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Original Article

Teratogenic Effect of Verbascoside, Main Constituent of *Lippia citriodora* Leaves, in Mice

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Abstract

Verbascoside (acteoside), a phenyl propanoid glycoside, comprises 0.5 to 3.5 % dry weight of *Lippia citriodora* leaves. A wide range of biological activities are attributed to verbascoside including anti-inflammatory, antioxidant, anti-bacterial, anti-tumor, anti-fungal, photoprotective as well as chelating effects. The objective of this study is to evaluate the effect of verbascoside on pregnancy outcome in mice. Timed-pregnant mice received doses of 1g/kg/day verbascoside or the vehicle control during organogenesis, intraperitoneally. Maternal body weights were measured throughout pregnancy. The litters were examined for external malformations and skeletal abnormalities. Then they were stained with Alizarin red S and Alcian blue. Maternal exposure to verbascoside throughout pregnancy did not influence the mean of maternal weight gain. Statistically significant difference was not found in mean number of implantation sites, live and resorbed fetuses between control and experiment groups. Our data demonstrate that the main component of *L. citriodora*, verbascoside using during organogenesis possesses no risk to fetuses. However, more research projects are needed to confirm these findings and determine the exact effects of verbascoside on human embryo development.

Keywords: *Aloysia triphylla;* Herbal medicine; *Lippia citriodora;* Teratogen; Verbascoside.

Introduction

Lippia citriodora (*Aloysia triphylla*, lemon verbena) is a species in the family *Verbenaceae* that grows spontaneously in South America. Spanish man brought it to Europe in the 17th century (1). In different languages the plant is known by various names e.g. lemon verbena (English), Lousia (Arabic), and Behlimoo in Persian. It is also cultivated in North region of Iran. It is cultivated mainly due to the lemon- like aroma emitted from its leaves. *L.citriodora* is a folk remedy for fever, colds, flatulence, spasms asthma, diarrhea, colic, indigestion, insomnia and anxiety(2). The essential oils from its leaves

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Table 1. Cesarean section parameters in BALB/c mice fetuses exposed to verbascoside.

	Treatment and dose (mg/kg/day)	
	Control (Normal saline)	**Treated group (1 g/kg)**
Pregnant mice, No	10	10
Number of implantation sites, Mean±SEM	118 (11±0.61)	112 (11.2±0.89)
Live fetuses, No(%)	115(97.45)	112(94.73)
Resorbed fetuses, No(%)	3 (2.54)	2 (1.78)
Fetal weight, Mean±SEM (g)	1.08± 0.03	1.22±0.47
Fetal length, Mean±SEM (mm)	18.58±0.42	21.3±0.61

Data are shown as mean ± SEM or percentage. The difference between two groups is not statistically significant (T-test or Fisher›s Exact Test).

are valuated to have digestive, antispasmodic, antipyretic, sedative and stomachic as well as antimicrobial activities (3). Essential oil and phenolic compounds (flavonoids) in most of the species of *Verbenaceae* are responsible for its pharmacological effects. Verbascoside (acteoside), a phenyl propanoid glycoside, comprises 0.5 to 3.5 % dry weight of *L.citriodora* leaves. It has been detected in more than 200 plant species in both root (e.g., primaryand secondary roots) and shoot system (e.g., stems, leaves and flowers) but at widely varying levels (4). In 1963, verbascoside was isolated from mullein (*Verbascum sinuatum* L.; *Scrophulariaceae*) by Italian scientists(5). A wide range of biological activities are attributed to verbascoside including anti-inflammatory, antioxidant, anti-bacterial, anti-tumor, anti-fungal, photoprotective as well as chelating effects (6). Recently it has also been applied in dermo-cosmetic and topical drug formulations. Verbascoside suppositories are used in treatment of inflammation in the intestinal mucosa as well (6).

