

Full Length Research Paper

A comparison study of the effects of *Echinacea purpurea* extract and mesna on cyclophosphamide-induced macroscopic fetal defects in rats

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Accepted 13 November, 2009

There are reports that the teratogenic effects of cyclophosphamide can be decreased by application of antioxidant drugs and stimulation of maternal immune system. *Echinacea* extract has being found to be antioxidative and immunomodulatory. Mesna (Sodium 2-mercaptoethane sulfonate) is used for decreasing side effect of cyclophosphamide, especially hemorrhagic cystitis. In this study, the prophylactic effects of mesna and *Echinacea* extract on teratogenic effects of cyclophosphamide were compared. This study was performed on 32 pregnant rats that were divided into four groups. The first group (control group) received normal saline and the other groups received cyclophosphamide (15 mg/kg intraperitoneally) at 13th day of gestation. Mesna and *Echinacea purpurea* extract were administrated at dose of 100 and 400 mg/kg intraperitoneally, respectively, 12 h later after cyclophosphamide injection, in two groups. Examination of fetuses was carried out in the 20th day of gestation and after determination of gross malformations, they were stained by Alizarin red - Alcian blue method. Cleft palate incidence was 38.46, 30.77 and 14.28% in fetuses of rats that received only cyclophosphamide, cyclophosphamide with mesna and cyclophosphamide with *Echinacea* extract, respectively. In addition, skeletal anomalies incidence including limbs, vertebra, sternum and scapula defects were decreased by *Echinacea* extract. Thus, *Echinacea purpurea* has better prophylactic effect than mesna on incidence of cyclophosphamide-induced cleft palate and some other anomalies.

Key words: Cyclophosphamide, macroscopic fetal defects, mesna, *Echinacea purpurea*, rats.

INTRODUCTION

Some chemical agents and drugs can induce teratogenic effects and abortion (Giavini and Menegola, 2004). Developmental defect is one of the major problems of health. In USA for example, 3 - 5% of fetuses have congenital abnormality. Nearly 2 - 3% of developmental defects in the general population are related to teratogenic agents (Finell, 1999) of which 1% of them are caused by use of drugs during pregnancy period (De santis et al., 2004). Although 40 agents have being found to be teratogenic to human fetuses, more agents are teratogenic in laboratory animals. Valproic acid, cyclophosphamide, methylnitrous urea and phenytoin are the

the best known teratogenic drugs in humans and laboratory animals (Orup et al., 2003; Prater et al., 2004).

Several studies have shown that, the stimulation of maternal immune system can decrease or prevent drug-induced embryonic abnormalities (Halody et al., 2000 and 2002). For example, in one study, macrophage activation decreased the incidence of cleft palate and digital and tail anomalies in fetuses of mice that received urethane and methylnitrous urea (Halody et al., 2002). In another study, interferon gamma reduced urethane - induced cleft palate and granulocyte-colony stimulating factor decreased cyclophosphamide -induced distal limb abnormalities in mice (Syska et al., 2004).

Cyclophosphamide as an alkalinizing agent is used for treatment of cancer and prevent of rejection of tissue transplantation. Cyclophosphamide has several toxic effects including hemorrhagic cystitis. Metabolites of cyclophosphamide, especially acrolein modulates its toxic effects

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(Hardman and Limbird, 2001). Mesna (sodium 2-mercaptoethane sulfonate) is used for decreasing toxic effects of cyclophosphamide in patients (Win et al., 1999). In addition, mesna was effective in lowering cyclophosphamide-induced malformation in rats (Slott and Hales, 1986).

Echinacea potentiates immune system and is applied in the prevention or treatment of some diseases including influenza and common cold. Echinacea extract activates macrophage, polymorphonuclear leukocytes and natural killer cells (Barrett, 2003). The beneficial effect of *E. purpurea* extract on decreasing phenytoin-induced cleft palate in mice was reported by Khaksary et al. (2006).

In the present study, the prophylactic effect of mesna and *E. purpurea* extract was compared on cyclophosphamide-induced macroscopic fetal defects in rats.

MATERIALS AND METHODS

Dried aerial parts of *E. Purpurea* were purchased from Goldaruo Co. Isfahan, Iran. The plant was taxonomically identified at the Department of Botany, School of Agriculture, Shahid Chamran University, Ahvaz, Iran. Plant was powdered using a grinder (MSE, England). 100 g of the powder was placed in a beaker and 1000 ml of 70% ethanol added. The mixture was left in room temperature for 3 days. The extract was separated and remaining plant was extracted by more ethanol after 2 days. The extract was filtered by Wattman (No. 10) filter paper and concentrated by vacuum evaporation and then concentrated extract was dried by oven at low temperature.

