

Protective Effect of *Sambucus elbus* Extract on Teratogenicity of Albendazole

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Abstract: Albendazole is utilized as an anthelmintic agent. One of its side effects is teratogenicity. The aim of present study was evaluation the protective effect of *Sambucus* extract on albendazole induced- fetal malformation. Four groups of female pregnant Wistar rats (8 rats each group) were used. One group was received normal saline (as control group). A single oral dose 30 mg/kg of albendazole was administered to rats on day 10 of gestation in group 2. Rats in group 3 were received albendazole similar to group 2 plus *Sambucus* extract at dose 600mg/kg. Rats in group 4 were received only *Sambucus* extract on day 10 of gestation. The rats were euthanatized on day 20 of gestation. The fetuses were harvested and their weight and length and also the weight of placenta were measured. The skeletal malformation of fetus was studied by stereomicroscope after staining by Alizarin red - Alcian blue. The length and weight of fetuses were significantly decreased by albendazole but *Sambucus* extract prevent this effect. In group that received only *Sambucus* extract, the length and weight of fetuses was similar to control group. *Sambucus* extract decreased albendazole effect on weight of placenta. The fetus resorption was decreased by *Sambucus* extract when co-administrated with albendazole. The incidence of skeletal malformations (mostly of the limbs, vertebrae and palate) decreased significantly by *Sambucus* extract. Thus, *Sambucus* extract may have protective effect on albendazole teratogenicity; but this subject needs more detailed evaluation.

Key words: Albendazole • *Sambucus* extract • Skeletal malformation • Pregnancy • Rats

INTRODUCTION

Albendazole is broad spectrum anthelmintic and affects nematodes, cestodes and trematodes. This drug belongs to benzimidazole group which act by binding to parasite -tubulin, inhibiting its polymerization and impairing glucose uptake and carbohydrate metabolism in parasites and cause their death [1]. Albendazole is drug of choice for treatment of microsporidiosis, ascariasis, enterobiasis, hookworm infections, cystic hydatid disease and neurocysticercosis [2]. Albendazole is safe in human and animals. The incidence of its side effects is very low, with gastrointestinal side effects (lesser than 1%) [3] and teratogenic effect especially in laboratory animals such as rat. Albendazole is initially oxidized to albendazole sulphoxide, an active metabolite and then to albendazole sulphone, which is inactive [4]. Albendazole sulphoxide is 70% protein bound and has a half-life of 9 h [2].

There are two *Sambucus* species (*Sambucus ebulus* L. and *Sambucus nigra* L.). Extracts from the root and leaves of *Sambucus ebulus* L. are used in traditional medicine for the treatment inflammatory joint diseases, rheumatic pain and sore throat [5]. Extracts of its aerial parts had protective effect in the carrageenan induced rat paw edema [6]. Leave extracts showed also affect on the concentration of cytokines (interleukin-1 α , interleukin-1 β , TNF α) [7]. Moreover, leaves of *Sambucus ebulus* are applied externally to treat burns, infectious wounds, edema, eczema, urticaria, rheumatism and inflammations [8, 9]. Some pharmacological effects such as antiinflammatory [6, 7], anti-*Helicobacter pylori* [10], antiviral [11], antibacterial and radical scavenging [12] activities were reported for *Sambucus* species. Ursolic acid is active compound of leave extract of *Sambucus ebulus* L.

The objective of the present study was to evaluate protective effect of *Sambucus* aerial parts extract on albendazole induced- fetal malformation in pregnant rats.

MATERIALS AND METHODS

Male and female healthy Wistar rats, 10-12 weeks of age, weighing 180-200g were purchased (Joundishapour laboratory animal center, Ahvaz, Iran) and housed individually (males) or at 10 per polycarbonate cage (female) for a 2-week acclimation period. Rats were fed *ad libitum* by standard laboratory pellet (Pars khurakdam, Shushtar, Iran.) and tap water. A 12-h light: 12-h dark cycle was maintained. Room temperature was at $23\pm 2^{\circ}\text{C}$ with a relative humidity of 45-55%. Albendazole was purchased from Sigma Co. U.S.A. Leaves of *Sambucus ebulus* L. were collected in Sari area. Herbarium identification was done at the agriculture of Shahid Chamran University, Ahvaz-Iran. The fresh leaves were air dried at room temperature in a shaded room. The *Sambucus* hydro alcoholic extract was prepared by maceration method according to study of Schwaiger *et al.* [5].

