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Study of teratologic occurrence in neonatal rat skeletal system under the effect of zonisamide and sodium valproate

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Abstract

Accordingly, in this study, the effects of teratology on a fetal rat skeletal system that received zunsyamide and valproate sodium were evaluated. In conjunction with zonisamide teratogenic effects on the fetus during pregnancy, there is not much information available. High dose of zonisimide (200 mg / kg) was gavaged by animal feeding method to the first intervention group on the ninth and twelfth days of gestation , 600 mg / kg of Sodium valproate was gavaged by animal feeding method (to the second intervention group on the ninth and twelfth days of gestation. The rats in the control group received normal saline on the same days. After the conception, all born babies were kept in an ethanol alcohol solution 96 °. In order to study the newborn's skeleton system, the red alizarin staining method was used. The obtained data were expressed as Mean ± SEM and analyzed using the ANOVA method. The Abnormalities of Wavy Rib, Abnormal position , scoliosis, and the deviation of the femur and fibula were observed. The position of the sternum and tail vertebrae were normal .Generally, the use of sodium valproate and zonisamide in the ninth to twelfth day of gestation and causes growth disorder in fetus such that creates the reducing impacts in the growth parameters of newborn rats. The use of zonisamide during pregnancy can be considered as a risk factor for the development of newborn that its consumption during organogenesis can cause malformations in the newborn rat.

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Introduction

Teratology is a branch of the embryology that studies the causes, mechanisms and patterns of abnormal development. The main topic of Teratology is that the particular stages of embryonic development are more vulnerable compared with other stages of teratogenesis. Some of the teratogenes pass through the placental barrier and cause adverse effects on the development; so, leading abnormalities Although, may improve the condition of the mother as a drug, it is toxic to the fetus, causing various effects (Moor *et al.*,1998, Orahilly *et al.*,1996). Zonisamide is one of the most effective anti-epileptic drugs that is frequently used.

In conjunction with zonisamide teratogenic effects on the fetus during pregnancy, there is not much information available. Low molecular weight and its inability of binding to plasma proteins strengthen the possibility of crossing the placental barrier by the drug. FDA has classified the drug in group C. This means that there isn't much evidence of teratogenic effects of this drug on humans. During pregnancy, the drug is placed in class C and its use is subscribed only when its benefit is more than its probable risks to the fetus. Any drug can have some risks for pregnancy and since all risks are not known, it must be avoided during pregnancy.

Valproic acid is an anti-epileptic drug which was allowed to use from 1976 in Europe and 1978 in the United States, and since then has been used extensively over the past two decades in treating patients with epilepsy. During recent years, a considerable number of clinical studies and researches have linked the use of sodium valproate with a low risk of fetal malformations (Ehlers *et al.,* 1992, Kaufman *et al.,* 1992). Some researchers also found that the drug caused some abnormalities in organs of mice (Ehlers *et al.,* 1992, Okada *et al.,* 1997). Experiences have suggested that the use of VPA as antiepileptic drug during pregnancy should be considered carefully. Previous studies have shown that the drug during early pregnancy can pass the placental obstacle and enters the blood circulation; so, can have adverse effects on the fetus body structure forming depending on the amount of received drug and its impact time (Meador *et al.,* 2006, Vajda *et al.,* 2005). Accordingly, in this study, the effects of teratology on a fetal rat skeletal system that received zonisamide and valproate sodium were evaluated.

Materials and methods

For this purpose, 36 intact Wistar rats were used. After intercourse and observation of vaginal plaque the zero day of pregnancy was determined (Cayohyeong *et al.,* 2004, Hafez *et al.,* 1970, Ognio *et al.,* 2003), then they were divided into 3 equal groups (Two experimental groups and one control group), they were kept under the standard conditions of place, enough food, water, light, and heat. The first group: intervention with zonisamide in one-day infants' samples, the second group: intervention with sodium valproate in one- day infants' samples, the third group: control using normal saline in infants samples born from normal mothers. The rats were caged in a laboratory animal's cage at ambient temperature of at 25 (2°c for 24 hours. High dose of zonisimide (200 mg / kg) was gavaged by animal feeding method to the first intervention group on the ninth and twelfth days of gestation (organigenesis stage) (Abdulrazzaq *et al.,* 1997, Ognio *et al.*, 2003). 600 mg / kg of Sodium valproate was gavaged by animal feeding method (Cayohyeong *et al.,* 2004) to the second intervention group on the ninth and twelfth days of gestation. The rats in the control group received normal saline on the same days. After the conception, all born babies were kept in an ethanol alcohol solution 96 °. in order to study the newborn's skeleton system, the red alizarin staining method was used.

After red alizarin staining in which all bones was seen in red color, the samples were placed under stereomicroscope and the long bones were measured using a calibrated lens.

