

TERATOGENIC AND EMBRYO-TOXIC EFFECTS OF LATEX AND LEAVES METHANOL EXTRACT OF *EUPHORBIA HELIOSCOPIA* L. IN MICE AND CHICKEN EMBRYOS

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ABSTRACT

Euphorbia helioscopia L. has been investigated for antibacterial, antifungal, antiviral and vasodepressor activities. The study was designed to probe into the reproductive toxicity (teratogenicity) potential of *Euphorbia helioscopia* L. in albino mice. Toxicity of latex and methanol extract of leaves (10, 50, 80, 100 and 120 µg/200 µL) was evaluated in white leghorn chicken embryos (5th day) using chorioallantoic membrane route (n = 30 for each experiment i.e. latex & extract, or 5 per dose and 5 as control group). Teratogenic potential of latex (n= 5) and extract (100, 300, 500, 700, 1000, 1500 and 2000 mg/kg body weight, n= 40, or 5 per concentration and 5 as control group) was evaluated in pregnant albino mice on 7th day of gestation using intra-peritoneal route. Both latex and leave extract checked in chicken embryos were safe. LD₅₀ calculated with TSK software was 1397 mg/kg body weight for methanol extract of leaves observed in albino mice. The rest of the experimental concentrations were non-lethal in mice without any observable malformation. It is concluded that the concentration (administered parentally) less than LD₅₀ is devoid of teratogenic effects in pregnant female mice and chicken embryos.

Key words: *Euphorbia helioscopia*, Chicken Embryo, Mice, Teratogenicity, Toxicity.

INTRODUCTION

A battery of experiments on animals (pre-clinical) and human beings (clinical) are required prior to the approval of new drug to be used as regular therapeutic agent in human beings. To ensure the safety of new drug in pregnant females, reproductive toxicity test "teratogenesis" is needed to be performed (Katzung *et al.*, 2008). Teratogenesis is derived from Greek word teratogen, an agent that has capability to induce malformations such as anencephaly, phocomelia, cleft palate and other morphological nonreversible changes at time of birth (Tuchmann-Duplessis, 1987).

Euphorbia helioscopia, belongs to medicinally rich family *Euphorbiaceae*, is an annual weed and used as folk medicine to treat warts in various parts of Pakistan. *Euphorbia helioscopia* whole plant has great medicinal importance, the leaves and stems are used as febrifuge and vermifuge (Wu *et al.*, 1991). The oil from the seeds has purgative properties, the roots are used as anthelmintic and the seeds mixed with roasted pepper have been used in the treatment of cholera (Panda *et al.*, 2004). A number of researchers had explored different pharmacological activities of various extracts of *Euphorbia helioscopia* such as insulin secretagogue (Hussain *et al.*, 2004), antibacterial (Ramezani *et al.*, 2008; Uzair *et al.*, 2009; Farhat *et al.*, 2011), antifungal (Uzair *et al.*, 2009; Farhat *et al.*, 2011),

phytotoxicity (Ramezani *et al.*, 2008), vasodepressor (Barla *et al.*, 2006), antiviral (Ramezani *et al.*, 2008), anticancer (Wang *et al.*, 2012), allelopathic (Tanveer *et al.*, 2010), antioxidant (Ramezani *et al.*, 2008; Nikolova *et al.*, 2011; Ben-Mohamed *et al.*, 2012), anti-allergic and anti-asthmatic (Park *et al.*, 2001), breast cancer resistant protein (BCRP) and P-glycoprotein modulators (ABCB1 and ABCG2) (Barile *et al.*, 2008), cytotoxic (Zhang and Guo, 2006) and molluscicidal action (Najia *et al.*, 2000).

The use of plants for treatment or cure of different diseases can be found 5000 years back (Goldman, 2001; Biazzi, 2003). Synthetic compounds have replaced natural medicinal plants in most areas with the advancement in organic chemistry (British medical association, 1993). WHO recognized the use of natural products in 1978, though these have been in human use since a long time back (Ministério, 2001). The major hazards with the use of herbs for medicinal purpose were populations' blind trust on natural plants and believe that all natural products are safe without any toxic effect (Rates, 2001; Gallo and Koren, 2001).

Thus, pharmacological screening of natural medicinal plants or any new drug for their toxic potential becomes imperative. Drug regulatory authorities require toxicity data of experiments on at least two species for approval of a new drug (Katzung *et al.*, 2008). Keeping this requirement in view, current experiments were

planned in mice and chicken embryos to investigate toxic effects and safety margin of *Euphorbia helioscopia*.

