progesterone receptor, thereby blocking the activity of progesterone and terminating the pregnancy [22]. The necrosis of decidua causes the release of endogenous prostaglandins and induces uterine contractions and cervical softening; as a result, miscarriage occurs [23].

Based on our results, SCA acted not only in all levels of follicular cells but also in the uterus. The main damage to the ovary was manifested as degeneration and necrosis of follicular cells at different levels. SCA also caused congestion, hemorrhage, and edema in the uterus. These results indicated that SCA elicited reproductive toxicity via two major mechanisms. In the ovary, SCA affected the metabolic processes in all stages of follicular cell development by changing ovarian development and function. The ovary in mammals is a polyphase organ and contains numerous follicular cells in different developmental stages. In SCA-induced toxicity, the metabolism in this reproductive organ is impeded, leading to degeneration and necrosis of follicular cells. In the uterus, SCA prevented implantation. The uterus is the part of the female reproductive system where a fertilized egg is implanted. As such, this part is important in fetal development. In SCA-induced toxicity, the En became abnormal, thereby impeding embryo development; as a result, implantation was prevented. Hyperemia was visible in My. Furthermore, En showed a significant increase in height. In SCA-treated mice, the implantation of fertilized egg was affected, thereby causing interrupted pregnancy or abortion.

Of note, the chemical compositions of traditional Chinese medicines are complex; as such, a chemical reaction occurs when the main component interacts with other substance, thereby affecting the efficacy of medicines to a certain extent. In the present study, we evaluated the reproductive toxicity of SCA, one class of the chemical extracts from Albizia. However, the chemical extracts from Albizia comprise of several components and affect multiple targets; these extracts also exhibit characteristics different from general chemical drugs. Therefore, compositions other than SCA in this type of extract could also elicit reproductive toxicity effects. This assumption requires further studies. Nevertheless, SCA should be further evaluated, alongside with other components, to provide a solid basis for rational clinical applications of this traditional Chinese medicine.

References


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