Regard to the therapeutic and non-therapeutic widespread use of *L.citriodora* and its active component (verbascoside) in traditional medicine and modern pharmacology and the belief by many that lemon verbena is an herbal remedy, it is estimated that many young women may utilize it during their pregnancy. Currently there is no available information to support the safety of verbascoside use in pregnancy. The objective of this study is to evaluate the effect of verbascoside on pregnancy outcome in mice (7).

Experimental

Material and animal treatment

Verbascoside was purchased from Xian Aladdin Biological Technolog. Alizarin red and Alcian blue was obtained from Merck (Darmstadt, Germany). BALB/c virgin female mice, 10-12 weeks of age, body weight 20-30 g, were provided from Avicenna Research Institute of Mashhad University of Medical Sciences and were adapted to natural light/darkschedule cycle (12h - 12h) and room temperature (18–22 °C) during two weeks. All animal experiments were performed with the approval of Mashhad University of Medical Sciences. They had free access to drinking water and food.

Two females were caged with one male of the same strain overnight and examined for a vaginal plug the next morning and the day of vaginal observation was considered as gestational day zero(GD0) (8). Twenty pregnant mice were randomly divided into two groups, one experimental group that received 1g/kg/day of verbascoside intraperitoneally during GD6–15 (organogenesis period) and one control group that received the same volume of normal saline on the same days. For dose selection, the median lethal dose (LD_{50}) and maximum tolerateddoses (MTD) of verbascoside were assessed. The MTD was about 3 g/kg/day due to the lethality that was observed at higher doses. 30 % of MTDs was chosen as the administered dose.

Maternal and fetuses observation

Maternal body weights were measured

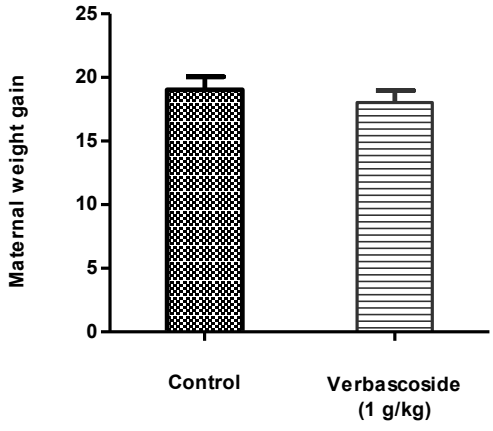

Figure 1. Effect of verbascoside on maternal weight gain during pregnancy. Data are shown as mean ± SEM. The difference between two groups is not statistically significant (T-test).

during the experiment. All animals were observed daily throughout gestation for mortality, morbidity, general appearance and behavior. Pregnant mice were sacrificed by cervical dislocation on the 18th day of pregnancy. The uteri were removed and the embryos were dissected and examined for numbers of dead and viable fetuses.

The degree of resorption was recorded to determine the relative time of death of the conceptus. Individual fetuses were evaluated carefully for external malformations and then their weight and crown-rump length were recorded. All fetuses were studied by stereomicroscope (model MZ 12, Leica, Solms, Germany) for skeletal malformations. After dissolving the soft tissues in 1 % KOH, skeletal structures had been double stained by Alizarin red S and Alcian blue according to Kimmel and Trammel techniques(9). As a result, bone and cartilage tissues become red and blue, respectively.

Statistics

The body weight and length were recorded as mean ± SEM. T-test was carried out between control and experimental group. To compare the difference in the frequency of the absorbed and live fetuses and external malformation between groups, Fisher exact probability test was performed. Data were analyzed using SPSS software (version 11.5).

Results

Maternal observation

All pregnant mice and fetuses were alive at the time of cesarean section. Maternal exposure to verbascoside throughout pregnancy did not influence mean maternal weight gain compared with the control group (19.89 ± 0.86 and 20.18 ± 1.01, respectively) (Figure 1). Food consumption, water intake and behavior signs in pregnant mice of both groups were not affected. Statistically significant difference was not found in mean number of implantation sites, live and resorbed fetuses between control and experiment groups (Table1).