Male and female healthy wistar rats, 6 - 8 weeks of age, weighing 180 - 200 g were purchased (Razi Institute, Karadje, Iran) and housed individually (males) or at 10 per polycarbonate cage (female) for a 2-week acclimation period. Rats were feed 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%.

Male and female rats were housed together. Pregnant females were divided into four groups of eight animals each. The first group received normal saline (10 ml/kg), the second group received cyclophosphamide (15 mg/kg) (Slott and Hales, 1986), the third group received cyclophosphamide (15 mg/kg) along with 12 h later mesna (100 mg/kg) (Slott and Hales, 1986) and the fourth group received cyclophosphamide (15 mg/kg) along with 12 h later extract of *E. purpurea* (400 mg/kg) (Khaksary et al., 2006). All drugs were diluted in distilled water, and then were administrated intraperitoneally.

The animals were sacrificed by cervical dislocation at the 20th day of gestation and fetuses were collected and numbered. The weight and length were measured and gross malformations determined. Fetuses were stained by Alizarin red-Alcian blue method (Yolanda, 1993) and investigated by stereomicroscope for skeletal defects. The incidence of macroscopic defects was determined and was compared in groups.

Statistical significance between groups was determined using SPSS program and compared by one factor analysis of variance (ANOVA). The minimum level of significance was $P < 0.05$.

RESULTS

The result showed that there were no observed macro

scopic anomalies in the control animals. In the control group, palatal closures of fetuses were normal (Figure 1A). Cyclophosphamide induced cleft palate at 38.46% incidence (Figure 1B). However, Mesna reduced incidence of cyclophosphamide induced cleft palate to 30.77%, while *E. purpurea* extract reduced it to 14.28%. In group two, 32.6% of fetuses died. Percentage of reabsorbed fetuses was 40.8, 51.85 and 55.3 in group 2, 3 and 4, respectively.

Exencephaly, omphalocele, open eye, brachygnathia and several anomalies in vertebrae, sternum, scapula and limbs were observed (Figure 2) with their incidence as shown in Table 1. Their incidences (except exencephaly) were decreased by Echinacea extract. Mean weight ($P < 0.043$) and length ($P < 0.02$) were significantly decreased in group which received only cyclophosphamide (group two). The means of weight ($P < 0.044$) in the group (group three) that received mesna were significantly greater than the group which received cyclophosphamide only (group two). The mean weight and length in group that received mesna did not significantly differ with control group. The mean weight ($P < 0.049$) and length ($P < 0.001$) were significantly decreased in the group (group four) that received Echinacea extract and significantly differed in comparison to the control group (Figures 3 and 4).

DISCUSSION

Several studies have verified that maternal immune stimulation can reduce teratogenic anomalies (Ivnitsky et al., 2001). Mechanisms of this effect remain unclear, but it is thought that the fetal gene expression could have been modulated (Halody et al., 2002). Alternatively, enhancing antioxidative effects can protect fetuses against phenytoin teratogenicity (Syska et al., 2004). Sharova et al. (2004) showed that interferon-gamma and Freund's complete adjuvant reduced severity of the urethane-induced cleft palate in mice (Sharova et al., 2002). In the present study, the prophylactic effects of mesna and Echinacea on cyclophosphamide-induced macroscopic fetal defects were compared in rats. Both mesna and Echinacea reduced the severity of incidence of cleft palate. Echinacea was able to induced greater decreased incidence of cleft palate than mesna, however, mesna had better protective effect than Echinacea on weight and length of fetuses.

Slott and Hales (1986) evaluated the effect of mesna on cyclophosphamide-induced teratogenicity. They administered cyclophosphamide at doses of 10 and 15 mg/kg in rats in 13th day of gestation. They observed that cyclophosphamide can induce teratogenicity in 50 and 100% of fetuses at a dose of 10 and 15 mg/kg, respectively (Slott and Hales, 1986). They determined fetal defects including hydrocephaly, omphalocele, open eye, brachygnathia and limb defects. These anomalies

