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Effect of *Curcuma longa* Alcoholic Extract on Mice Embryos Development

Doha Ali*
Department of Biology, College of Sciences for Women, University of Baghdad, Baghdad, Iraq.

Zainab Karim
Department of Biology, College of Sciences for Women, University of Baghdad, Baghdad, Iraq.

***Corresponding Author:** doha.ali1202@csw.uogaghdad.edu.iq

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Abstract

This study focused on determining the effect of the alcoholic extract of *Curcuma longa* on mouse embryos. Twenty-four adult white Swiss mice were used in this study. Experimental animals were divided into four groups, with six mice in each group. For mating to occur, three females and a male were placed in the cage. We prepared three sublethal concentrations of *Curcuma longa* and applied them to the experimental groups except the control group: 10 mg/kg, 50 mg/kg, and 100 mg/kg. From 7–14 days of gestation, we gave the pregnant mice 0.1 ml per 10 g of body weight. After 18 days of gestation, we extracted the embryo to study the effect of the alcoholic extract, the weights and lengths of the embryos, the embryo malformation, as well as the weight of mice before and after the experiment. This study shows a significant ($P<0.01$) decrease in the body weight of mice treated with three concentrations (10, 50, and 100) mg/kg compared with a control group, but there is no significant difference in the lengths and weights of mouse embryos. Concentrations of 50 mg/kg and 100 mg/kg show many malformations induced in the mice compared with embryos of the control group, including cleft lips, hemorrhage, and Micromelia, as well as absorbed embryos at 50 mg/kg and 100 mg/kg. This study concluded that an alcoholic extract of (*Curcuma longa*) has teratogenic effects on the embryos of mice.

Keywords: *Curcuma longa*, embryonic malformation, Micromelia, Teratogenic effects.

1. Introduction

As medical tools and alternative therapeutic, those medical plants are excessively used for the treatment and protection of many diseases [1]. Medical plants have natural products that are used in pharmaceutical preparations; these compounds may be pure or extracted [2]. *Curcuma longa*

belongs to the Zingiberaceae family, which is an herbal plant family that has about 50 genera and around 1600 known species [3]. *Curcuma longa* is a perennial plant with wide leaves and yellow flowers (4). *Curcuma longa* is also known as "Kurkum" in Arabic, "Haldi" in India, and "turmeric" worldwide [5]. *Curcuma longa* is also one of the important compounds in curries that give them their yellow pigment in India, Malaysia, China, and Thailand (6). The yellow pigment and healing properties of turmeric are related to compounds in it [7]. The most commonly used part of these plants is the rhizome. The volatile oil and nonvolatile curcuminoids are the main active compounds in the rhizomes [8]. The largest country that produces turmeric is India, which supplies more than 90% of the world's demand [9]. According to the World Health Organization (WHO), about 80% of people in developed countries use this tradition [10]. The major component in *Curcuma longa* is curcumin, which is responsible for biological activity [11]. Curcumin is the most important polyphenol compound that is present in and isolated from the rhizome [12]. Curcumin also has various pharmacological activities, such as antioxidant, anti-inflammatory, antifungal, and antibacterial [13]. Curcumin is the main curcuminoid found in turmeric. Desmethoxycurcumin and bis-desmethoxycurcumin are other curcuminoids [14]. The aim of this study is to determine the effect of an alcohol extract of *Curcuma longa* on mice's embryonic development during the gastation period.

2. Materials and Methods

Twenty four mice with ages from 8 to 6 weeks and weights from 29-22 grams were used. We obtained the animals from the Animals House of Biotechnology Research Center at Al-Nahrain University. The animals (females) were divided into four groups (6 mice per group), and we placed three females and one male in each cage. In the early morning, they observed the vaginal plug being placed after mating; this day was considered the zero day of pregnancy, followed by the day considered the first day of gastritio [15].We selected three sub lethal concentrations of *Curcuma longa*, 10mg/kg, 50mg/ 100 mg/kg the LD50 of *Curcma Longa* is 2000 mg/kg [16]. We used the gavage tube for oral administration. Between 7-14 days of gastation, the mice were orally administered 0.1 ml per 20 units of weight every day.

The mice in the control group were also administered 0.1 ml per 20 units of weight of distilled water. In this study, we took the weights of females before and after the end of the experiment. Pregnant mice were dissected after 18 days of pregnancy; the fetuses were extracted from the uterus to study the fetal malformation, and lengths and weights were recorded. We used *Curcuma longa* powder and alcohol methanol in ratio of 1:10 (weight to size) at 60-80 °C about 6-8 hours by soxhlet extactor, then used filter paper to extract the liquid and left it to dry out of sun.