Data analysis

The obtained data were expressed as Mean ± SEM

and analyzed using the ANOVA method. The multiple Tukey's comparison test was used to compare differences between groups. P <0.05 was considered to determine the significance between groups.

Results

The morphology results of the neonatal rats' long bones.

The average length of the humerus

The average length of the Humerus in the zonisamide intervention group, sodium valproate group, and control group was 5.752±0.02, 4.544±0.03, and 6.156±0.03, respectively, such that there was no significant difference between intervention groups (P> 0.05), but a significant difference was seen between intervention and control groups (P< 0.0001) (Fig. 1).

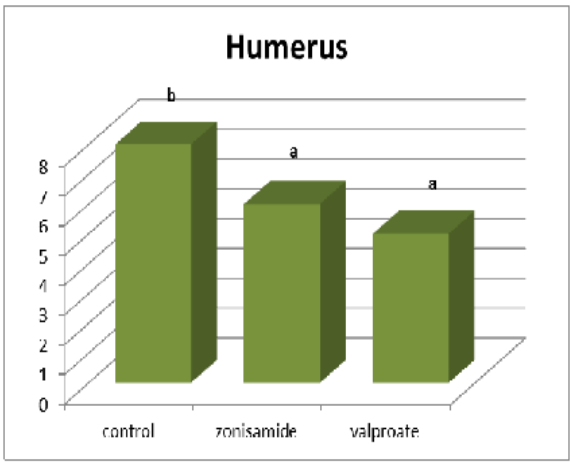

Fig. 1. Comparison between the newborns' humerus in the zonisamide, sodium valproate and control groups.
*The different letters Indicate a significant difference between the groups (P < 0.0001).

The average length of the radius

The average length of the Humerus in the zonisamide intervention group, sodium valproate group, and control group was 4.980±0.07, 4.544±0.03, and 6.156±0.03, respectively, such that there was no significant difference between intervention groups (P> 0.05), but a significant difference was seen between intervention and control groups (P< 0.0001) (Fig. 2).

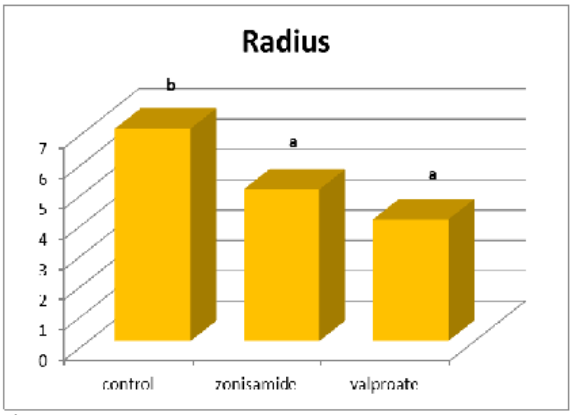

Fig. 2. Comparison between the newborns' radius in the zonisamide, sodium valproate and control groups.
*The different letters Indicate a significant difference between the groups (P < 0.0001).

The average length of the ulna

The average length of the ulna in the zonisamide intervention group, sodium valproate group, and control group was 5.566±0.04, 5.190±0.18, and 7.140±0.09, respectively, such that there was no significant difference between intervention groups (P> 0.05), but a significant difference was seen between intervention and control groups (P< 0.0001) (Fig. 3).

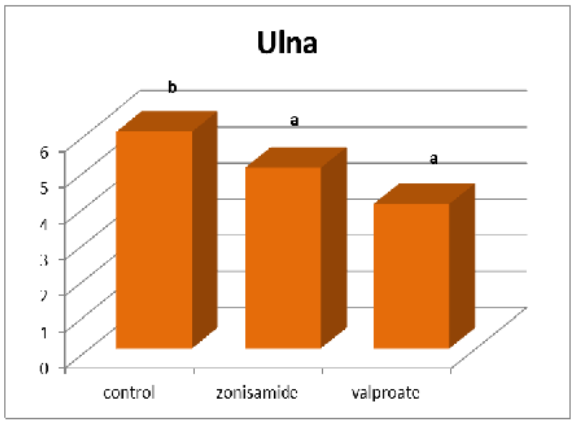

Fig. 3. Comparison between the newborns' ulna in the zonisamide, sodium valproate and control groups.
*The different letters Indicate a significant difference between the groups (P < 0.0001).

The average length of the femur

The average length of the femur in the zonisamide intervention group, sodium valproate group, and control group was 5.846±0.04, 5.216±0.02, and 6.470±0.15, respectively, such that there was no significant difference between intervention groups

(P> 0.05), but a significant difference was seen between intervention and control groups (P< 0.0001) (Fig. 4).

