

## Evaluation of *In Vivo* Teratogenic Effect of *Ocimum lamiifolium* Hochst. Ex Benth. (Lamiaceae) and *Zehneria scabra* (Linn. f.) Sond. (Cucurbitacea) Leaves in Mice

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### Abstract

**Background:** Herbal medicine is practiced throughout the world. Herbal medicine use is common among pregnant women because of a variety of pregnancy related ailments. Exposure of pregnant women to herbal substances may affect their fetus and contribute to maternal and fetal morbidity and mortality. The leaves of *Ocimum lamiifolium* and *Zehneria scabra* are traditionally used in Ethiopia to treat pregnancy related ailments, but the safety of the plants during pregnancy is not scientifically studied. Evaluation of the teratogenic effect of medicinal plants is very important to identify teratogenic medicinal plants and to prevent their use during pregnancy.

**Objective:** To evaluate teratogenicity of the hydromethanol extracts of *Ocimum lamiifolium* and *Zehneria scabra* leaves in mice.

**Methods:** The leaves of *Ocimum lamiifolium* and *Zehneria scabra* were extracted with 80% methanol, and teratogenicity of the extracts was evaluated on mice based on the recommendations of OECD guideline. Data were analyzed by one way ANOVA followed by Tukey's posthoc test using SPSS version 20 software.

**Result:** The administration of *Ocimum lamiifolium* methanolic extract caused insignificant changes in body weight and consumption of food and water in pregnant mice. Additionally, the plant did not affect the mean number of implantation, live and resorbed fetuses compared to the control group. *Ocimum lamiifolium* leaf extract treated group showed increased mean fetal body weight and crown-rump length compared to the control group, but the difference was not statistically significant ( $P > 0.05$ ). There was no detected limb, craniofacial, skeletal, vertebral column and growth abnormality in *Ocimum lamiifolium* leaf extract treated group. Similarly, *Zehneria scabra* leaf extract administration did not cause notable changes in food and water intake and behavior during the pregnancy period. Moreover, treatment with *Zehneria scabra* did not increase the incidence of skeletal and external malformations.

**Conclusion:** The methanolic leaf extracts of *Ocimum lamiifolium* and *Zehneria scabra* didn't show a statistically significant teratogenic effect on mice fetuses up to 800 mg/kg and 400 mg/kg dose, respectively.

**Keywords:** *Ocimum lamiifolium*; *Zehneria scabra*; Teratogenic Effect; Mice

### Introduction

WHO defines traditional medicine as including diverse health practices, approaches, knowledge and beliefs incorporating plant, animal, and/or mineral based medicines, spiritual therapies, manual techniques and exercises applied singularly or in combination to maintain well-being, as well as to treat, diagnose or prevent illness [1].

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In Africa, pregnant women use herbal remedies to treat pregnancy related problems due to cost-effectiveness of therapy and easy access to the product [2]. Herbal medicine use during pregnancy raises particular concerns of safety. Exposure to herbal products during pregnancy period may affect the fetus and contributes to maternal and fetal morbidity and mortality. For instance, a study in rats found an association between prenatal exposure to ginger in high quantities with increased fetal loss, increased fetal weight and bone maturation [3]. Additionally, poor regulatory framework for importation, manufacturing and distribution of herbal medicines in Africa makes herbal medicine use risky. Although registered herbal products exist, they do not adhere to good manufacturing practices, principles of safety and efficacy as is required for conventional medicines [4]. Moreover, the use of herbal medications does not have strict regulations like modern medicines; thus, the use of these products particularly during pregnancy is a matter of concern [5].

Different studies in Ethiopia indicated a wide use of herbal medicines among pregnant women for a variety of ailments [2,6]. A study conducted at Nekemte hospital, western Ethiopia reported that *Zingiber officinale* (44.36%), *Allium sativum* (37.32%), Eucalyptus (9.15%) and *Ruta chalepensis* (9.15%) were used among pregnant women to treat ailments during pregnancy [2]. Another study conducted at University of Gondar referral and teaching hospital, northeast Ethiopia revealed that *Zingiber officinale* (40.7%), *Ocimum lamiifolium* (38.40%), *Allium sativum* (19%), fenugreek (26%), flax seeds or *Linum usitatissimum* seeds (18.60%), *Zehneria scabra* (8.5%), *Ruta chalepensis* (3.40%), peppermint (1.70%) and others (2.80%) were used among pregnant women to treat pregnancy related ailments [6]. In Ethiopian traditional medicine, the leaves of *Ocimum lamiifolium* have been used for the treatment different disorders such as oropharyngitis, wound, pain, fever, and other inflammatory disorders [7,8]. The leaf juice of *Zehneria scabra* is used to treat fever, headache, Paralysis and external wounds [9,10]. But, the safety of these medicinal plants specifically *Ocimum lamiifolium* and *Zehneria scabra* during pregnancy is not scientifically studied.