3. Results and Discussion

Table 1 shows that there is a significant ($p > 0.01$) decrease in body weight in pregnant mice that were treated with 10mg/kg, 50 mg/kg and 100 mg/kg concentrations of alcoholic extract of *Curcuma longa*.

Table 1. Effect of different concentrations of alcoholic extract of *Curcuma longa* in body weight of pregnant mice.

Concentration (mg/kg)	Mean ± SE of Weight of the Mothers (gm)	
	Before	After
Control	26.68 ±1.15 a	46.13 ±1.76 a
G: 10	24.85 ±0.33 a	40.62 ±1.56 b
G: 50	24.45 ±0.55 a	41.92 ±1.03 b
G: 100	24.95 ±0.36 a	39.27 ±0.49 b
LSD value	2.267 NS	3865 **
P-value 0.174	0.0085	

Similar letters in the same column mean that there are no significant differences at (P <0.01)

The body weight decrease in pregnant mice that were treated with 10 mg/kg, 50 mg/kg, and 100 mg/kg concentrations of alcoholic extract of *Curcuma longa*, compared with pregnant mice in the control group that were untreated. This agrees with a previous study that showed the weight of pregnant rats after being administered for 6–15 days of gestation decreased the weight gain of female rats, which was comparable to gabapentin. Gabapentin (50 mg/kg bw) and *C. Mangga* extract (1000 mg/kg bw) also caused resorption. Resorption might arise due to disruption in morphological development, which causes malformations and death [17].

Table 2. Effect of different concentrations of alcoholic extract of *Curcuma longa* in body Weight and Length of pregnant mice

Concentration (mg/kg)	Group Mean ± SE of weights and lengths of embryos	
	Weight (g)	Length (cm)
Control	1.151 ±0.03	1.65 ±0.02
G: 10	1.140 ±0.03	1.68 ±0.02
G: 50	1.182 ±0.04	1.601 ±0.03
G: 100	1.150 ±0.03	1.631 ±0.02
LSD value	0.102 NS	0.078 NS
P-value 0.875	0.307	

The teratogenic substance has the mildest effect on the size and weight of the body [18]. The size and weight of the fetus indicate nutritional and developmental support through the pregnancy [18]. Previous studies show the mean litter size of the fetus was 6.80 at 6–15 days of pregnancy. These results indicate that curcuma had no effect on the size of embryos compared to normal (p >0.005) [20]. In Figure 1, the control fetus at 18 days of gestation has eyes, an ear pinna, a mouth, a trunk, and a tail. Figer 2 shows the difference between the control fetus and the treated fetus with 10 kg/mg of *Curcuma longa* which has micromelia (M.m.).

Table 3. Effect of Concentration in percentage of dead embryos.

Percentage of dead embryos ± SE	Concentration (mg/kg)
Control	0.00 ± 0.00 b
G: 10	0.00 ± 0.00 b
G: 50	5.56 b ± 3.51 b
G: 100	3.58 ± 20.04 a
7.406 *	LSD value

Means having with the different letters in same column differed significantly. * (P≤0.05).

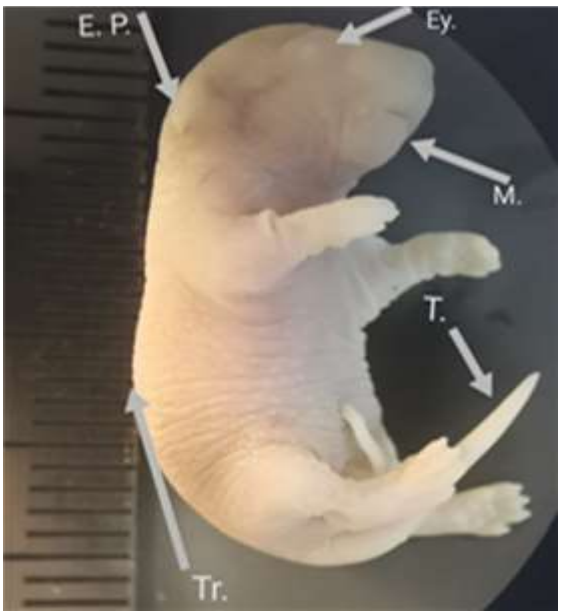

Figure 1. lateral View of control fetus showing: Eye (Ey.), Ear Pinna (E.p.), Trunk (Tr.), Tail (T), and Mouth (M.).

