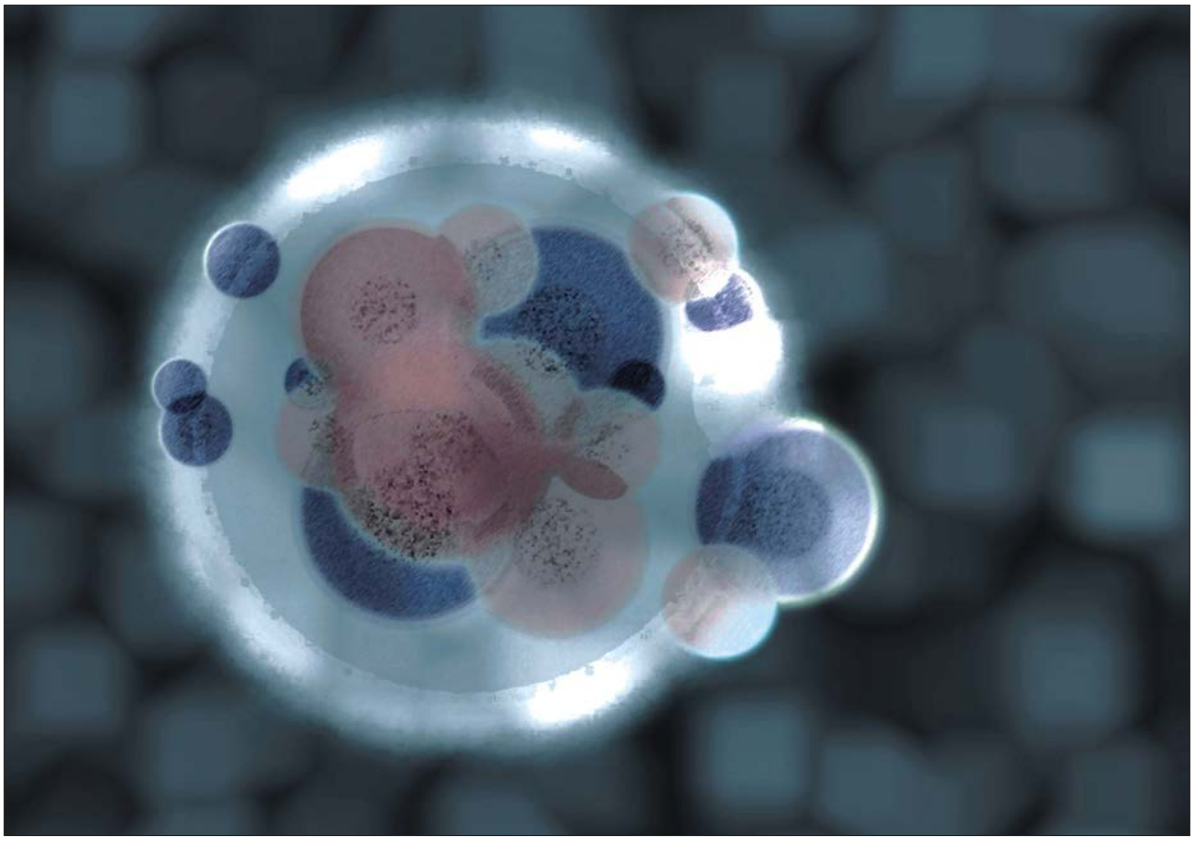

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A Comparison Study of Effects of Vitamin E and Silymarin on Phenytoin-Induced Cleft Palate in Rats

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Abstract: In this study, the prophylactic effects of vitamin E and silymarin on teratogenic effects of phenytoin was compared. This study was performed on 32 pregnant rats that were divided into four groups. The first group (control group) received normal saline intraperitonealy and the other groups (test groups) received phenytoin (75 mg kg^{-1} b.wt.) intraperitonealy) at 10-11th day of gestation. Vitamin E and silymarin were administrated at dose of 100 mg kg^{-1} b.wt. intraperitonealy, respectively, in along with, in two groups. Fetuses were carried out in 20th day of gestation and after determination of weight and length; they were stained by Alizarin Red-Alcian Blue method. Cleft palate incidence was 16.66, 5.55 and 2.77% in fetuses of rats that received only phenytoin, phenytoin with silymarin and phenytoin with vitamin E, respectively. Mean weight and length of fetuses of animals that received normal saline was significantly greater than those received vitamin E and silymarin. It is concluded that vitamin E can decrease oxidative stress more than silymarin and has better prophylactic effect on incidence of phenytoin- induced cleft palate.

