

Effects of fish oil supplementation against formaldehyde-induced congenital skeletal anomalies in Wistar rats

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Abstract

It has been shown that formaldehyde (FA) as a common organic compound causes developmental anomalies and fetal defects. Fish oil is recommended for the normal growth and development of the fetus due to its fatty acid docosahexaenoic acid. The aim of this study was to investigate the potential of FO in protecting fetal growth and preventing skeletal anomalies caused by treatment of pregnant rats with FA. For this thirty pregnant Wistar rats were randomly categorized into five groups of control, sham (Normal saline; Orally and intraperitoneally), fish oil (0.5 mg/kg/bw; Orally), formaldehyde (10 mg/kg/bw; intraperitoneally), formaldehyde + fish oil. The treatment period was from day 0 to 20 of pregnancy. On the 20th day of gestation, the animals underwent anesthesia, and followed by a laparotomy to determine the weight and crown-rump length (CRL) of the fetuses. Skeletal stereomicroscopic assessments were conducted on the fetuses using the alizarin red / alcian blue staining method. Additionally, the expression of Runx2 and BMP4 was assessed through qPCR. The findings indicated that exposure to formaldehyde (FA) during prenatal development significantly reduced fetal weight and CRL, as well as the expression of Runx2 and BMP4 genes. Furthermore, FA heightened the occurrence of congenital skeletal abnormalities, including cleft palate, spina bifida, and non-ossification of fetal bones. Nevertheless, co-administration of fish oil (FO) with FA injection in pregnant rats improved fetal bone growth and mitigated skeletal anomalies. Fish oil demonstrated the potential to alleviate the teratogenic effects of exposure to formaldehyde by enhancing the expression of genes related to osteogenesis.

Key words: Congenital skeletal anomalies, Formaldehyde, Fish oil, Fetus, Rat

Introduction

Formaldehyde (FA) is a simple organic compound that belongs to the aldehyde family of compounds. It is the simplest member of this family, with the chemical formula CH₂O (El-Maghrabey et al, 2022).

Low levels of formaldehyde can be detected in food, fruit and water. On the other hand, exogenous sources of formaldehyde include vehicle exhaust, power plants, fire and cigarette smoke, and photochemical

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oxidation of hydrocarbons (Zhang et al, 2021). Formaldehyde has a wide range of applications, including its use in the manufacture of building materials, such as particleboard, plywood, and fiberboard. It is also used in many household products, such as paints, adhesives, and cleaning agents (Gonzalez-Rivera et al, 2020). Furthermore, formaldehyde is used as a disinfectant and preservative in pathological laboratories, anatomy mortuaries, hospitals, and plastic industries (Soltanpour et al, 2022). Despite its many uses, exposure to formaldehyde at high levels can cause adverse health effects. The International Agency for Research on Cancer (IARC) and National Agencies have classified formaldehyde as carcinogenic to humans (group 1) (McGregor et al, 2006). Populations exposed to formaldehyde include not only adult workers who are occupationally at risk, but also the elderly, pregnant women, and young children (Lam et al, 2021). Recently, it has been found that the exposure of women to formaldehyde during pregnancy causes damage to the body of the pregnant woman and disrupts the development of the fetus (Zhang et al, 2021).

There is strong evidence that exposure to formaldehyde during pregnancy may be associated with an increased risk of birth defects in infants (Haffner et al, 2019). Studies have shown evidence of an association between formaldehyde exposure among pregnant women who worked in jobs such as embalmers, pathologists, or histology technicians, and an increased risk of miscarriage (Duong et al, 2011). Another study found that formaldehyde in pregnant women affects fetal development and causes congenital heart anomalies (Zhang et al, 2021). Exposure to formaldehyde during pregnancy can lead to oxidative stress in the fetus. Oxidative stress caused by formaldehyde can lead to adverse effects on fetal development, including impaired growth, developmental anomalies, and changes in gene expression (Pidoux et al,

2015). It has been suggested that exposure to formaldehyde during the first trimester of pregnancy can have the most adverse effects on fetal development and increase the possibility of congenital anomalies leading to spontaneous abortion (Thrasher and Kilburn, 2001). The results of studies have shown that the adverse effects of formaldehyde during pregnancy lead to a variety of congenital anomalies, including congenital heart disease, obstruction of the digestive tract, organ defects, and cleft palate, which ultimately lead to low birth weight and fetal death (Motoki et al, 2019). While these studies provide some evidence for a link between formaldehyde exposure and birth defects, they have not definitively established a causal relationship.

During pregnancy, the developing fetus needs significant amounts of omega-3 and other essential fatty acids to support the growth of rapidly dividing cells. These fatty acids are important for the development of the fetal brain, nervous system, and other organs and tissues (Nnamonu et al, 2020). Fish oil is recommended for the normal growth and development of the fetus due to its fatty acid docosahexaenoic acid (Massari et al, 2020). Docosahexaenoic acid is a family of long-chain polyunsaturated fatty acids (LC-PUFAs) or omega-3 fatty acids found in the phospholipid membrane of most cells. This fatty acid is one of the essential fatty acids that the body is not able to synthesize and must enter the body through diet (Roszkos et al, 2020). These fatty acids are transferred from the mother to the fetus through the placenta during pregnancy and are essential for fetal growth. Most reports indicate that a diet rich in these fatty acids during pregnancy prevents placental abruption, fetal growth retardation and preterm delivery (Basak et al, 2020). These fatty acids protect the tissue against free oxygen radicals by preventing lipid peroxidation. Fish oil with omega-3 fatty acids, such as eicosapentaenoic acid and docosahexaenoic acid, has antioxidant

properties and its consumption in rats reduces MDA activity (Gould et al, 2013).