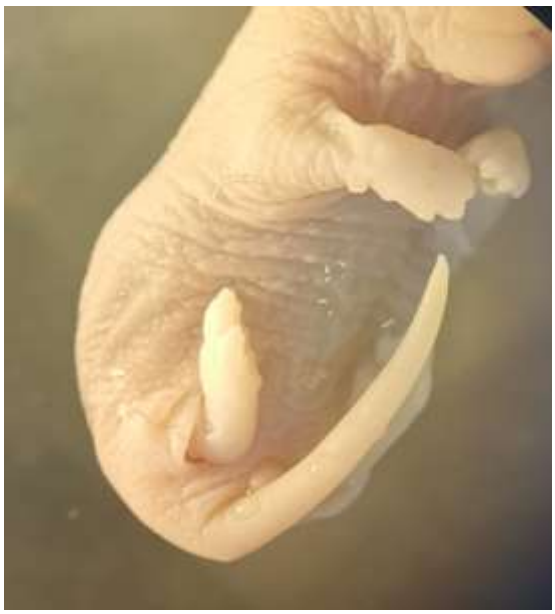

Figure 2. lateral View on the right and show fetus treated with 10 kg/mg of *Curcuma Longa* in with micromelia (M.m.)

Figure 3 shows a fetus on day 18, which trated 50 kg/mg of *Curcuma longa* and has a lip clef (L.C.). **Figure 4** lateral view of a fetus treated with 50 kg/mg of *Curcuma longa* has hemorrhage in his leg compared with the control fetus. **Figure 5** shows the right lateral view of the absorbed embryo treated with 100 kg/mg of *Curcuma longa*.

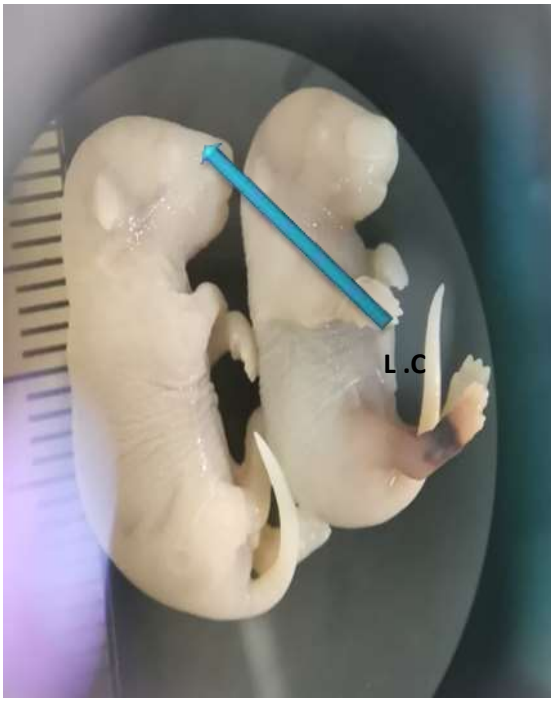

Figure 3. ventral View of the l fetus treated with 50 kg/mg of *Curcuma longa* with lip clef (L.C).

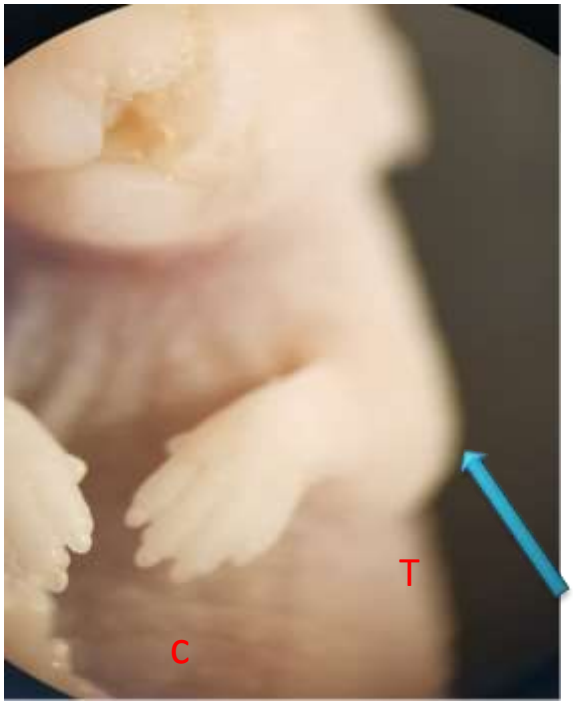

Figure4. lateral View of the control fetus on the right and left show fetus treated with 50 kg/mg of *Curcuma Longa* has hemorrhage in leg

Figure 5. Lateral View of the control embryo on the right and left show aborted embryo (Em.) treated with 100 kg/mg of *Curcuma Longa*

Figure 6 shows the ventral view of the uterus horns and embryos of a pregnant mouse.

