Vol. 20, No. 1, Spring, 2024, 24-34 *Original Article*
DOI: 10.22055/ivj.2023.406180.2596
Journal Homepage: http://www.ivj.ir/

The effect of Prosopis Farcta extract on teratogenic effects of valproic acid and expression of BMP4 and Runx2 in skeletal system of rat embryo

Arezoo Banaei[1], Reza Ranjbar[2]*, Mahmood khaksary mahabady[3], Mohammad-Reza Tabandeh[4] and Javad Jamshidian[5]

[1] PhD Student in Comparative Anatomy and Embryology, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran
[2] Professor, Department of Basic Sciences, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran
[3] Professor, Anatomical Sciences Research Center, Institute for Basic Sciences, Kashan University of Medical Sciences, Kashan, Iran and Department of Basic Sciences, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran
[4] Associate Professor, Department of Biochemistry and Molecular Biology, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran and Member of Excellence Center of Warm Water Fish Health, Shahid Chamran University of Ahvaz, Ahvaz, Iran
[5] Assistant professor, Department of Basic Sciences, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran

Received: 09.07.2023 Accepted: 14.10.2023

Abstract

Sodium valproate (SV), as a common anti-epileptic drug, causes teratogenic effects on skeletal system by reactive oxygen species (ROS) generation. Herbal extract of Prosopis Farcta (PF), as a natural antioxidant necessary for many physiological activities, can probably ameliorate the teratogenic effects of SV during pregnancy. This study aimed to investigate the possible anti-teratogenic role of PF and Vitamin E (VE) on skeletal anomalies caused by SV in rat fetuses. Adult female rats (n=30) were categorized into 6 groups including control, SV (400 mg/kg), SV+VE (100mg/kg), and three doses of PF (50, 100, and 150 mg/kg) + SV. Each male rat mated with three adult female rats. The rats received SV, PF and VE at the 8th and 9th days of pregnancy by intraperitoneal injection. The animals were anesthetized and the laparotomy was applied at the 20th day of pregnancy. Skeletal abnormalities were analyzed using Alizarin red and Alcian blue staining. The expression of Runx2 and BMP2 genes was assessed using qPCRTM analysis in limbs bones. SV showed significant teratogenic effects including decrease in the rate of animal weight, Crown-rump length (CRL), various skeletal anomalies. The mRNA expression of Runx2 and BMP2 was also reduced in SV exposed animals. Administration of PF (especially 100mg/kg) in SV-exposed animals increased the weight of animals, CRL index, expression of Runx2 and BMP2, and reduced skeletal anomalies. The body weight, CRL index, Runx2 and BMP2 mRNA expression significantly increased, and skeletal anomalies decreased in VE group compared to the SV group. The results showed that PF could ameliorate the skeletal abnormalities and thus decreased osteogenic associated genes induced by SV in rat offsprings.

Key words: Sodium valproate, Skeletal anomaly, Prosopis Farcta, Teratogenicity, Runx2, BMP2

Introduction

Sodium valproate (SV) is an anti-epileptic drug derived from valproic acid and is used for treatment of various types of epilepsy. This molecule was identified as an

* **Corresponding Author**: Reza Ranjbar, Professor, Department of Basic Sciences, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran
E-mail: rranjbar@scu.ac.ir

antiepileptic substance in 1964 and was approved by the US Food and Drug Administration as an antiepileptic agent in 1978 (Pitetzis et al, 2017). Teratogenicity of SV including neural tube defects and congenital abnormalities (Gruppuso et al, 2022) has been reported in humans and animals in several in-vivo or in-vitro studies. Some exogenous substances can damage embryonic cell membranes causing intra-cellular antioxidant system defect and ROS generation. These pathological conditions directly lead to various malformations such as skeletal anomalies. SV generates high levels of ROS as one of the critical teratogenic agent during pregnancy (Adewole et al, 2023; Saeed et al, 2020). Since the teratogenic side effects of SV have been confirmed in many studies (Nie et al, 2016; Safdar and Ismail, 2023), deep assessment of molecular mechanism of SV along with explanation of new modern therapy seems necessary. According to the previous studies, intraperitoneal (IP) administration of SV at dose of 400mg/kg on 7^{th} and 8^{th} days of pregnancy can induce skeletal anomalies in mice fetuses (Padmanabhan and Ahmed, 1996). Thus, a comprehensive investigation of molecular mechanism of SV and innovation of antioxidant procedure against teratogenic features of SV in pregnancy seems necessary. In this regard, several previous reports indicate teratogenic effects of SV on mice skeletal system (Gebuijs et al, 2020; Holmes, 2002; Macfarlane and Greenhalgh, 2018; Nie et al, 2016; Safdar and Ismail, 2023). According to the Menegola's findings, subcutaneous injection of SV with the doses of 150, 300 and 400 mg/kg on the pregnancy days of 9-11 in female mice can potentially induce skeletal deformities of sternum, vertebrae and ribs (Menegola et al, 2002). Khaksary et al, (2011) reported teratogenic effects of SV (300 mg/kg, IP) on the 8^{th} and 9^{th} days of pregnancy including cleft palate, spina bifida, and exencephaly abnormalities in rat.

Prosopis Farcta (PF) is a widespread herb in Middle East with many applications in traditional medicine. Although this plant is generally known as a pest in agriculture and is cultivated only in medicinal plant centers, it has strong medicinal and antioxidant properties. The protective effects of PF have been approved against oxidant agents (Jahromi et al, 2018). Cell membranes peroxidation is highly inhibited by PF in oxidative micro-environments. Mohammed et al, (2020) reported the beneficial features of PF on abnormal alteration of hematology and lipid profiles caused by alloxan exposure.

RUNX2 is a key transcription factor associated with osteoblast differentiation. It has also been suggested that Runx2 plays a cell proliferation regulatory role in cell cycle entry and exit in osteoblasts. RUNX2 is required for bone formation, physiologically. BMP-2 like other bone morphogenetic proteins plays an important role in the development of bone and cartilage. Insufficient expression of the above mentioned genes can lead to defects in bone formation (Kirihata et al, 2018).