Key words: Phenytoin, cleft palate, vitamin E, silymarin, rat

INTRODUCTION

Some chemical agents and drugs can induce teratogenic effects and abortion (Giavini and Menegola, 2004). Developmental defects are a major health problem as in the USA 3-5% of fetuses has congenital abnormality. Nearly 2-3% of developmental defects in the general population are related to teratogenic agents (Finnell, 1999). De Santis *et al.* (2004) also estimated that defects attributable to drug therapy represent about 1% of congenital defects of known etiology. Although, 40 agents are teratogenic for human fetuses, more agents are teratogenic in laboratory animals. Valperoic acid, cyclophosphamide, methylnitrous urea and phenytoin are the best known teratogenic drugs in human and laboratory animals (De Santis *et al.*, 2004; Orup *et al.*, 2003; Prater *et al.*, 2004; Syska *et al.*, 2004).

Phenytoin as hydantoin derivative is used for control of epilepsy. It is believed that phenytoin produces anomalies in 34% of fetuses which are exposed to it (Winn and Wells, 1999). It has been suggested that phenytoin teratogenicity is induced by embryonic hypoxia with vascular disruption as tissue necrosis as a result of ischemic damage and/or reactive oxygen species generation at reoxygenation (Damelsson *et al.*, 1997). Phenytoin is thought cause chorionic intrauterine hypoxia/ischemia and embryo-fetal toxicity via., reactive molecular oxygen intermediates. The pathology of oxidative stress can be preventing by antioxidants known to be effective in treating conditions associated with oxidative damage (Brogaard and Clausen, 1997; Syska *et al.*, 2004; Wells and Winn, 1996).

In the other hands, vitamin E, a natural antioxidant, is believed to help prevent diseases associated with oxidative stress (Kappus and Diplock, 1992). Vitamin E is considered safe in pregnancy, although experiments evaluating the safety of high-doses vitamin E treatment in pregnancy have not been reported by Cohen-Kerem and Koren (2003).

Silymarin, the mixture of flavonolignans extracted from blessed milk thistle (*Silybum marianum*) is a scavenger of radicals, such as hydroxyl, superoxide and hydrogen peroxide (H_2O_2) and increases SOD and decrease lipid peroxidation (Oliveira *et al.*, 2001).

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In present study, prophylactic effect of silymarin and vitamin E was compared on phenytoin-induced cleft palate in rats.

MATERIALS AND METHODS

This research project was conducted from starting date 2007/5/1 to 2008/3/10 ending date.

Silymarin powder (Sigma, USA) and phenytoin and vitamin E (Darupakhsh, Iran) were purchased.

Male and female healthy rat of Wistar strain, 10-12 weeks of age, weighing 200-250 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 (Shushtar Co., Iran) and tap water and kept in animal house of Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz. A 12 h light: 12 h dark cycle was maintained. Room temperature was at 23±2°C with a relative humidity of 45-55% which measured by commercial apparatus Jual Co.

One male rat was housed with four females overnight between 1700 and 0900 h. Pregnancy was ascertained the next morning by presence of a vaginal plug and this time was designated as gestational day (GD) 1. Pregnant animals were divided into four groups (n = 8) and treated as follow:

First group received normal saline (10 mL kg^{-1} b.wt.), the second group received phenytoin (75 mg kg^{-1}) (Zhu and Zhou, 1989), the third group received phenytoin (75 mg kg^{-1} b.wt.) and along with it silymarin (100 mg kg^{-1} b.wt.) (Oliveira *et al.*, 2001) and the fourth group received phenytoin (75 mg kg^{-1}) and along with it vitamin E (100 mg kg^{-1} b.wt.) (Viana *et al.*, 1996). All drugs were administrated intraperitonealy.

The animals were sacrificed by cervical dislocation at 20th day of gestation. Following laparatomy, the uterus was exteriorized and the number and location of fetuses and resorptions were noted, then their weight and length (crown-rump length) were measured. Fetuses were stained by Alizarin red-Alcian blue method (Kimmel and Trammel, 1981) and investigated by stereomicroscope (Nikon, Japan) for cleft palate. The incidence of cleft palate was determined.