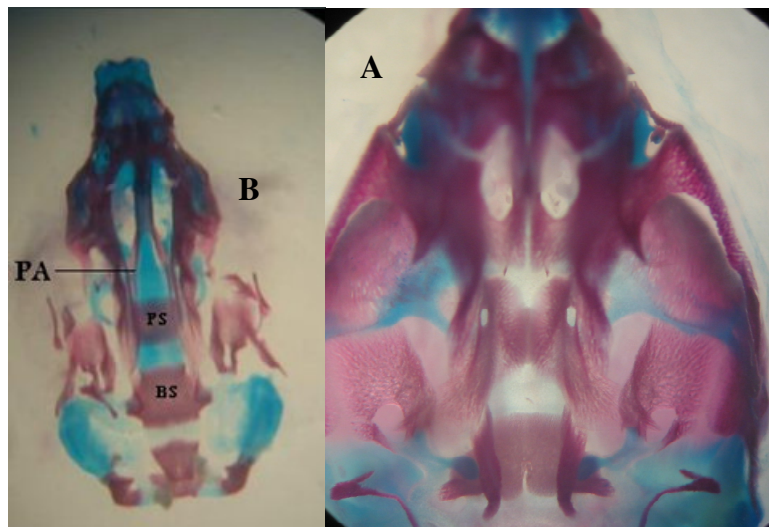


Figure 1. Ventral view of the skull of GD 20 fetal rats. A) Normal palatine bone B) Cleft palate induced by phenytoin (arrow) which stained with Alizarine red-Alcian blue. PS = palatine; BS = sphenoid.

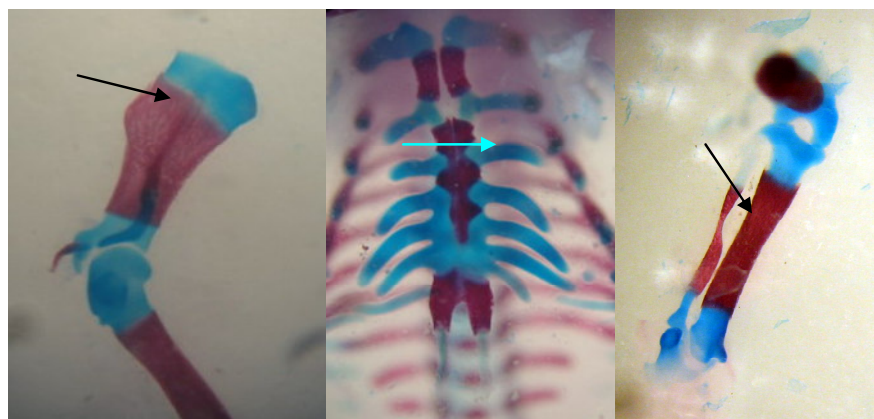


Figure 2. Some skeletal defects in fetuses of rats. Curved spine of scapula and absence of the deltoid tuberosity (left); split xiphoid process (middle); curved fibula (right).

Table 1. Incidence of anomalies in fetuses of groups. Group1 (control) without macroscopic anomalies; Group2 was received cyclophosphamide; group3 was received cyclophosphamide + mesna; group4 was received cyclophosphamide + Echinacea extract.

Anomaly	Incidence (%)		
	Group 2	Group 3	Group 4
cleft palate	38.46	30.77	14.28
Exencephaly	62.06	92.3	100
Omphalocele	11.11	7.69	4.76
Open eye	23.7	84.6	-
Brachygnathia	23.07	53.8	-
sternum defects	37.93	53.8	33.3
Vertebral defects	65.5	100	61.9
Limbs defects	100	100	76.19

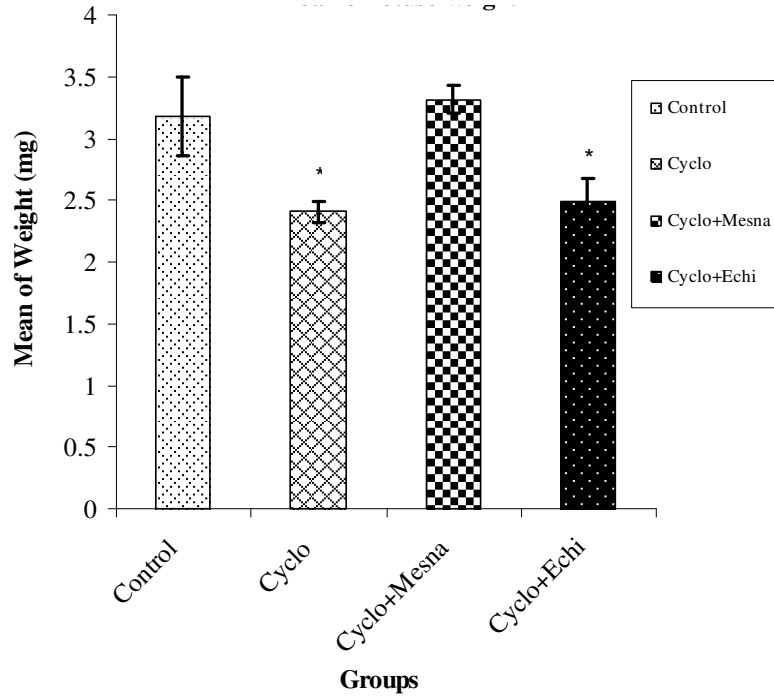


Figure 3. Weight (mean \pm SE) of fetuses treated with normal saline and test compounds. Control = normal saline (1 ml/100 g IP); Cyclo = cyclophosphamide (15 mg/kg IP); Cyclo + Mesna = cyclophosphamide + mesna (100 mg/kg IP); Cyclo + Echi = cyclophosphamide + Echinacea (400 mg/kg IP). n = 8; * Significant difference with phenytoin group ($p < 0.05$).