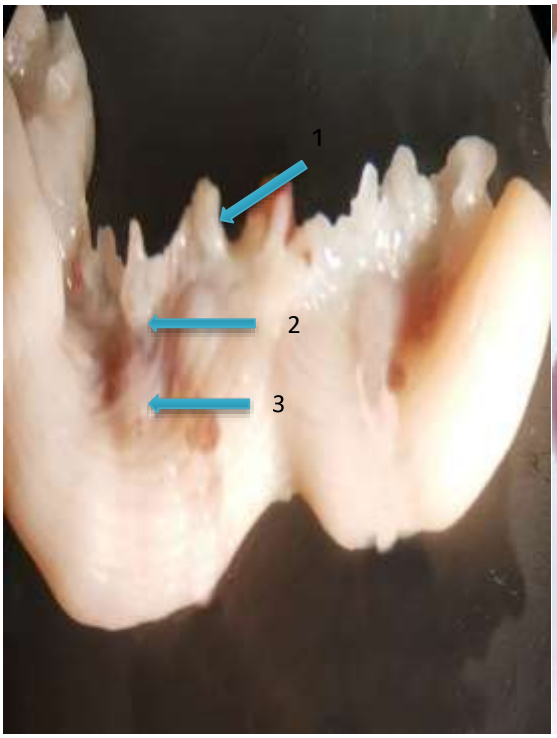

Figure 6. Uterus horns of control pregnant mouse showing: ovary (1), uterine horns (2), and embryo (3).

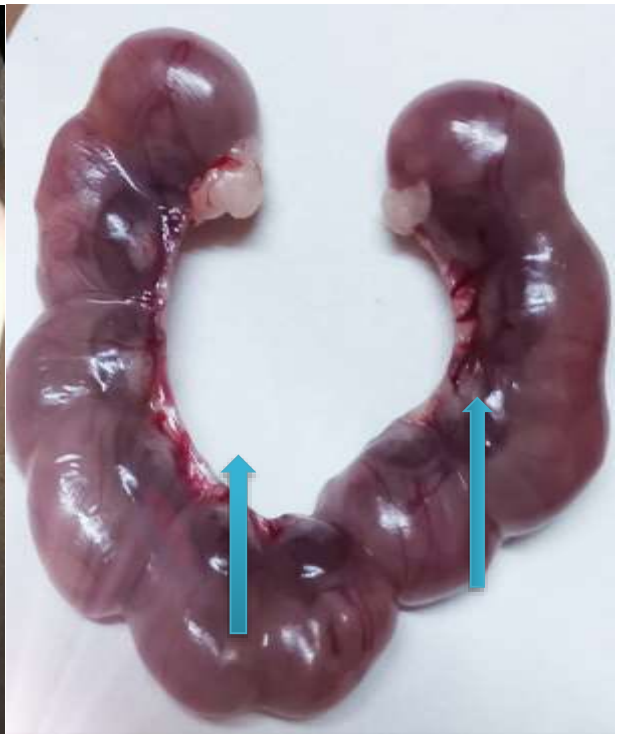

Figure 7. View the uterus the and Resorption (R) of embryo mouse treated with 100 kg/mg of *Curcuma Longa*

Figure 7 depicts the uterus with embryo adoption in a mouse treated with 100 kg/mg *Curcuma longa*. The organogenesis period in pregnancy is a critical period. In this period, cell differentiation occurs to build tissue and organs. Therefore, malformations occur in this period if exposed to toxic materials [19].

Saponins, steroids, terpenoids, and flavonoids are second-metabolites present in curcuma rhizomes. These compounds are active, have pharmacological activities, and might also be toxic in high concentrations. Previous studies showed a flavonoid derivative drug (hydroxyethylrutoside) caused congenital abnormality syndrome [21]. In addition, reproductive toxicity effects appeared in the female mice because of saponin presence [19]. In a previous study, curcumin also showed the ability to inhibit chondrogenesis by stimulating apoptosis and also impair bone development by reducing actin cytoskeleton reorganization [22]. Distruption in morphology development that leads to malformation and death may raise resorption [19].

4.Conclusions

This study concluded that an alcoholic extract of (*Curcuma longa*) has teratogenic effects on the embryos of mice.

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