Thus, in the present experimental study, the authors aimed to investigate the protective therapeutic effects of PF against teratogenic changes caused by SV during rat pregnancy.

Material and Methods

All animal manipulations were conducted in accordance with the ethical principles and under the supervision of the University's Ethics Committee (Ethic NO. EE/97.24.3.93643/scu.ac.ir). The animals were kept in a standard condition including 12/12 photocycle, 37ºC of temperature, and 55% of humidity.

The root of PF plant was prepared and confirmed by a university-based botanist. The roots were powdered by electric grinder and 50gr of root powder was dissolved in 1L of ethanol 80% (Merck, Germany) and kept in room temperature for one day. Then,

the filtration was applied through the filter paper followed by placement in an oven (40°C) for 7 days. The settled sediment was collected and stored at -20ºC for the future experiment. In the present study, the powdered of PF root extraction was dissolved in normal saline (0.9%) with three doses of 50, 100, and 150 mg/kg. All PF solutions were filtered using microbiologic filters and were administrated (50 Insulin Unit) intraperitoneally (Najafzadeh and Khaksari, 2009).

To prepare and mate animals, male (n=10) and female (n=30) Wistar rats were purchased from the center of laboratory animals of Jondishapur university of Medical Sciences. The mean age and weight of the adult animals were 3-4 months and 200±20 gr, respectively. The animals were kept in laboratory for two weeks for adaptation to the new environmental condition. For sexual stimulation and initiating of estrous cycle, the male and female animals were placed in the same cage (with the ratio of 3 females/ 1 male) divided by metal fence for two weeks (Abdolmaleki, et al., 2021). Then, the rats were met together freely to perform mating. On the next day, the successful mating was approved using detection of vaginal plaque (defined as the 0 day of pregnancy) (Abdolmaleki et al, 2021).

Following observation of vaginal plaque as a visual sign of successful pregnancy induction, the female rats were randomly divided into 6 groups as follows; control group (pregnant rats with no treatment), SV group (pregnant rats received intraperitoneal injection of SV at dose of 400 mg/kg), SV+VE group (pregnant rats received 400 mg/kg SV (Mohammed and Kakey, 2020) followed by intraperitoneal injection at dose of VE (100 mg/kg), SV+PF50 group (pregnant rats received 400 mg/kg SV followed by intraperitoneal injection of PF at dose of 50 mg/kg), SV+PF100 (pregnant rats received 400 mg/kg SV followed by intraperitoneal injection of PF at dose of 100 mg/kg), and SV+PF150 group (pregnant rats received 400 mg/kg SV followed by intraperitoneal injection of PF at dose of 150 mg/kg). All treatments were performed at days 8 and 9 of pregnancy (Menegola et al., 2002). SV was obtained (Rouz Daroo Co., Tehran, Iran) and VE vial was prepared (Osveh Pharmaceutical Co., Batch No: 9009, Iran).

Eighteen days after treatment, all pregnant rats were anesthetized by 50 IU of intraperitoneal injection/rat (ketamine. 150 mg/kg/xzylazin. 15 mg/kg). Then, euthanasia was induced by ketamine/Xylazine intramuscular injection during the surgery (Procópio et al, 2021). Midline laparotomy was performed and the uterus was dissected completely and placed in a petri dish containing normal saline. After uterus dissection and embryos excision from the amniotic sac, the number of viable or the scare of absorbed embryos was counted in both right and left horns. Then, the weight and length (Crown-rump length, CRL) of each fetus were also measured and recorded using scales and calipers, respectively. Then, the embryos available in the right horn were eviscerated and fixed in alcohol 96% for staining and macroscopic evaluations and the embryos located in left uterine horn were used for gene expression assessment. The embryos located in the right uterine horn were fixed in ethanol 96% for 3 days.

The samples were placed in a mixture of Alcian blue (14.5%), Alizarin red (12.5%), ethanol (96%) and Glacial Acetic Acid for 12 h for each group. The samples were washed in Potassium solution (0.1%) and were placed in pure glycerin solution for macroscopic assessment. The samples were carefully examined using a stereomicroscope (Nikon SMZ 800 Japan) in terms of skeletal system abnormalities including; cleft palate, thoracic anomalies (slipping rib, stuck ribs, extra rib, and incomplete rib), anomalies of spine (vertebral adhesion and spins bifida) and limbs anomalies (syndactyly and polydactyly) (Ovchinnikov, 2009).

The embryos implanted in left horn were used for gene expression assessments. The Total RNA was extracted from frozen tissue using RNX^{TM} isolation reagent according to the manufacturer's procedure (SinaClon, Tehran, Iran). Then, the purity and quality of the extracted RNA were assessed using aEppendorf µCuvette G1.0 microvolume measuring cell (Eppendorf BioPhotometer D30, Eppendorf, Germany). Reverse transcription was performed with the YTA cDNA synthesis kit (Yekta tajhiz, Iran) and Eppendorf Thermal Cycler (Germany) using 1 µg of RNA and random hexamer as recommended by the manufacturer. The expression of Runx2 and BMP2 was evaluated using qPCR^{TM} Green Master Kit for SYBR Green I® (Yektatajhiz, Iran). Specific sets of primers (Pishgam BioTech, Co, Tehran, Iran) designed for this study. Table 1 shows the characteristics of primers used in the current study. Real-time PCR was performed using the Roche Light-Cycler detection system (Basel, Switzerland) using YTA SYBR Green qPCR Mix (Yektatajhiz, Iran) as recommended by the manufacturer. The PCR reactions used consisted of a denaturation step at 95ºC for 5 min followed by amplification cycles (45 cycle) at 90ºC for 15s and 60ºC for 30s. All qPCR reactions were carried out in triplicates. GAPDH was used as a housekeeping gene. Relative quantification was performed according to the comparative $2^{-\Delta\Delta Ct}$ method using Lightcycler 96® software.