### **Aim of the Study**

The aim of this study was to evaluate teratogenicity of the hydromethanol extracts of *Ocimum lamiifolium* and *Zehneria scabra* leaves in mice.

### **Materials and Methods**

#### **Collection of plant materials**

The fresh leaves of *Ocimum lamiifolium* and *Zehneria scabra* was collected from Gubalafto District located in North Wollo Zone, Amhara region, in October, 2020. The plants was authenticated by Botanists in Department of Biology, College of Natural and Computational Sciences, Wollo University.

#### **Chemicals and instruments**

Methanol, distilled water, water for injection, Analytical balance, What man filter paper No.1, beakers, funnels, measuring cylinder, rotary evaporator, Deep freezer, Lyophilizer, glass rod, vacuum pump, spatula, pipettes, oral feeding syringe, animal Cages and desiccators were used.

#### **The experimental animals**

Swiss albino mice of both sex (20 - 30g and 8 - 10 weeks of age) were obtained from animal house of Wollo University and Ethiopian Public Health Institute. The animals were housed in cages under standard conditions with 12 hours light and dark cycles. They were

provided with standard pellet diet and water *ad libitum*. They were acclimatized to the laboratory condition for a week before initiating the experiment [11]. Animals were handled according to the international laboratory animal use and care guidelines [12] throughout the experiment.

After seven days of adaptation period, the animals were allowed to mate overnight by placing sexually experienced albino male mice into a cage containing two albino female mice. After an overnight mating, each female mouse was assessed for the presence of a vaginal plug, and the presence of vaginal plug was considered as day-0 of gestation [13].

### **Preparation of plant extracts**

The fresh leaves of *Ocimum lamiifolium* and *Zehneria scabra* were collected from the vicinity of Dessie town and fleshed with tap water so as to remove dirt materials. The plant materials were dried under shade at room temperature. The dried leaves of *Ocimum lamiifolium* and *Zehneria scabra* were coarsely powdered using mortar and pestle. The Leaf powder of each plant (1000g) weighted and soaked with 8000 ml of 80% hydro-methanol and macerated for three days in a conical flask with occasional shaking. Then after, the extracts were separated from the residue by using muslin cloth and further filtered by what man filter paper No.1. The residues were re-macerated twice by adding fresh solvent and filtered in the same manner to maximize the yield. The collective filtrates were concentrated by a rotary evaporator at 40°C. Then, the concentrated extracts were frozen overnight using deep freezer followed by drying with a lyophilizer at -50°C with a vacuum pressure of 200 mBar to remove water. Finally, the dried extracts were put in screw cap vials, and stored in a refrigerator at -4°C until used for the experiment [14].

### **Acute toxicity study**

The oral toxicity study was carried out on the hydro-methanol extracts based on the recommendation of the Organization for Economic Cooperation and Development (OECD) guideline no 425. One female mouse was first fasted for four hours, and the fasting body weight was measured. Then, the extract was administered to the mouse at a dose of 2 g/kg. Then, the mouse was kept under strict observation of physical and behavioral changes for 24 hr, with special attention during the first four hr. Following the observation of the first mouse, another four mice were fasted for four hours and body weight for each mouse was measured. The extract was administered to each mouse at a dose of 2 g/kg and observed in the same manner. The observation was continued for fourteen days for any signs of overt toxicity. The test was done for each extract separately.

### **Teratogenicity testing**

Pregnant mice were divided randomly into six groups, each comprising 10 animals per group. Three Groups were selected for evaluation of *Ocimum lamiifolium* leaf extract and the remaining three groups for evaluation of *Zehneria scabra* leaf extract. Group one was given distilled water in both cases and considered as control group. Group II and III were given an oral dose of 600 mg/kg and 800 mg/kg of *Ocimum lamiifolium* extract and 200 mg/kg and 400 mg/kg of *Zehneria scabra* extract, respectively. The treatment was given daily from the sixth to the fifteenth day of pregnancy. The dose selection was based on previous pharmacological activity studies [15,16]. On the eighteenth day of pregnancy, the mice were weighed and then euthanized using chloroform and a caesarean section was performed to remove the uterus and its contents.