Fetus observation

The results revealed that there were no statistically significant differences on body weight and crown- rump length in the verbascoside exposed fetuses (Table1). The incidence rates and types of malformations and growth retardation are shown in Table 2. The total number of birth defects was lower than the significance level. Skeletal abnormalities are observed in < 2 % of cases and include vertebral column deformity (<1 %) and limb deformity (<1 %). Also, minor skeletal malformation (wavy ribs) was observed in verbascoside-treated groups.

Discussion

The goal of this study was to provide the

Table 2. Skeletal malformations in BALB/c mice fetuses exposed to verbascoside.

	Treatment and dose (mg/kg/day)	
	Control (Normal saline)	**Treated group (1 g/kg)**
Dams, No	10	10
Fetuses examined, No	115	112
Minor skeletal malformations,No (%)	0	1 (0.89)
Vertebral column deformity, No (%)	0	1 (0.89)
Limb deformity, No (%)	0	1 (0.89)
Mandible and calvaria, No (%)	0	0 (0)
Growth retardation, No (%)	0	2 (1.78)
Total number of birth defect, No (%)	0	3 (2.67)

Data are shown as mean ± SEM or percentage. The difference between two groups is not statistically significant (Fisher›s Exact Test).

potential teratogenic effects of verbascoside in pregnant mice. Verbascoside exhibits a number of biological activities including anti-inflammatory, anti-oxidative, anti-bacterial, anti-androgen and anti-tumor actions(10). Besides having widespread therapeutic use, lemon verbena has also been used traditionally to relieve menstrual pain (primary dysmenorrhea) in Mexico. It was revealed that *L.citriodora* hexane extract inhibited the contractile response induced by PGF2 thus uterine relaxation (16). But, there is not yet enough information available to estimate the side effects and risk of *L.citriodora* and derivatives in pregnancy. Our results showed that administration of 1g/kg of verbascoside to pregnant mice on GD 6–15 exert no significant toxicity on the mothers or in the development of fetuses.

Funes *et al* also found that orally administration of lemon verbena extract (containing verbascoside) up to 2000 mg/kg cannot induce acute oral toxicity or adverse effects in rat (11). However, a recent study has indicated that long time exposure to high verbascoside level on oocyte fertilization in-vitrocan cause toxic effects (reduction in blastocyst formation) through their pro-oxidant activity. According to this study, verbascoside or verbascoside derived products had a toxic effect on oocyte cytoplasmic competence and consequently on embryo development resulting from generation of H_2O_2. It is noticeable that the concentrations and exposure time are mentioned as key factors in amounts of H_2O_2 production. In other words, verbascoside at lower level may be able to exert differential protection against oxidative stress (12-14). In living system, verbascoside has indirect antioxidant activities via endogenous antioxidant enzymes induction or/and activation and inactivation of pro-oxidant enzymes (4). However, there are various studies that emphasize on antioxidant activity of *L.citriodora* (15, 16). Cytogenotoxic evolution of aqueous infusion and decoction from *L.citriodora* through cellular proliferation kinetics, mitotic index, single-cell gel electrophoresis assay, sister chromatid exchanges and micronucleustest also did not show genotoxic effect (15). IFRA (International Fragrance Association) is stated that the essentialoil from the fresh herb extract might sensitize the skin to sunlight (phototoxicity) (17). No other concerning adverse effects have been reported yet.

Scientific explorations have indicated that some of extensively used herbal remedies may have toxic effects on fetal development. Animal study showed that intraperitoneal injection of crocin or safranal, main constituents of saffron, during organogenesis, can induce embryonic abnormalities noticeably skeletal malformations (18). Also *Salvia leriifolia* Benth consuming during pregnancy might cause some malformations such as spina bifida, limb abnormalities, abdominal bleeding, and bone abnormalities (19).

Taken together, our data demonstrate that the main component of *L. citriodora*, verbascoside using during organogenesis poses no risk to fetuses. However, more research projects are needed to confirm these findings and determine

the exact effects of verbascoside on human embryo development.

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