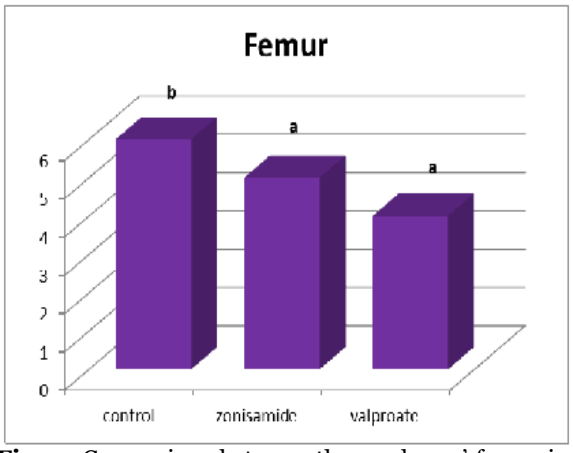

Fig. 4. Comparison between the newborns' femur in the zonisamide, sodium valproate and control groups. *The different letters Indicate a significant difference between the groups (P < 0.0001).

The average length of the tibia

The average length of the tibia in the zonisamide intervention group, sodium valproate group, and control group was 5.846±0.04, 5.216±0.02, and 6.470±0.15, respectively, such that there was no significant difference between intervention groups (P> 0.05), but a significant difference was seen between intervention and control groups (P< 0.0001) (Fig. 5).

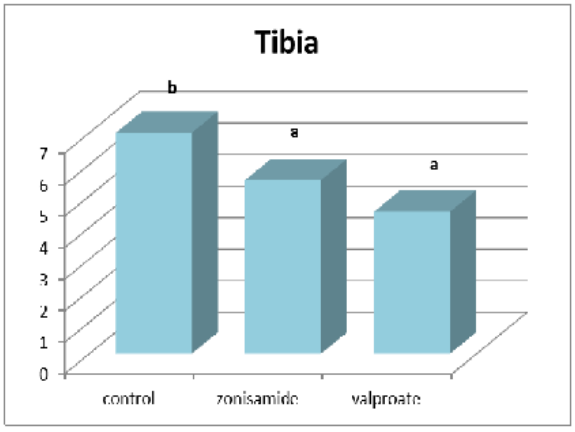

Fig. 5. Comparison between the newborns' tibia in the zonisamide, sodium valproate and control groups. *The different letters Indicate a significant difference between the groups (P < 0.0001).

The average length of the fibula

The average length of the fibula in the zonisamide intervention group, sodium valproate group, and control group was 5.570±0.04, 5.074±0.06, and 7.11±0.07, respectively, such that there was no significant difference between intervention groups (P> 0.05), but a significant difference was seen between intervention and control groups (P< 0.0001) (Fig. 6).

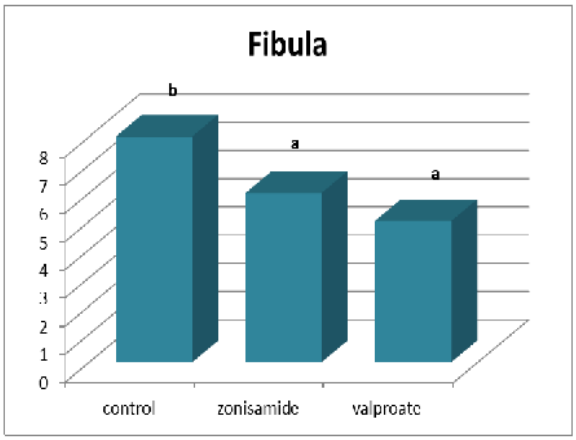

Fig. 6. Comparison between the newborns' fibula in the zonisamide, sodium valproate and control groups. *The different letters Indicate a significant difference between the groups (P < 0.0001).

Observations of the fetus and newborns' skeletal system morphology stained with rule-alizarin method.

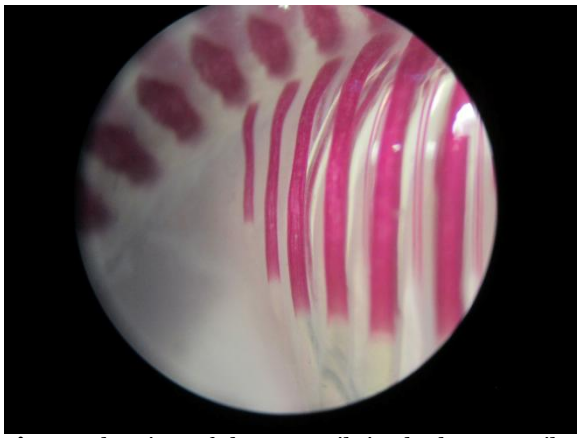

Fig. 7. The view of the wavy rib in the last two ribs (ribs 12 and 13). In sodium valproate Intervention group- red alizarin- magnification 25x.