Table 1: Characteristics of primers used in the current study

Gene	Primer Sequence (F:forward; R: reverse)	Accession number
BMP2-F	5′- CTTCCATCACGAAGAAGCCATC-3′	NM_017178.1
BMP2-R	5′-AGCTTCCTGCATTTGTTCCC-3′	
Runx2-F	5′-*ACTCTGCCGAGCTACGAAAT*-3′	NC-007299
Runx2-R	5′- AAGTGAAACTCTTGCCTCGTC-3′	
GAPDH-F	5′-AGTTCAACGGCACAGTCAAG-3′	XM_017593963.1
GAPDH-R	5′- TACTCAGCACCAGCATCACC-3′	

The data analysis was conducted using SPSS Statistical Software version 16 (SPSS Inc., Chicago, IL). All data were presented as mean ± standard deviation (SD). The one-way ANOVA was used to determine the statistical difference between experimental groups. The Tukey post hoc test was used to determine the differences between the groups. A *p*-value ≤ 0.05 was considered statistically significant.

Results

Number of alive and absorbed embryos

The total number of alive and absorbed embryos was not significantly different ($p<0.05$) between different experimental groups (Table 2).

Weight and length of embryos

The results revealed that the weight of embryos in SV group was decreased significantly compared to the control animals ($p\leq0.05$). The treatment groups represented higher weight gain in VE and PF groups than untreated animals significantly ($p\leq0.05$). The weight gain was found significantly higher in SV+VE animals than SV+PF50 and SV+PF groups ($p\leq0.05$). The weight of embryos was higher in SV+PF150 group in comparison with SV+VE, SV+PF50 and SV+PF100 groups (Figure 1). The CRL value significantly decreased ($p\leq0.05$) in SV embryos than control group. Treatment with VE or PF could restore the CRL reduction induced by SV administration. The CRL value was significantly higher ($p\leq0.05$) in SV+VE group than SV+PF50 and SV+PF100 groups. CRL significantly increased ($p\leq0.05$) in SV+PF150 than other VE and PF treated animals (Figure 2).

Table 2: Total number of alive, absorbed and incidence rate of abnormal embryos in different groups

	Variables	Groups					
		Control	SV	SV+VITAMINE E	SV+PF50	SV+PF100	SV+PF150
Embryos (%)	Alive	97.2	81.3	94.2	92.4	93.9	96.1
	Absorbed	2.8	18.7	5.8	7.6	6.1	3.9
Skeletal Anomalies (%)	Palatine Cleft	0	66.66*	18.18λ	23.33	15.62	2.77χ
	Fused Ribs	0	70.37*	30.30λ	40.00	28.12	8.33χ
	Extra Ribs	0	6*	3λ	5	5	2χ
	Non-ossification of sternum	0	48.14*	15.15λ	23.33	12.5	5.55χ
	Non-ossification of 13th rib	0	44.44*	9.09λ	16.66	6.25	0χ
	Spina Bifida	0	51.85*	12.12λ	20.00	9.37	0χ
	Syndactyly	0	4*	4λ	5	3	0χ
	Polydactyly	0	4*	3λ	3	4	0χ
	Delay in Fore-limb Ossification	0	40.74*	21.21λ	26.66	18.75	2.77χ
	Delay in Hand Ossification	0	40.74*	21.21λ	26.66	18.75	2.77χ
	Delay in Hind-limb Ossification	0	48.14*	24.42λ	23.33	21.87	5.55χ
	Delay in Foot Ossification	0	48.14*	24.42λ	23.33	21.87	5.55χ

SV; sodium valproate, VE; vitamin E, PF; Prosopis Farcta, * represented significant level of SV than Control, λ represented significant alteration of SV+VE than SV+PF50 and SV+PF100, χ represented significant changes of SV+PF150 than SV group. All statistics were represented in percentage. Significant level was considered p<0.05.

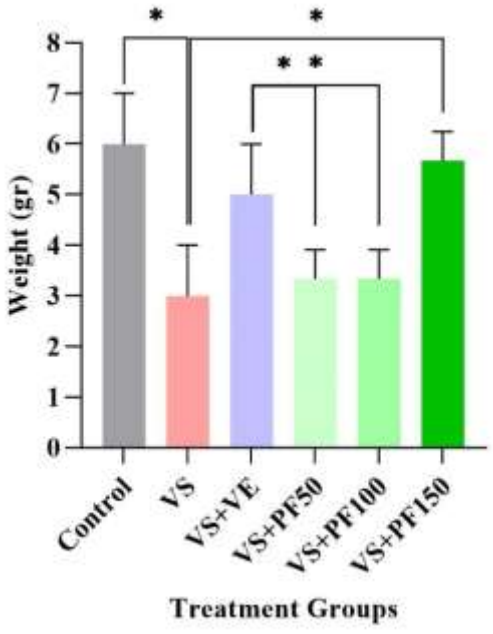

Figure 1: Weight changes (gr) in control and treatment groups. SV; sodium valproate, VE; vitamin E, PF; Prosopis Farcta. * represented significant changes (p<0.05) among the groups.

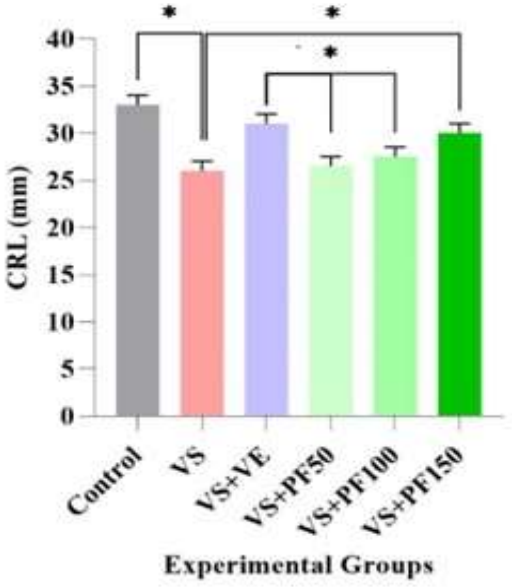

Figure 2: CRL changes (mm) in control and treatment groups. CRL; Crown-rump length, SV; sodium valproate, VE; vitamin E, PF; Prosopis Farcta. * represented significant changes (p<0.05) among the groups.