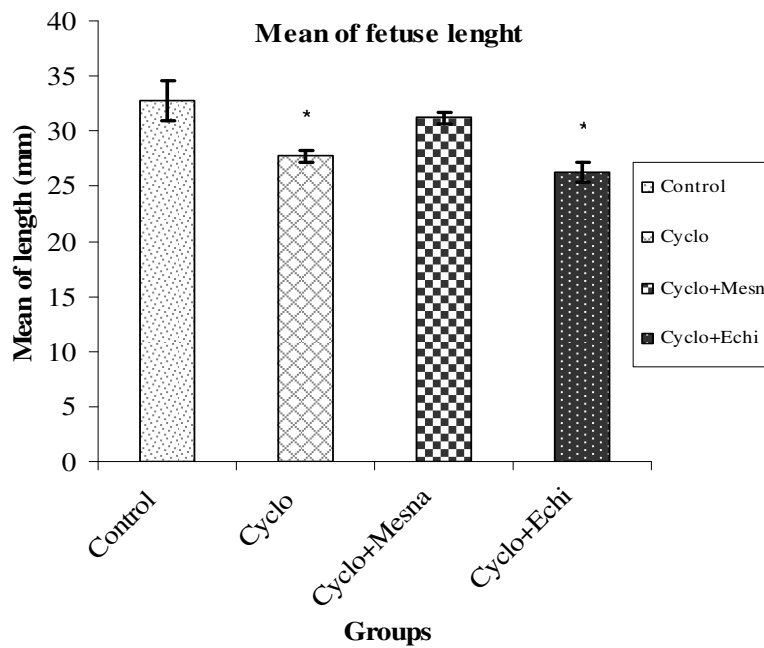


Figure 4. Length (mean \pm SE) of fetuses treated with normal saline and test compounds. Control = normal saline (1 ml/100 g IP); Cyclo = cyclophosphamide (15 mg/kg IP); Cyclo + Mesna = cyclophosphamide + mesna (100 mg/kg IP); Cyclo + Echi = cyclophosphamide + Echinacea (400 mg/kg IP). n = 8; * Significant difference with phenytoin group ($p < 0.05$).

were decreased by mesna with 30 mg/kg.

Cytokines have been shown to mediate cyclophosphamide-induced neurotoxicity (Halody et al., 2000). Granulocyte-macrophage colony stimulating factor (GM-CSF) as cytokine and injection of leukocytes decreased cyclophosphamide-induced teratogenicity including limb defects (Halody et al., 2000; Sharova et al., 2002).

E. purpurea stimulates various immune cells including macrophages and natural killer cells. It has anti-inflammatory effects (Barrett, 2003). In one study, *E. purpurea* root increased levels of interleukin 1 and 6, tumor necrosis factor and antibody production more than extracts of *Echinacea angustifolia* and *Echinacea pallida* (Sharek et al., 1996). Bukovsky et al. (1995) reported that ethanolic extract of *E. purpurea* increased activity of mouse peritoneal macrophage following 5 days exposure (Bukovsky et al., 1995). In one double-blinded study using 24 men, oral administration of *E. purpurea* extract increased polymorphonuclear (PMN) phagocytic activity for 5 days that reached its peak levels at the 5th day. Moreover, Echinacea has anti-oxidative and free-radical scavenging activity (Mishima et al., 2004). Echinacea is used for treatment of acute upper respiratory infections including common cold and influenza (Barrett, 2003).

In the present study, *E. purpurea* extract had prophylactic effect on incidence of cyclophosphamide-induced skeletal anomalies. Mesna metabolites of cyclophosphamide including acrolein and reduces its side effects. Effect of cyclophosphamide on teratogenicity is mediated indirectly by inducing oxidative stress. Thus, *E. purpurea* can decrease anomalies from cyclophosphamide by antioxidative or immunostimulant effect.

ACKNOWLEDGEMENT

The authors wish to express their gratitude to the research council of Shahid Chamran University for their financial support.

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