MATERIALS AND METHODS

Plant collection: *Euphorbia helioscopia* was collected from suburbs of Lahore city of Pakistan. After identification and authentication by a taxonomist, its voucher specimen (1501) was deposited in herbarium of Botany Department Government College University, Lahore-Pakistan. Leaves and stem were separated and ground after drying under shade. Fine powder was used in extraction. Latex was collected by cutting stem into dried bottle and stored at room temperature till used in experimentation.

Preparation of extracts: Extract was prepared by two methods; 1) maceration [solvents: water and ethanol], 2) hot soxhlet extraction [solvents: petroleum ether, chloroform, and methanol]. Solvent was removed with rotary evaporator at 40°C. Methanol extract of leaves and latex were chosen for this study on the basis of their promising *in-vitro* antioxidant activity in our earlier study (Uzma *et al.*, 2014).

Preparation of samples: Samples of methanol leaves extract were prepared using distilled water. Latex was also diluted with distilled water into 1:1(v/v) ratio. Total solid contents and density of latex were determined and this calculation was used to prepare different concentrations of latex.

Teratogenicity in pregnant mice: The study was carried out after approval from Animal Ethics Committee of University College of Pharmacy (UCP), University of the Punjab (PU), Lahore-Pakistan, (Approval No.: AEC/UCP 1009/ 4313 Ph) on albino female virgin mice (n = 45) of 8-12 weeks old, weighing 25-30 grams. One male of the same strain was caged with five females. The animals were provided standard animal pellet feed and water *ad libitum*. The animals were provided 12 hours light and dark cycle, with $22 \pm 1^\circ\text{C}$ temperature and $55 \pm 5\%$ relative humidity (Nasrin *et al.*, 2012). Mice were kept in pathogen free cages with saw-dust bedding. The female mice were examined daily, to see the vaginal plug, in the early morning to see the occurrence of conception (Runner and Ladman, 1950). Those animals showing vaginal plug were isolated and the day of pregnancy was recorded. Then pregnant mice were given different doses (100, 300, 500, 700, 1000, 1500 and 2000 mg/kg, (groups I-VII) of aqueous solution of methanol extract of leaves (L.MT) and latex (group VIII) of *Euphorbia helioscopia* L by intra-peritoneal (IP) route on 7th day of gestation. The rationale behind selecting IP route was to achieve higher bioavailability over oral route of administration (Michael *et al.*, 2010). Control group (IX) was kept on

water and normal feed only. All the pregnant mice were allowed to give birth to litters which were observed to detect cleft palate, septal defect, limbs ectrodactyly/syndactyly and any other sign of abnormality.

Toxicity in chicken embryos: Five days old fertilized embryos (n = 60, or 5 per dose and 5 as control) of white leghorn chicken were purchased from Big Bird hatchery, Raiwind road, Lahore, Pakistan. Infertile eggs and dead embryos were discarded by observing in egg Candler. All the eggs were disinfected with 70% ethanol using single swipe of cotton. Under laminar flow hood, a window of approximately 3 inches (over the air sac, the broader end of egg) was made in each egg and 4 – 5mL albumin was removed from the egg with 10mL disposable syringe and 200 μL of different doses (10, 50, 80,100 and 120 μg , each) of L.MT (groups A-E) and latex (group G-K) were injected with micropipette along the inner side of egg shell by chorioallantoic membrane route. Five chicken embryos were inoculated with each dose of plant extract and then sealed with wax. In control groups, (F and L for L.MT and latex respectively) same dose of phosphate buffer saline was injected (Singh and Gupta, 1972). Five days old chicken embryos were selected to study developmental abnormalities as described by Singh and Sinha (1973). All the test sample solutions were passed through syringe filter (0.2 μm pore size) under laminar flow hood. After completing this procedure, eggs were placed in sterilized incubator that was preset at 37.5°C and having 60–70 percent humidity. The eggs were auto-rotated inside the incubator. After 48 hours incubation, eggs were removed from incubator and chilled in freezer. Live embryos were separated and observed for any abnormality.

Statistical analysis: For Statistical analysis, Trimmed spearman-karber (TSK) software, version 1.5 was used to calculate LD₅₀ of test samples.