Skeletal anomalies

Total skeletal anomalies including palatine cleft, fused ribs, extra ribs, non-ossification of sternum, non-ossification of 12th rib, spina bifida, syndactyly, polydactyly, delay in fore-limb ossification, delay in hand ossification, delay in hind-limb ossification, delay in foot ossification significantly increased in SV embryos compared with the control group ($p \leq 0.05$). The presence of these anomalies was significantly lower ($p \leq 0.05$) in SV+VE animals than SV+PF50 and SV+PF100 groups. Total skeletal anomalies significantly decreased ($p \leq 0.05$) in SV+PF150 than SV group (Table 2 and Figures 3, 4, and 5).

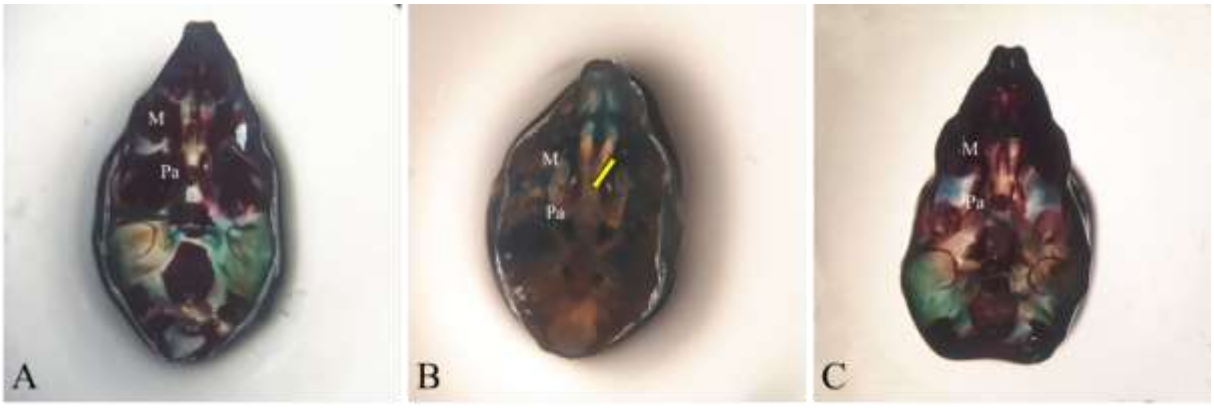

Figure 3. Ventral view of skull of rat fetuses of gestation day 20 (GD20), stained with alizarin red S-alcian blue. A) Normal palatine bone (control); B) Cleft palate induced by SV (Yellow arrow); C) Normal palatine bone in group received SV along with PF 150 mg/kg. M: Maxilla; Pa: Palatine.

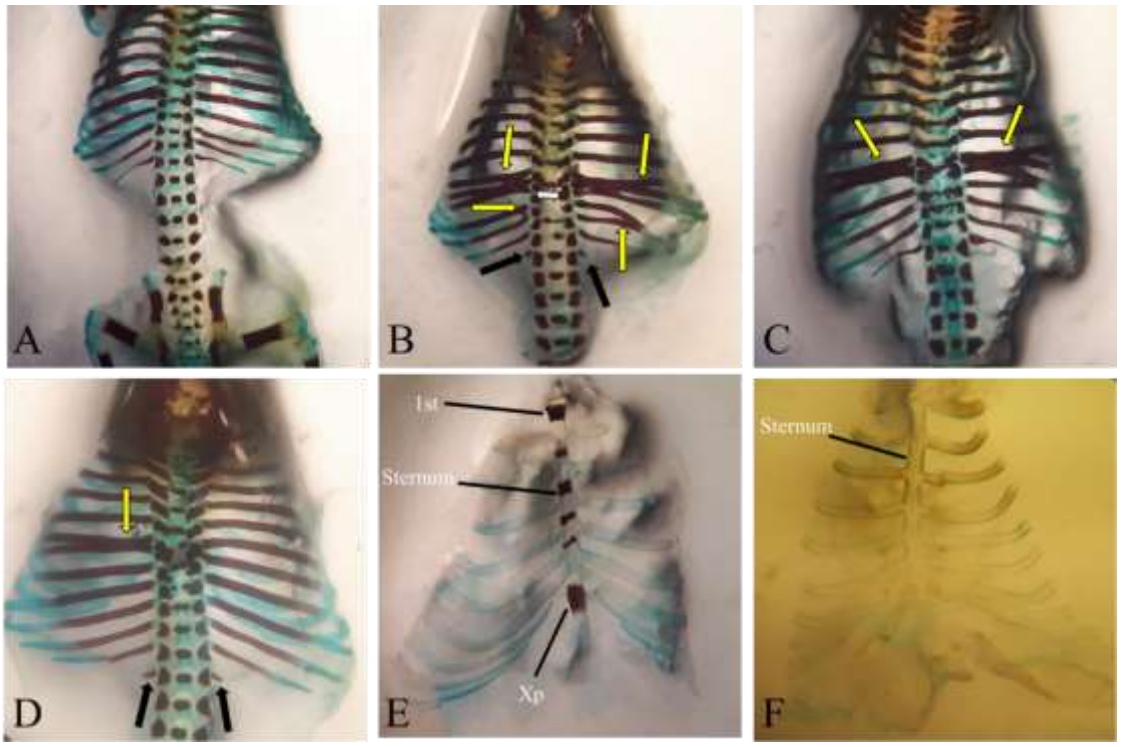

Figure 4. A-D: Dorsal view of vertebral column of rat fetuses of gestation day 20 (GD20), stained with alizarin red S-alcian blue. A) Normal vertebral column and ribs (control); B-D) Spina bifida (white arrow), fused ribs (Yellow arrow) and extra ribs (Black arrows) induced by SV. E-F: Dorsal view of sternum of gestation at 20th day fetal rat, stained with alizarin red-alcian blue. E) Normal sternum; F) Sternum with non-ossification of the sternum in the SV group (1st: First sternum; Xp: Xiphoid process).