In the studies of the sample was prepared by the red alizarin method the intervention control groups had some abnormalities. The Abnormalities of Wavy Rib (Fig.7), Abnormal position (Fig.8), scoliosis, and the deviation of the femur and fibula were observed. The

position of the sternum and tail vertebrae was normal (Fig.9).

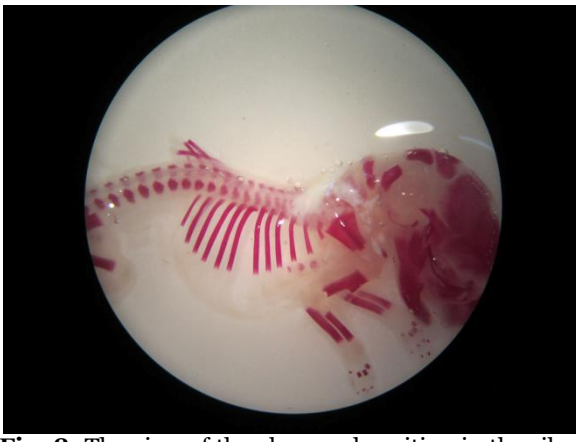

Fig. 8. The view of the abnormal position in the ribs 7 and 8. In sodium valproate Intervention group- red alizarin- magnification 15x.

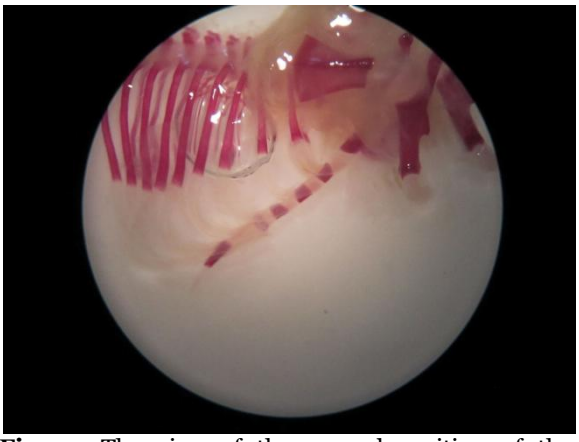

Fig. 9. The view of the normal position of the sternum. In Intervention group- red alizarin- magnification 20x.

Discussion

Considering the obtained morphometric results from the Long bones, there was a significant reduction in the average of the mean bone length in anterior and posterior extremities in the effect of zonisamide and sodium valproate. It can be said that, sodium valproate and zonisamide affected the cartilage model and/or early limb bud mesenchyme. In general, considering the average height and weight of the experimental group, it can be concluded that the reduction of the bone height was related to the height and weight reduction. In fact, the volume of cartilage and bone were less than the cartilage volume and bone of the control group. Indeed, the skaletal status of the organs was such that the organs' growth was delayed in intervention groups because of using zonisamide and valproate. In general, administration of medicine in organogenesis stages cause newborn's growth disorders. Furthermore, the results obtained from the average length of long bones in newborns between the two interventional had no significant defference. Researches demonstrate that embryonic skeletal disorders from mothers that received anti-epilepsy drugs during pregnancy can be attributed to the role of these drugs in the metabolism of calcium, vitamin D, and alkaline phosphatase. Vitamin D plays a role in Ca absorption and excretion of phosphate, and its deficiency will lead to reduced precipitation of calcium salts during osteogenesis (Farhat *et al.*, 2002). Based on the obtained results of the newborns' skeletal system morphology that was prepared by red alizarin staining, the effects of skeletal abnormalities such as wavy rib, scoliosis, femur and fibula deviation, and impaired position of the ribs in the intervention group was seen. The obtained results are consistent with the other studies that have been conducted on antiepileptic drugs. Both antiepileptic drugs used in this study (zonisamide and sodium valproate) demonstrated almost similar effects on disorder induction. In a study conducted by Baran *et al.*, 2006 on the effect of sodium valproate on rat newborns' skeletal system they pointed out the results similar to the present study. The Effects were abnormal position of the ribs and the wavy rib (Baran *et al.*, 2006). In a study, the main reson of congenital malformations following the use of antiepileptic drugs has been expressed in the creation of free radicals by these drugs. Furthermore, genetic disorder in hydrolysis of these metabolites increases abnormalities created by the drugs (Mark *et al.*, 2004). From the results obtained from the weight and length of the rat newborns it seems that the use of zonisamide and sodium valproate have no significant differences in growth parameters.

Conclusion

Generally, the use of sodium valproate and zonisamide in the ninth to twelfth day of gestation and causes growth disorder in fetus such that creates

the reducing impacts in the growth parameters of newborn rats. The use of zonisamide during pregnancy can be considered as a risk factor for the development of newborn that its consumption during organogenesis can cause malformations in the newborn rat.

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