### Maternal and fetus observation

Maternal body weights were recorded daily throughout the pregnancy period and weight gain was calculated by taking the difference between weight of pregnant mice on day-0 and day-18 of pregnancy. All pregnant animals were observed for abortion, morbidity, mortality, general appearance and behavior from day-0 to day-18 of pregnancy.

The dams were sacrificed under anesthesia on day 18 of gestation for cesarean section as mentioned above. The fetuses were carefully removed from uterus, and each uterus was investigated for embryonic resorption. Number of live and dead fetuses, weight and length of body (crown-rump length) were recorded. Each fetus was assessed for external malformation (abdominal hernia, polydactyl, anencephaly, cleft palate and other structural changes) under dissecting stereomicroscope. Additionally, all fetuses were assessed for any skeletal malformation. The detection of skeletal deformities was performed by whole mount skeletal staining with Alizarin red S and Alcian blue using Kimmel and Trammell methods [17].

### Ethical clearance

The laboratory work was done in accordance with the guide for the care and use of laboratory animals [18], and the study was submitted and approved by the ethical review committee of college of medicine and health sciences, Wollo University before initiating the study.

### Statistical analysis

The data were expressed as mean  $\pm$  standard error of the mean. Means of all parameters among groups and within a group were compared using one-way ANOVA followed by Tuckey's post hoc multiple comparison test. P-values  $< 0.05$  were considered statistically significant. SPSS Version 23 Software was used for statistical analysis.

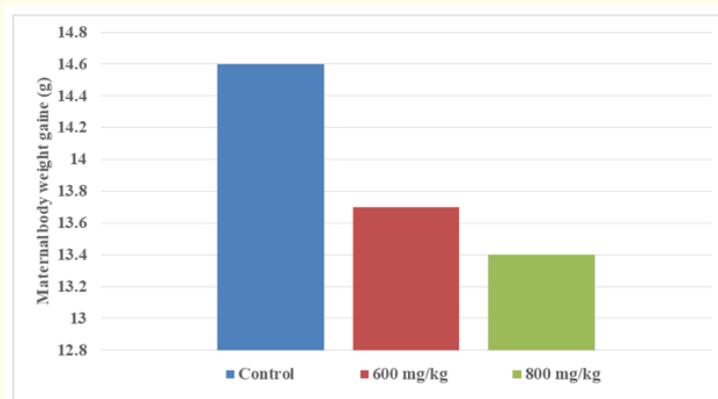
## Results

### Acute toxicity study

All mice treated with the leaf methanolic extract of *Ocimum lamiifolium* and *Zehneria scabra* (2000 mg/kg) remained alive during 14 days of observation. Oral administration of the methanolic leaf extract of each plant with a dose of 2000 mg/kg did not produce observable changes in behavior, breathing, skin effects, defecation, yellowing or loss of hair, postural abnormalities, impairment in food intake and body weight in the female mice.

### Effect of *Ocimum lamiifolium* extract on maternal and fetal parameters in mice

All Pregnant mice were alive at the time of the cesarean section. The Maternal mean weight gain during pregnancy were  $14.6 \pm 0.62g$ ,  $13.7 \pm 0.51g$  and  $13.4 \pm 0.40g$  in control, 600 mg/kg and 800 mg/kg of *O. lamiifolium* extract treated groups, respectively. The administration of *O. lamiifolium* methanolic extract during pregnancy resulted in no significant change in maternal body weight (Figure 1).



**Figure 1:** Maternal weight gain comparisons during pregnancy between groups that received distilled water, 600 mg/kg and 800 mg/kg dose of *Ocimum lamiifolium* methanolic leaf extract.

Food and water consumption of pregnant mice did not differ significantly among groups. Behavior signs in dams of either doses of *Ocimum lamiifolium* methanolic extract did not change compared to the control group. No significant difference was observed in the mean number of implantation, live and resorbed fetuses between the control and treatment groups. *Ocimum lamiifolium* leaf extract administration at doses of 600 and 800 mg/kg increased the mean fetal body weight and crown-rump length as compared to control group but the change was not statistically significant ( $P > 0.05$ ) (Table 1).