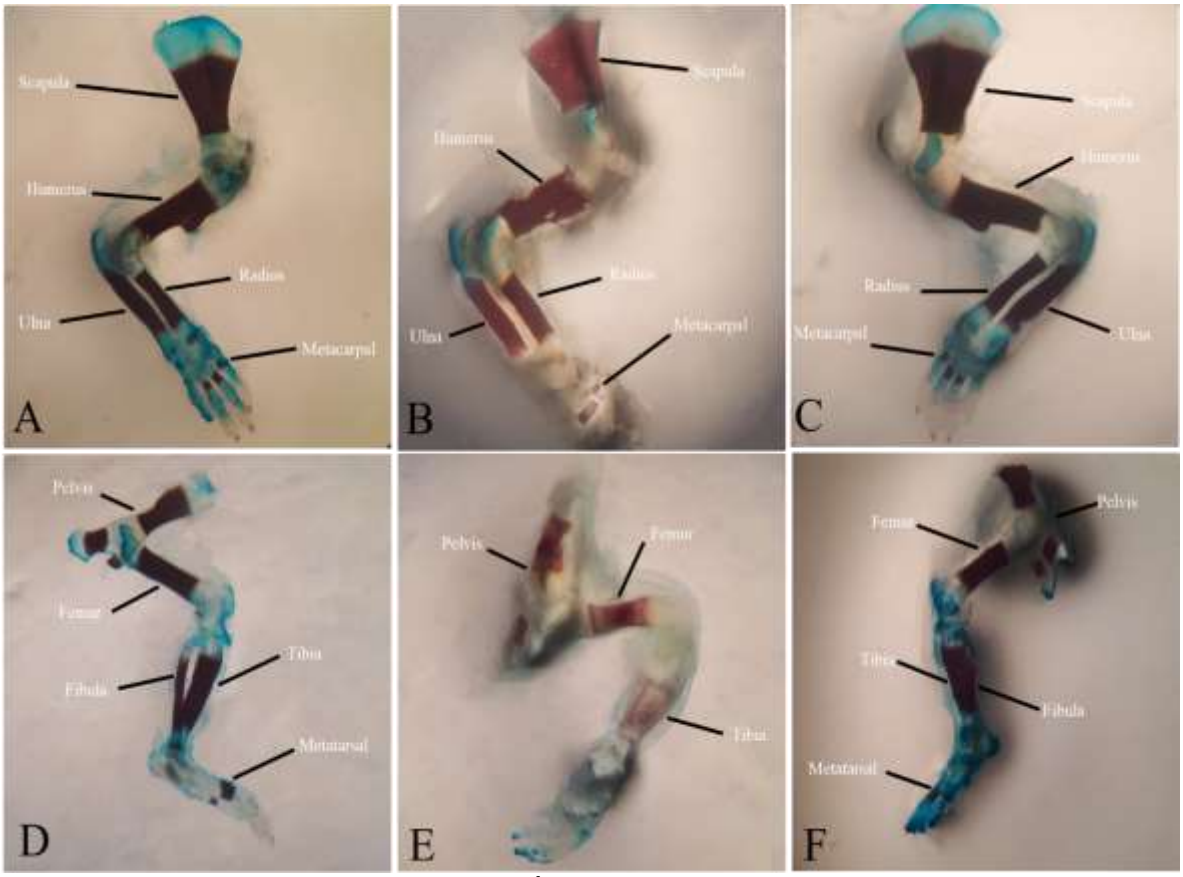

Figure 5. Lateral view of limbs of gestation 20th day fetal rat, stained with alizarin red-alcian blue. A) Normal forelimb (control); B) Delay ossification of forelimb in the SV group; C) Normal forelimb in group that received SV along with PF 150 mg/kg; D) Normal hindlimb (control); E) Delay ossification of hindlimb in the SV group; F) Normal hindlimb in group that received SV along with PF 150 mg/kg.

Expression of Runx2 and BMP2 in different experimental groups

As indicated in Figure 2, the expression of BMP2 and Runx2 genes significantly decreased ($p \leq 0.05$) in SV embryos compared with the control animals. The decreased mRNA levels of *Runx2 and BMP2 genes in SV groups were significantly attenuated in animals that received* Vitamin E and PF. On the other hand, the expression levels of Runx2 and BMP2 genes were significantly higher in VE and SV+PF treated embryos than SV group (Figure 6 and 7).

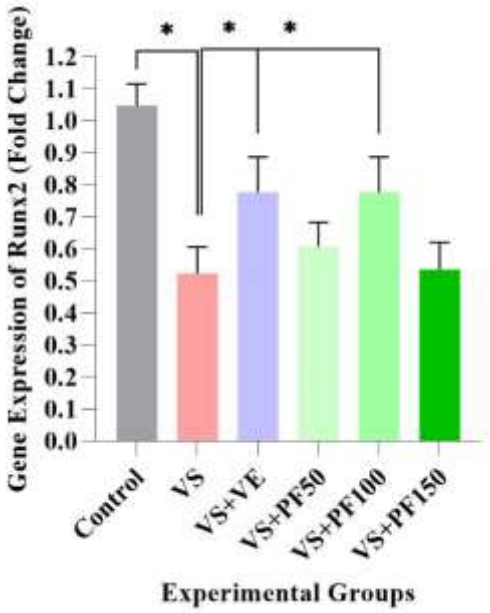

Figure 6. Gene expression of Runx2 (fold change) in control and treatment groups. SV; sodium valproate, VE; vitamin E, PF; Prosopis Farcta. * represented significant difference between different experimental groups at p≤0.05.

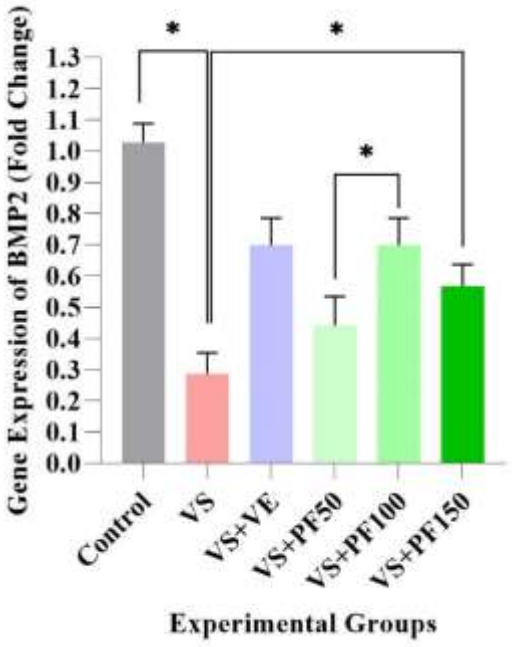

Figure 7. Gene expression of BMP2 (fold change) in control and treatment groups. SV; sodium valproate, VE; vitamin E, PF; Prosopis Farcta. * represented significant changes (p≤0.05).