Male and female rats were housed together. Pregnant females were divided into four groups (n=8) and treated as follow on day 10 of pregnancy:

First group received normal saline (5 ml/kg), the second group received albendazole (30mg/kg) orally, the third group received albendazole (30mg/kg) and along with it *Sambucus* extract (600mg/kg) intraperitoneally and the fourth group received *Sambucus* extract (600mg/kg).

The animals were sacrificed by cervical dislocation at 20th day of gestation and fetuses were collected and numbered, then weight and length of them were measured and gross malformations were determined. The weight of placenta was measured. Fetuses were stained by Alizarin red-Alcian blue method [13] and investigated by stereomicroscope (Nikon, Japan) for skeletal defects. The incidence of macroscopic defects was determined and compared among the groups.

Statistical significance between groups was determined using SPSS program. The minimum level of significance was $p<0.05$.

RESULTS

There were not any aborted or absorbed fetuses from normal saline group. Total number of collected fetuses from groups 1, 2, 3 and 4 were 26, 64, 64 and 44, respectively. It seems that the number of aborted fetuses decreased in control.

There were not observed macroscopic anomalies in the control animals. In the control group palatal closures of fetuses were normal at day 20 of gestation (i.e., palatal shelves had grown vertically on the sides of the tongue, then horizontally to meet and fuse). Albendazole induced cleft palate (46%). *Sambucus* extract reduced incidence of albendazole-induced cleft palate to 22%. Double ossification center in vertebral column was observed by albendazole in 23% of fetuses. Its incidence was prevented by *Sambucus* extract to zero percent.

Mean weight and length were significantly ($P<0.05$) decreased in albendazole treated group. The means of weight and length of fetuses in the group that received albendazole along with *Sambucus* extract were not significantly greater than the group received only albendazole. The mean weight and length in the group that received *Sambucus* extract did not significantly differ with control group (Fig. 1, 2).

DISCUSSION

Pregnant rats were received 30 mg/kg of albendazole on gestational days 10. This dose of albendazole was toxic for embryos. Some embryos were absorbed and some had growth reduction which characterized by reduced fetus body weight. The growth reduction was very considerable, so the fetuses were seen immature on day 20 of pregnancy. The mean of fetus length in group 2 was significantly lesser than control group. Similar effect of albendazole was reported in several studies. Embryo lethality and growth reduction was reported by Mantovani *et al.* [14]. They seen this effect was dose dependent. At 20 and 30 mg/kg, more than 20% of embryos showed morphologic alterations including shape abnormalities and the development of forelimb buds [14].

Albendazole affected ossification process in fetuses. In our study, the skeletal malformation was included cleft palate (in 46% fetuses) and double ossification center in vertebral column (in 23% fetuses). In addition, albendazole directly acts on the embryogenesis causes malformations, like agenesis of the tail and hydropic fetuses [15]. The incidence of external and skeletal malformations (mostly of the tail, vertebrae and ribs, gross external and skeletal abnormalities in the thoracic region and limbs) was reported with albendazole sulphoxide [16].

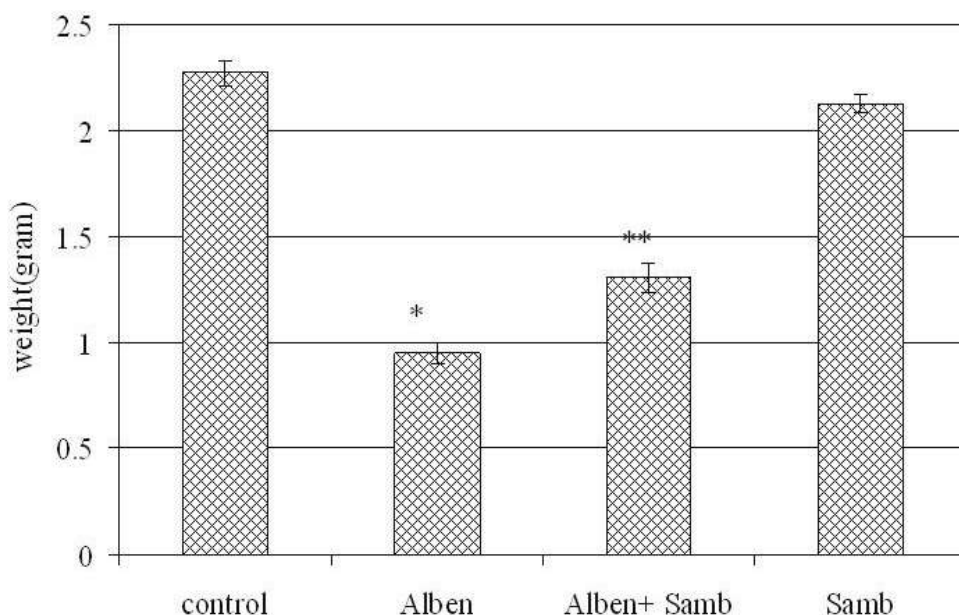


Fig. 1: Weight (mean±SE) of fetuses in normal saline and test groups: control: normal saline (1 ml/100g IP); Alben: albendazole (30 mg/kg po); Alben+Samb: albendazole +*Sambucus* extract (600 mg/kg IP); Samb: *Sambucus* extract (600 mg/kg IP).n=8. * represents significant difference to other groups. ** represents significant difference to control and *Sambucus* group by $p<0.05$