	Control	600 mg/kg of OLME	800 mg/kg OLME
Total number of Dams (pregnant mice)	10	10	10
Total number of fetuses	115	109	104
Mean fetal number ± SEM	11.5 ± 0.72	10.9 ± 0.77	10.4 ± 0.58
Total Live fetuses, No (%)	113 (98.26%)	105 (96.33%)	101 (97.11%)
Mean fetal weight ± SEM (g)	1.18 ± 0.06	1.21 ± 0.03	1.23 ± 0.03
Mean fetal length ±SEM (mm)	18.90 ± 0.42	19.32 ± 0.75	19.72 ± 0.62

**Table 1:** Effect of hydromethanolic *Ocimum lamiifolium* leaf extract on albino mice fetuses.

OLME: *Ocimum lamiifolium* Methanolic Extract; Data are shown as mean ± SEM.

The difference between the groups is not statistically significant ( $p > 0.05$  compared to the control group, one-way ANOVA followed by Tuckey's post hoc multiple comparison test).

All live fetuses from the vehicle, 600 mg/kg and 800 mg/kg leaf extract treated groups were examined for external and skeletal malformations, and there was no detected limb, craniofacial, vertebral column and skeletal abnormality (Table 2).

	Control	600 mg/kg of OLME	800 mg/kg of OLME
Number of pregnant mice	10	10	10
Number of examined fetuses	113	105	101
Minor skeletal malformations	0/113	0/105	0/101
Vertebral column deformity, No	0/113	0/105	0/101
Limb deformity, No	0/113	0/105	0/101
Craniofacial abnormalities, No	0/113	0/105	0/101

**Table 2:** Skeletal and external malformations in albino mice fetuses treated with 600 mg/kg and

800 mg/kg of *Ocimum lamiifolium* leaf extract.

OLME: *Ocimum lamiifolium* Methanolic Extract.

### Effect of *Zehneria scabra* methanolic leaf extract on maternal and fetal parameters in mice

Mortality was not observed among pregnant animals and all dams were alive at the time of cesarean section. The maternal weight gain didn't show a statistically significant difference among groups ( $p > 0.05$ ) (Table 3). During the pregnancy period, there were no notable changes in food and water intake and behavior among treated and control groups. The mean fetal weight was lower in the treated groups when compared with the control, but the difference was not statistically significant. Moreover, there was no a significant change in the mean body length of fetuses in the treated groups when compared with the control group ( $p > 0.005$ ). Additionally, there was no significant difference in mean fetal body weight and length between the two treated groups. The Comparison between treated and control groups showed no significant differences ( $p > 0.05$ ) in the mean number of implantation, and live and resorbed fetuses.

	Control	200 mg/kg of ZSME	400 mg/kg ZSME
Number of Dams ( pregnant mice)	10	10	10
Mean maternal weight gain $\pm$ SEM (g)	16.54 $\pm$ 0.61	15.25 $\pm$ .72	14.34 $\pm$ 0.64
Number of total fetuses	112	99	94
Mean fetal number $\pm$ SEM	11.2 $\pm$ 0.53	9.9 $\pm$ 0.64	9.4 $\pm$ 0.56
Total live fetuses, No (%)	107	97	92
Mean fetal weight $\pm$ SEM (g)	1.15 $\pm$ 0.06	1.1 $\pm$ 0.05	1.03 $\pm$ 0.06
Mean fetal length $\pm$ SEM (mm)	19.84 $\pm$ 0.69	18.86 $\pm$ 0.70	18.63 $\pm$ 0.85

**Table 3:** Effect of *Zehneria scabra* methanolic leaf extract on albino mice fetuses.

ZSME: *Zehneria scabra* Methanolic Extract.

Data are shown as mean  $\pm$  SEM. The difference between the groups is not statistically significant ( $p > 0.05$  compared to the control group, one-way ANOVA followed by Tuckey's post hoc multiple comparison test).

All live fetuses in each group were examined for limb, craniofacial, skeletal, vertebral column malformations and growth malformations. The treatment with *Zehneria scabra* methanolic leaf extract did not increase the incidence of skeletal malformations. As shown in table 4, there was no malformations detected in fetuses born from groups treated with methanolic leaf extract of *Zehneria scabra* at doses of 200 mg/kg and 400 mg/kg.