Discussion

The SV is one of the commonly used drugs for seizures treatment. SV with three different mechanisms can lead to inhibition of the onset of seizures including acting on GABA (γ aminobutyric acid) levels in the CNS, blocking voltage-gated ion channels, and inhibiting histone deacetylase activity (Safdar and Ismail, 2023). Based on the several published articles, SV causes the production and release of a large amount of ROS leading to many cellular and tissue side-effects (Saeed et al, 2020; Safdar and Ismail, 2023). SV can also affect the fetus depending on the stage of fetal development prescription. Thus, the SV is considered as a teratogenic agent and it is recommended to be replaced with another drug during pregnancy (Ornoy et al, 2020). PF is one of the herbal antioxidants with ROS scavenging activity. Following the application of PF, total antioxidant capacity of the several tissues accelerates leading to toxin detoxification and body health (Noroozi et al, 2019).

Previous investigations showed that SV has teratogenic properties, while it cannot lead to fetal death (Adewole et al, 2023; Safdar and Ismail, 2023). Sultana et al, (2019) also approved the teratogenic effects of SV in chick embryos with no fatal effects. The weight and CRL of embryos were the critical factors affected by SV application (Gebuijs et al, 2020; Nie et al, 2016). Our results showed that SV could decrease the weight of animals and the CRL index. These changes may be associated to the skeletal degeneration induced by SV. In accordance with our results, Khan et al, (2013) reported the significant weight loss following SV application in male mice. They also stated that the weight of testes decrease after SV prescription. Tissue degeneration and apoptosis have been considered as possible mechanism leading to the considerable weight loss following SV treatment (Khan and Jena, 2013). Vafaee-Shahi et al also reported the significant weight loss in children under SV

treatment (Vafaee-Shahi et al, 2022). Our results showed that the weight loss trend decreased in SV+PF groups leading to the weight gain in SV-animals treated by PF. This finding represented that the PF has weight-gain property in animals under SV treatment. The reduction of CRL value was also reported in the experimental study of Pratibha Shakya on fetal mice treated by SV (Shakya et al, 2020). Although the SV is the first-line antiepileptic drug, the teratogenicity of SV has been approved in many experiments. This drug is directly associated with the neurodevelopmental delay in the children of women exposed to the drug during pregnancy (Duncan, 2007). There are various ranges of skeletal anomalies associated with the SV exposure including palatine cleft, ribs deformities, non-ossification of bones, spina bifida, syndactyly and polydactyly. In the present experimental investigation, various anomalies were detected in animals exposed to SV; also the treatment effects were seen in SV group after PF administration. In accordance with our findings, Valentina et al. assessed the probable skeletal malformations following SV administration in experimental animal model systems with focusing on the cervical malformations. They reported that the vertebral fusions and the presence of cervical ribs can be observed in animals exposed to the SV in a dose dependent manner (Massa et al, 2005). Kirihata et al, (2018) assessed the repair ability of skeletal alterations induced by sodium valproate in rats. In this experiment, the SV was used at dose of 400 mg/kg to pregnant Sprague-Dawley rats between days 9 to 11 of gestation period. Fetuses and pups were examining at day 21 of gestation period and also at day 11 after birth to determine the skeletal abnormalities by using Alizarin red S and Alcian blue staining. In agreement with our findings, they also reported that the SV can induce costal and vertebral alterations in the fetuses including discontinued rib cartilage, fused rib, full our short supernumerary rib, bipart ossification of thoracic centrum, supernumerary lumbar vertebrae, and lumbarization (Kirihata et al, 2018). RUNX2 protein is a key transcription factor associated with osteoblast differentiation. It has also been suggested that Runx2 plays a cell proliferation regulatory role in cell cycle entry and exit in osteoblasts (Komori, 2019). The BMP2 protein belongs to the superfamily of Transforming growth factor beta (TGF-β). This protein is critically involved in bone and cartilage development and osteogenesis (Cai et al, 2021). As we detected in the present study, SV can potentially decrease the expression of Runx2 and BMP2 genes leading to the abnormal skeletal development and skeletal anomalies. Our findings showed that the down regulation of Runx2 and BMP2 genes was attenuated following PF administration. It can be concluded that the PF can probably lead to the proper osteogenic genes expression in SV-affected animals. Kim et al. proposed a new bone-associated anomalies therapy using Runx2-modifying enzymes. They concluded that by the application of procedures influencing Runx2 gene expression, the skeletal malformations can be actively inhibited (Kim, et al, 2020). As we found in this study, the application of herbal antioxidant agent is a novel natural drug for activation of Runx2 gene expression in SV-affected animals. So, SV down regulates genes by ROS generation, but PF causes up regulation by antioxidant effect (Mohammed and Kakey, 2020).

The findings of this study showed that SV has teratogenic effects following consumption on days 8 and 9 of pregnancy period in rat. This drug can lead to inhibition of proper osteogenesis by reducing the expression of Runx2 and BMP2 genes. The macroscopic and molecular analysis showed that PF with its therapeutic properties can increase the expression of genes involved in osteogenesis and lead to the attenuation of

the skeletal defects induced by SV treatment. More experimental studies are needed to investigate the exact molecular mechanism of PF on reduction of teratogenic complications caused by SV exposure.

Acknowledgment

The authors express their gratitude to the Research Council of Shahid Chamran University of Ahvaz for its financial support.

Conflict of interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

Funding

This research was supported by the Vice Chancellor for Research of Shahid Chamran University of Ahvaz.