Statistical significance between groups was determined using SPSS program and compared by one way analysis of variance (ANOVA) with post hock LSD. Binomial data were examined using the chi-square test. The minimum level of significance was $p < 0.05$.

RESULTS AND DISCUSSION

There were not any absorbed and death fetuses from the treated animals. Total number of collected fetuses from groups 1, 2, 3 and 4 were 64, 81, 77 and 61, respectively.

In control group, palatal closures of fetuses were normal (Fig. 1a). Phenytoin induced cleft palate at 16.66% incidence (Fig. 1b). Silymarin reduced incidence of pheytoin-induced cleft palate to 5.55%, but vitamin E reduced it to 2.77 % (Fig. 2). The means of weight in group that received vitamin E was lesser than the groups received normal saline (Fig. 3). The means of weight in group that received vitamin E was greater than the groups received silymarin (Fig. 3). The means of weight in groups that received silymarin was lesser than the

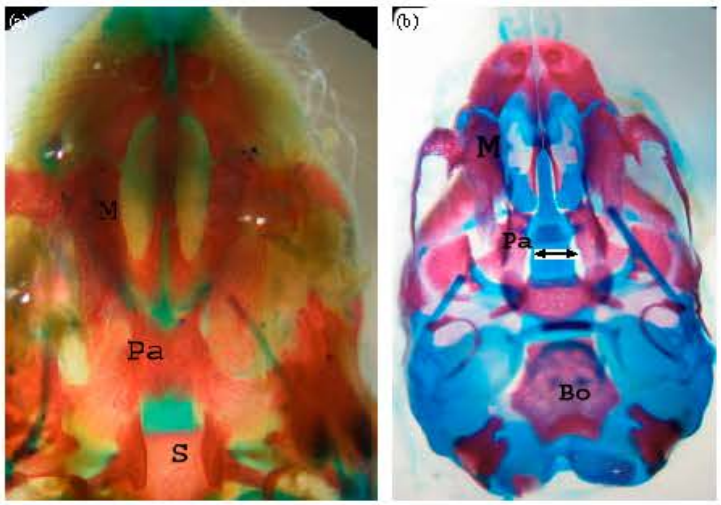

Fig. 1: Ventral view of skull of GD 19 fetal rat. (a) Normal palatine bone and (b) Cleft palate induced by phenytoin (arrow) which stained with Alizarin red- Alcian blue. M: Maxilla, Pa: Palatine, S: Sphenoid and Bo: Basioccipital

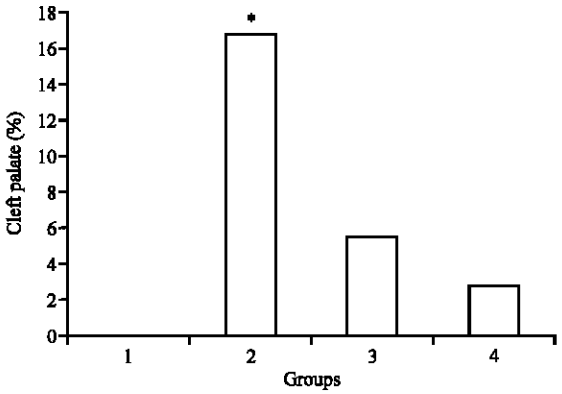

Fig. 2: Incidence (%+ SE) of cleft palate of normal saline and test groups. 1: Normal saline (10 mL kg^{-1} i.p.); 2: Phenytoin (75 mg kg^{-1} i.p.); 3: Phenytoin+silymarin (100 mg kg^{-1} i.p.); 4: Phenytoin+vitamin E (100 mg kg^{-1} i.p.). n = 8; *Significant difference when compared with other groups ($p<0.05$)