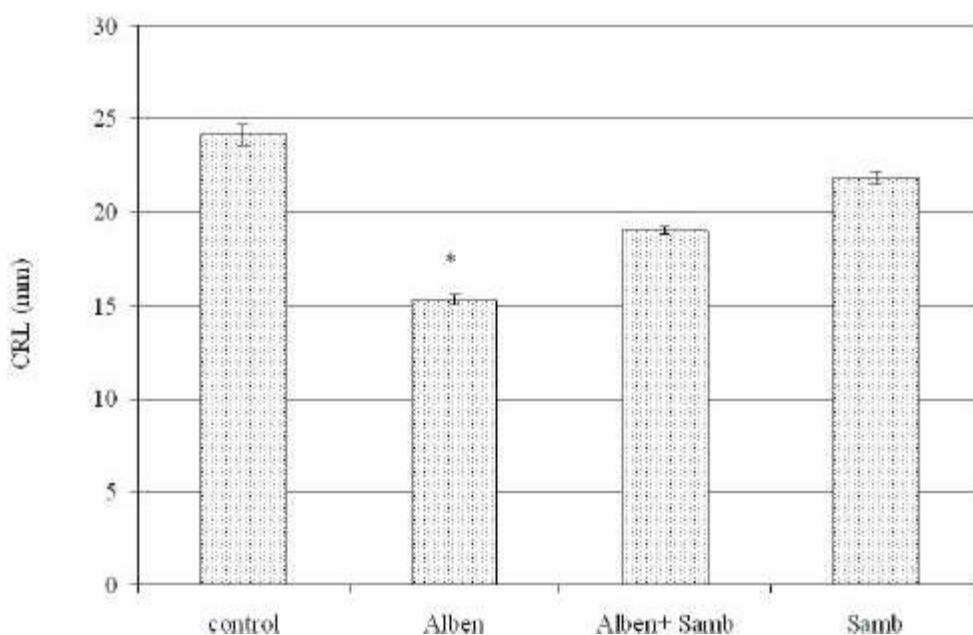


Fig. 2: Length (CRL) (mean±SE) of fetuses in normal saline and test groups: control: normal saline (1 ml/100g IP); Alben: albendazole (30 mg/kg po); Alben+Samb: albendazole +*Sambucus* extract (600 mg/kg IP); Samb: *Sambucus* extract (600 mg/kg IP).n=8. * represents significant difference to other groups by $p<0.05$

It appears the embryo toxicity and teratogenicity of albendazole relates its major active metabolite as known albendazole sulfoxide. Albendazole is normally not

detectable in human plasma since it is rapidly metabolized [4]. Both albendazole and its sulfoxide metabolite produce embryotoxic effects in this rat model [17].

Data of resorptions, placental and fetal characteristics and fetal skeletal malformations by albendazole sulfoxide were recorded. Resorption and decreasing of placenta weight and smaller size fetuses by albendazole sulfoxide was reported by Teruel *et al.* [18]. Also they observed reductions in ossification process and malformations or fetal death when albendazole sulfoxide was orally administered to pregnant rats [18].

We administrated *Sambucus* extract with albendazole by this thought the oxidative stress induced by albendazole or its metabolites was reduced with *Sambucus* extract. Consequently, its embryo toxicity and teratogenic effect may reduce. Co- administration of *Sambucus* extract prevented effect of albendazole on placenta weight. Also, it decreased albendazole -induced skeletal malformation such as cleft palate and vertebrate ossification. Flavonoids of *Sambucus* extract have several therapeutic effects such as antioxidant [19, 20]. Inhibition of lipid peroxidation effect by flavonoids, is supposed to increase the viability of collagen fibrils, by activating the DNA synthesis and preventing the cell damage [21]. Therefore, preventive malformation effects of *Sambucus ebulus* may be attributed to the phytochemicals exist in the leaves.

In summary, the *Sambucus* extract as herbal antioxidant can decrease some teratogenic effects of albendazole. Although, we proposed its flavonoids compounds are evaluated by more details.

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