References

Abdolmaleki, A., Jalili, C., Mansouri, K., & Bakhtiari, M. (2021). New rat to mouse xenograft transplantation of endometrium as a model of human endometriosis. *Animal Models and Experimental Medicine, 4*(3), 268-277.

Adewole, K. E., Attah, A. F., & Osawe, S. O. (2023). Exploring phytotherapeutic approach in the management of valproic acid-induced toxicity. *Advances in Traditional Medicine, 23*(2), 347-367.

Cai, H., Zou, J., Wang, W., & Yang, A. (2021). BMP2 induces hMSC osteogenesis and matrix remodeling. *Molecular Medicine Reports, 23*(2), 1-1.

Duncan, S. (2007). Teratogenesis of sodium valproate. *Current opinion in neurology, 20*(2), 175-180.

Gebuijs, I. G. E., Metz, J. R., Zethof, J., Carels, C. E. L., Wagener, F. A. D. T. G., & Von den Hoff, J. W. (2020). The anti-epileptic drug valproic acid causes malformations in the developing craniofacial skeleton of zebrafish larvae. *Mechanisms of Development, 163*, 103632.

Gruppuso, P. A., Ahmed, R., & Adashi, E. Y. (2022). Valproate Teratogenicity: A Moving Target. *Obstetrics & Gynecology, 140*(3), 408-411.

Holmes, L. B. (2002). Teratogen-induced limb defects. *American journal of medical genetics, 112*(3), 297-303.

Jahromi, M. A. F., Etemadfard, H., & Zebarjad, Z. (2018). Antimicrobial and antioxidant characteristics of volatile components and ethanolic fruit extract of Prosopis farcta (Bank & Soland.). *Trends in Pharmaceutical Sciences, 4*(3), 177-186.

Khan, S., & Jena, G. B. (2013). Effect of sodium valproate on the toxicity of cyclophosphamide in the testes of mice: influence of pre- and post-treatment schedule. *Toxicol Int, 20*(1), 68-76. doi:10.4103/0971-6580.111562

Kim, W.-J., Shin, H.-L., Kim, B.-S., Kim, H.-J., & Ryoo, H.-M. (2020). RUNX2-modifying enzymes: therapeutic targets for bone diseases. *Experimental & Molecular Medicine, 52*(8), 1178-1184.

Kirihata, Y., Ban, Y., Nakamori, C., Takagi, H., Hashimoto, T., & Tsutsumi, S. (2018). Repairability of skeletal alterations induced by sodium valproate in rats. *Congenital Anomalies, 58*(3), 99-101.

Komori, T. (2019). Regulation of proliferation, differentiation and functions of osteoblasts by Runx2. *International Journal of Molecular Sciences, 20*(7), 1694.

Macfarlane, A., & Greenhalgh, T. (2018). Sodium valproate in pregnancy: what are the risks and should we use a shared decision-making approach? *BMC pregnancy and childbirth, 18*(1), 1-11.

Massa, V., Cabrera, R. M., Menegola, E., Giavini, E., & Finnell, R. H. (2005). Valproic acid-induced skeletal malformations: associated gene expression cascades. *Pharmacogenetics and genomics, 15*(11), 787-800.

Menegola, E., Broccia, M. L., Di Renzo, F., & Giavini, E. (2002). Comparative study of sodium valproate-induced skeletal malformations using single or double staining methods. *Reproductive Toxicology, 16*(6), 815-823.

Mohammed, I. H., & Kakey, E. S. (2020). Effect of Prosopis farcta extracts on some complications (hematology and lipid profiles) associated with alloxan induced diabetic rats. *Iraqi J Vet Sci, 34*(1), 45-50.

Najafzadeh, V. H., & Khaksari, M. M. (2009). A comparison study of the effects of Echinacea purpurea ethanolic extract and mesna on cyclophosphamide-induced macroscopic fetal defects in rats.

Nie, Q., Su, B., & Wei, J. (2016). Neurological teratogenic effects of antiepileptic drugs during pregnancy. *Experimental and therapeutic medicine, 12*(4), 2400-2404. doi:10.3892/etm.2016.3628

Noroozi, R., Sadeghi, E., Yousefi, H., Taheri, M., Sarabi, P., Dowati, A., . . . Ghafouri-Fard, S. (2019). Wound healing features of Prosopis farcta: in vitro evaluation of antibacterial, antioxidant, proliferative and angiogenic properties. *Gene Reports, 17*, 100482.

Ornoy, A., Becker, M., Weinstein-Fudim, L., & Ergaz, Z. (2020). S-adenosine methionine (SAME) and valproic acid (VPA) as epigenetic modulators: Special emphasis on their interactions affecting nervous tissue during pregnancy. *International Journal of Molecular Sciences, 21*(10), 3721.

Ovchinnikov, D. (2009). Alcian blue/alizarin red staining of cartilage and bone in mouse. *Cold Spring Harb Protoc, 2009*(3).

Padmanabhan, R., & Ahmed, I. (1996). Sodium valproate augments spontaneous neural tube defects and axial skeletal malformations into mouse fetuses. *Reproductive Toxicology, 10*(5), 345-363.

Pitetzis, D. A., Spilioti, M. G., Yovos, J. G., & Yavropoulou, M. P. (2017). The effect of VPA on bone: From clinical studies to cell cultures—The molecular mechanisms revisited. *Seizure, 48*, 36-43.

Procópio, I. M., Pereira-Sampaio, M. A., Costa, W. S., Sampaio, F. J. B., & Souza, D. B. d. (2021). Histomorphometric comparison of the corpus cavernosum of rats submitted to euthanasia with ketamine and xylazine or isoflurane. *Acta Cirúrgica Brasileira, 36*, e361103.

Rahgazar, O., Ranjbar, R., Varzi, H. N., & Mahabady, M. K. (2011). Prophylactic effects of melatonin on sodium valproate-induced neural tube defects and skeletal malformations in rat embryos. *American Journal of Applied Sciences, 8*(5), 413.