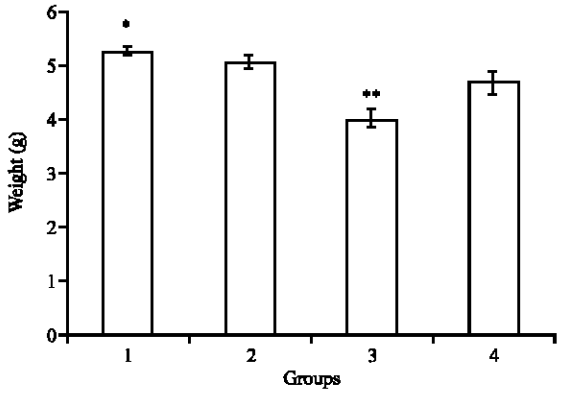

Fig. 3: Weight (Mean±SE) of fetuses of normal saline and test groups: 1: Normal saline (10 mL kg^{-1} i.p.); 2: Phenytoin (75 mg kg^{-1} i.p.); 3: Phenytoin+silymarin (100 mg kg^{-1} i.p.); 4: Phenytoin+vitamin E (100 mg kg^{-1} i.p.). n = 8; *Significant difference when compared with phenytoin+silymarin and phenytoin+vitamin E groups; **Significant difference when compared with other groups ($p<0.05$)

groups received only phenytoin and normal saline (Fig. 3). The mean of length in group that received normal saline was greater than the other groups (Fig. 4).

In the present study for first time, the prophylactic effects of vitamin E and silymarin on phenytoin- induced cleft palate were compared in rats. Both vitamin E and silymarin reduced the frequency of incidence of clefting. Vitamin E was greater decreased incidence of cleft palate than silymarin, but the difference was not significant.

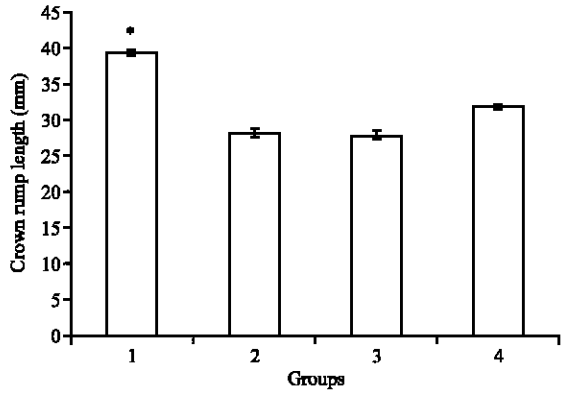

Fig. 4: Crown rump length (Mean±SE) of fetuses of normal saline and test groups: 1: Normal saline (10 mL kg^{-1} i.p.); 2: Phenytoin (75 mg kg^{-1} i.p.); 3: Phenytoin+silymarin (100 mg kg^{-1} i.p.); 4: Phenytoin+vitamin E (100 mg kg^{-1} i.p.). n = 8; *Significant difference when compared with other groups ($p<0.05$)

In vivo and *in vitro* studies indicated that phenytoin initiated teratogenesis may involve, at least in part, peroxidase-catalysed bioactivation of phenytoin to a reactive free radical intermediate. If not detoxified, it may initiate oxidative stress leading to oxidation of embryonic lipids, proteins and DNA (Winn and Wells, 1997).

A number of observation suggest that detoxification of a xenobiotic free radical intermediate with antioxidants may provide important embryo protection (Wells *et al*., 1997). Winn and Wells (1999) demonstrated the teratologic importance of antioxidant balance: maternal administration of the antioxidative enzyme catalase enhanced embryonic activity and inhibited phenytoin teratogenicity. In the other hand, enhancing antioxidative effects can protect fetuses against phenytoin teratogenicity (Winn and Wells, 1999).

Administration of vitamin E to pregnant diabetic animals decreases the rate of embryonic malformations and increases their body weight and enhances their maturation (Viana *et al*., 1996). Boskovic *et al.* (2005) reported that consumption of high doses of vitamin E during the first trimester of pregnancy was not associated with an increased risk for major malformations, but may be associated with a decrease in birth weight. While the other hand, vitamin E supplementation of the ewe resulted in a significant increase in lamb birth weight (Capper *et al*., 2005).

In present study, vitamin E and silymarin had prophylactic effect on incidence of phenytoin-induced cleft palate, could be related to its antioxidant effect.

In conclusion, probably phenytoin influences immune system that produces teratogenic effects including cleft palate. Effects of phenytoin on immunosuppression are mediated indirectly by inducing oxidative stress (Winn and Wells, 1999). While, vitamin E is more effective than silymarin in decreasing incidence phenytoin-induced cleft palate in fetuses of rats, but it is not significant.

The present study compared the effects of silymarin and vitamin E for the first time in rats. The results show that silymarin and vitamin E produce a similar reduction in phenytoin-induced cleft palate in rats. The protective effect of silymarin in phenytoin-induces cleft palate in rat may, at least in part, be due to its antioxidant activity.

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