	Control	200 mg/kg of ZSME	400 mg/kg of ZSME
Number of Pregnant mice	10	10	10
Number of examined fetuses	107	97	92
Minor skeletal malformations	0/107	0/97	0/92
Vertebral column deformity, No	0/107	0/97	0/92
Limb deformity, No	0/107	0/97	0/92
Craniofacial abnormalities, No	0/107	0/97	0/92

**Table 4:** Skeletal and external malformations in albino mice fetuses received 200 mg/kg and 400 mg/kg dose of *Zehneria scabra* methanolic leaf extract.

ZSME: *Zehneria scabra* Methanolic Extract.

## Discussion

A WHO survey revealed that about 70 - 80% of the world population rely on non-conventional medicine mainly of herbal medicine in their primary healthcare [19]. Pregnant women practice herbal medicine due to the presence of minor illnesses and prior herbal medicine use experience [5]. Although *Ocimum lamiifolium* [15] and *Zehneria scabra* [20] are the commonly used medicinal plants in Ethiopian traditional medicine, the risk of their use during pregnancy is not scientifically studied.

The findings of this study revealed that administration of different of methanolic extract of *Ocimum lamiifolium* and *Zehneria scabra* leaves during the period of organogenesis has not been associated with fetal malformations, and no sign of maternal toxicity was observed. *Ocimum lamiifolium* leaf extract caused an increase in mean fetal body weight and length compared to the control group but the difference was not statistically significant. Similarly, *Zehneria scabra* methanolic leaf extract caused statistically insignificant reduction in

mean fetal body weight and length compared to the control group. In contrast to findings of this study, previous scientific study on many herbal remedies has revealed teratogenic toxicity on animal models. For instance, Saffron (a widely used herb) induced embryonic abnormalities particularly skeletal malformations when administered to pregnant mice [21]. Additionally, administration of methanolic extract of *U. massaica* leaves and *C. megalocarpus* stem bark extracts to pregnant mice showed teratogenic effect [22].

One of the main mechanisms that have been hypothesized to clarify the cause of teratogen-induced fetal malformations is excessive formation of reactive oxygen species or impaired antioxidant defense [23]. It has been shown that supplementation of antioxidants can prevent and protect from teratogen-induced oxidative damage and thus reduce fetal damage. The commonly used medically important herb such as ginger has no teratogenic effects because of its high antioxidant activity [24]. The available experimental evidence and the safety of vitamins C and E in pregnancy suggest that experimental use of antioxidants in alcohol-consuming mothers should be seriously considered to reduce fetal alcohol damage [25].

Previous studies on *Ocimum lamiifolium* [15,26] and *Zehneria scabra* [27,28] leaves have reported strong anti-inflammatory and antioxidant activity. Previous phytochemical screening of *Ocimum lamiifolium* methanolic leaf showed the presence of terpenoids, flavonoids, tannin, saponins and steroids [29]. Previous studies on the hydro-methanol extract of the leaves of *Zehneria scabra* revealed the presence of tannins, saponins and glycosides [16,30]. It is likely that the presence of these biologically active phytochemicals like tannins, flavonoids, saponins and glycosides might contribute to the preventive antioxidant and anti-inflammatory activities of the plants [31-33]. Therefore, the antioxidant and anti-inflammatory activity of the experimental plants may partly explain the findings of this study. The findings of this study is in agreement with the reports of previous studies conducted on teratogenic evaluations of the leaf aqueous extracts of *Ocimum suave* [34] and *L. citriodora* [35].

## **Conclusion**

The methanolic leaf extracts of *Ocimum lamiifolium* and *Zehneria scabra* have no a statistically significant teratogenic effect on mice fetuses up to 800 mg/kg and 400 mg/kg doses, respectively. However, additional study is required to determine the teratogenic effects of *Ocimum lamiifolium* and *Zehneria scabra* on human embryo development.

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## **Conflict of Interest**

The authors declare that they have no conflict of interest.

## **Data Availability Statement**

The data used to support the findings of this study are included within the article.

## **Authors' Contributions**

All authors made substantial contributions to conception and design, acquisition of data, analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published and agree to be accountable for all aspects of the work. AA and YMB conducted the actual experiment.

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