Saeed, M., Saleem, U., Anwar, F., Ahmad, B., & Anwar, A. (2020). Inhibition of Valproic Acid-Induced Prenatal Developmental Abnormalities with Antioxidants in Rats. *ACS Omega, 5*(10), 4953-4961. doi:10.1021/acsomega.9b03792

Safdar, A., & Ismail, F. (2023). A comprehensive review on pharmacological applications and drug-induced toxicity of valproic acid. *Saudi Pharmaceutical Journal, 31*(2), 265-278.

Shakya, P., Shakya, N. K., & Mohanty, C. (2020). Teratogenic effects (resorptions and reduction in weight and crown rump length) of valproate on fetal mice. *National Journal of Clinical Anatomy, 9*(2), 39.

Sultana, S., Doshi, M., Jayasree, N., & Chandrupatla, M. (2019). Comparative study of drug damage caused on Cerebrum in Chick Embryos administered with Drugs-Cyclophosphamide and Sodium Valproate. *Journal of Pharmaceutical Sciences and Research, 11*(7), 2774-2777.

Vafaee-Shahi, M., Soheilipour, F., Mohagheghi, P., Riahi, A., Borghei, N.-S., & Talebi, A. (2022). Effect of Sodium Valproate on Weight, Body Mass Index, Uric Acid, Vitamin D3, Blood Insulin, and Serum Lipid Profile in Children. *The Open Neurology Journal, 16*(1).

Received: 09.07.2023
Accepted: 14.10.2023

DOI: 10.22055/ivj.2023.406180.2596

تأثیر عصاره کهورک بر اثرات تراتوژنیک والپرویک اسید و بیان BMP4 و Runx2 در دستگاه اسکلتی جنین موش صحرایی

آرزو بنائی[۱]، رضا رنجبر[۲*]، محمود خاکساری مهابادی[۳]، محمدرضا تابنده[۴] و جواد جمشیدیان[۵]

[۱] دانشجوی دکتری تخصصی آناتومی و جنین‌شناسی مقایسه‌ای، دانشکده دامپزشکی، دانشگاه شهید چمران اهواز، اهواز، ایران

[۲] استاد گروه علوم پایه، دانشکده دامپزشکی، دانشگاه شهید چمران اهواز، اهواز، ایران

[۳] استاد مرکز تحقیقات علوم تشریح، پژوهشکده علوم پایه، دانشگاه علوم پزشکی کاشان، کاشان، ایران و استاد گروه علوم پایه، دانشکده دامپزشکی، دانشگاه شهید چمران اهواز، اهواز، ایران

[۴] دانشیار گروه علوم پایه، دانشکده دامپزشکی، دانشگاه شهید چمران اهواز، اهواز، ایران و عضو قطب بهداشت و بیماری‌های ماهیان گرمابی دانشگاه شهید چمران اهواز، اهواز، ایران

[۵] استادیار گروه علوم پایه، دانشکده دامپزشکی، دانشگاه شهید چمران اهواز، اهواز، ایران

تاریخ دریافت: ۱۴۰۲/۴/۱۸ تاریخ پذیرش: ۱۴۰۲/۷/۲۲

چکیده

والپروات سدیم به عنوان یک داروی ضد صرع متداول به وسیله تولید گونه‌های فعال اکسیژن (ROS) اثرات تراتوژنیک را روی دستگاه اسکلتی دارد. عصاره گیاه کهورک به عنوان یک آنتی‌اکسیدان طبیعی فعالیت‌های فیزیولوژیک متعددی دارد و احتمال دارد که اثرات جانبی تراتوژنیک والپروات سدیم در طول بارداری را بهبود دهد. مطالعه حاضر با هدف بررسی نقش ضد تراتوژنیک احتمالی عصاره کهورک و ویتامین E بر اختلالات اسکلتی ناشی از والپروات سدیم در جنین موش صحرایی انجام شد. در این پژوهش تعداد ۳۰ سر موش صحرایی ماده بالغ به شش گروه شامل کنترل، والپروات سدیم (۴۰۰ mg/kg)، والپرویک اسید+ ویتامین E (۱۰۰mg/kg) سه دوز کهورک (mg/kg ۵۰ ، ۱۰۰ و ۱۵۰) همراه با والپروات سدیم تقسیم شدند. به ازای هر ۳ موش ماده یک موش نر برای جفت‌گیری قرار داده شد. موش‌ها والپروات سدیم، عصاره کهورک و ویتامین E را به صورت تزریق درون صفاقی در روزهای هشتم و نهم بارداری دریافت کردند. حیوانات در روز بیستم بارداری بی‌هوش و لاپاراتومی شدند. اختلالات استخوانی با استفاده از رنگ‌آمیزی با آلیزارین قرمز و آلسین آبی بررسی شدند. بیان ژن‌های Runx2 و BMP2 با استفاده از تکنیک Realtime-PCR در استخوان‌های اندام تحتانی اندازه‌گیری شد. نتایج نشان داد که والپروات سدیم اثرات تراتوژنیک معنی‌داری از قبیل کاهش وزن حیوانات، کاهش CRL و اختلالات اسکلتی مختلفی را باعث می‌شود. همچنین والپروات سدیم موجب کاهش بیان Runx2 mRNA و BMP2 شد. تزریق عصاره کهورک (به ویژه ۱۰۰ mg/kg) وزن، شاخص CRL و بیان Runx2 و BMP2 را افزایش و اختلالات اسکلتی را به طور معنی‌داری کاهش داد. تزریق ویتامین E، وزن، شاخص CRL و بیان Runx2 mRNA و BMP2 را نسبت به گروه والپروئیک اسید به طور معنی‌داری افزایش و اختلالات اسکلتی ناشی از والپروئیک اسید را کاهش داد. نتایج کلی این پژوهش نشان داد که عصاره کهورک، اختلالات اسکلتی و کاهش بیان ژن‌های Runx2 و BMP2 ناشی از والپروات سدیم را در جنین‌های موش صحرایی بهبود می‌دهد.

کلمات کلیدی: والپروات سدیم، اختلالات اسکلتی، کهورک، ناهنجاری‌زایی، Runx2، BMP2

* نویسنده مسئول: رضا رنجبر، استاد گروه علوم پایه، دانشکده دامپزشکی، دانشگاه شهید چمران اهواز، اهواز، ایران

E-mail: rranjbar@scu.ac.ir