RESULTS AND DISCUSSION

In present experiment conducted on pregnant mice, highest mortality (100%) was recorded in group that received 2000 mg/kg methanol leave extract intraperitoneally within 7 days following injection. In other two groups, treated with 1500 and 1000 mg/kg methanol extracts of leaves, mortality was 40 and 25 percent, respectively. Other doses used were safe and mortality was not observed. LD₅₀ (the concentration/dose that kills 50 % of the treated animals) was 1397 mg/kg with 95 % confidence limit (1139 mg/kg – 1714 mg/kg) calculated with trimmed spearman-karber (TSK) software. Different routes of drug administration may vary value of LD₅₀. The parameters observed in mice, Cleft Palate, Septal defect, Upper limb ectrodactyly, Lower limb ectrodactyly, Upper limb syndactyly and Lower limb syndactyly, showed no sign of abnormality.

Healthy and active litters were born from all lived pregnant mice (Fig. 1).

In case of chicken embryos, no mortality was observed at all selected doses of methanol extract of leaves and latex. On close observation of each inoculated chicken embryo, no abnormality was seen. Eyes, heart, limbs, crown and backbones of all the treated chick embryos were same and comparable with control group embryos (Fig. 2).

Pregnant females and baby feeding mothers consume natural products without consulting physician or health care personnel owing to trust in safety of herbs (Clarke *et al.*, 2007). The National Health Surveillance Agency (ANVISA) makes legislation, according to that each pharmaceutical company is bound to provide data on safety and efficacy of natural medicines (Turolla and Nascimento, 2006). During drug development, safety data of reproductive toxicity on at least two species is required (Katzung *et al.*, 2008). The chicken and mice embryos are best models for teratogenicity studies (George and McLone, 1995). However, chick embryo is the most suitable model to evaluate the teratogenic effects of various drugs (Gebhardt, 1972).

The safe, wise and rational use of drugs is of great interest in the current era. This interest is evident from the preclinical and clinical testing of new drugs

(either natural or synthetic) or medicinal agents throughout the world. Before the thalidomide disaster, no study was conducted on toxic potential of drugs in pregnant females. But now the importance of conducting research on teratogenic potential of drugs is well understood to prevent the big disaster again in the world.

The animals are best model for screening of teratogenic potential of new drugs in preclinical studies because the drug that is teratogenic in animals can cause teratogenicity in man also. Mice or rats are easily available and reproduce large number of litters in short time period; this is another reason for selecting them in understudied plant teratological studies. No earlier data are available on teratogenic effect of. Results of the present study revealed no indication of external malformation in litters or embryo of both species i.e. mice and chick embryos. Thus data clearly demonstrated absence of embryo-toxic as well as teratogenic effects of *Euphorbia helioscopia* in both species. All the registered marketed drugs are required to be tested in animals in pre-clinical study. Then for any therapeutic or adverse effects (phase III clinical trials) of drug development, a new drug is given to human beings to confirm and reproduce its effects that were observed in animals (Katzung *et al.*, 2008).

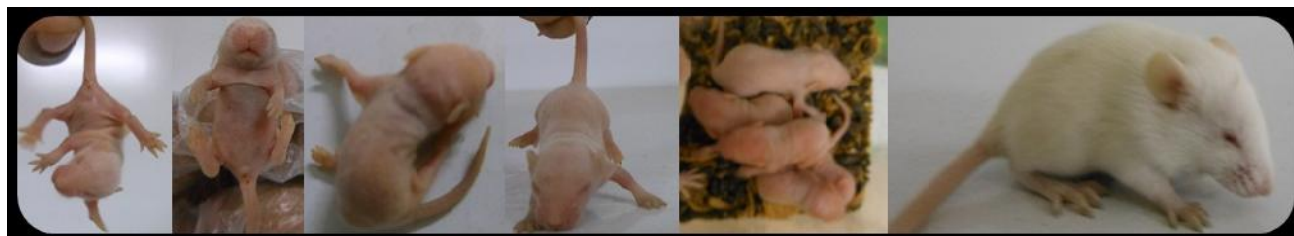


Fig. 1: Litters of mice-groups treated with methanol leaves extract and latex of *Euphorbia helioscopia* L, presenting normal growth and physical activity (Mixed Representative Figures)



Fig. 2: Seven days old chick embryos treated with different doses (10, 50, 80, 100 and 120 $\mu\text{g}/200 \mu\text{L}$) of aqueous solution of leaves methanol extract (Groups A-E & F was control group) and latex (Groups G-K & L was control group) of *Euphorbia helioscopia* L, showing normal growth.

Conclusion: It is concluded from the study that *Euphorbia helioscopia* L lacks the potential to cause teratogenic and embryotoxic effects in mice and chicken embryos at doses applied in the experimentation.

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