PUFAs are important for cellular signaling, gene expression, and synthesis of second messengers that affect membrane function. They also act as ligands for transcription factors and other intracellular proteins, regulating gene expression, bone formation, and cellular product production (Basak et al, 2020). PUFAs are important for bone growth and development as they regulate inflammation in bone tissue, which is crucial for bone regeneration and repair. Additionally, PUFAs are known to enhance the absorption of calcium and other minerals essential for bone mineralization (Martyniak et al, 2021). They can also regulate osteoblast and osteoclast activity, affecting the equilibrium of bone formation and resorption. Adequate intake of long-chain PUFAs is therefore crucial for maintaining optimal bone health and preventing conditions such as osteoporosis (Bao et al, 2020).

Therefore, this study aimed to evaluate the effect of prenatal formaldehyde injection on fetal development and congenital skeletal anomalies and the protective role of fish oil against formaldehyde - induced developmental toxicity.

Materials and Methods

Study design

In this experimental study, 30 adults female Wistar rats and 10 adults male Wistar rats weighing 220 ± 20 g were used for the experiments. Animals were purchased from the authorized laboratory animal breeding center of the Faculty of Veterinary Medicine (Laboratory Animals House, Shahid Chamran University of Ahvaz, Ahvaz, Iran). The rats were kept in a controlled environment that was free of pathogens and maintained at a temperature of 22.00 ± 2.00 °C, with a relative humidity of $50.00 \pm 10.00\%$ and a light cycle of 12 h light/dark. Rats were fed with a standard pellet diet and had free access to water. All

experimental assays were conducted in compliance with Guidelines for the Humane Care and Use of Laboratory Animals using protocols approved by the Shahid Chamran University of Ahvaz (EE/1401.2.24.204168/scu.ac.ir). Two weeks before the experiment, the rats were placed in the polypropylene rat cage to adapt to the experimental situation. Three female and one male adult rat were kept overnight in a cage for mating. The next morning when a vaginal plug was seen; so that day was considered as the zero day of pregnancy.

Animals grouping

A total number of 30 pregnant Wistar rats were randomly divided equally into five groups. Group 1 served as the control with no treatment. In group 2, sham group, normal saline was administered by gavage and IP. Group 3 was treated orally with fish oil at a dose of 0.5 mg/kg/bw via intragastric gavage (Albert et al., 2017). Group 4 received formaldehyde (Merck, Darmstadt, Germany) at a dose of 10 mg/kg/bw IP (Monfared, 2014). Group 5 received formaldehyde IP along with fish oil via intragastric gavage. The treatment period of the animals was from days 0 to 20 of gestation.

Fetus preparation and morphometric analysis

On the 20th day of gestation, the pregnant rats anesthetized through intraperitoneal injection of a ketamine-xylazine combination, followed by laparotomy. Subsequently, the uterus was dissected, and embryos were excised from the amniotic sac. The weight and Crown-rump length (CRL) as well as count of viable and absorbed fetuses were evaluated in both the right and left uterine horns. Thorough examination for visible congenital anomalies was performed.

Evaluation of the fetal skeletal anomalies

Typically, fetal skeleton staining involves using Alizarin Red S for bones and Alcian Blue for cartilage (Banaei et al., 2024). In the examination of congenital skeletal anomalies, the fetuses were skinned and soaked in acetone for one day, followed by 96% ethanol for seven to ten days. The specimens were then immersed in a solution containing 0.12% Alizarin Red S and 0.14% Alcian Blue in ethanol and glacial acetic acid. Subsequently, fetuses underwent maceration in 2.00% KOH, followed by clearing and hardening in a mixture of 1:1 glycerin and distilled water, and were finally stored in pure glycerin (Mahabady et al., 2016). The occurrence of congenital skeletal anomalies was meticulously assessed using a stereo-microscope. The evaluation of congenital skeletal anomalies, including cleft palate (CP), spina bifida (SB), fused ribs, non-ossification of the sternum (NO-St), delayed ossification of the forelimb (DO-FL), and delayed ossification of the hindlimb (DO-HL), was conducted using a stereomicroscope (Nikon, SMZ 800, Japan).

Expression of BMP4 and Runx2 genes

Total RNA isolation and cDNA synthesis

RNA extraction and cDNA synthesis procedures were conducted on fetuses located in the left uterine horn to evaluate the expression of BMP4 and Runx2 genes. For this study, total RNA was extracted (RNXTM) according to the manufacturer's protocol. The concentration of RNA was determined using a nanodrop device at 260 and 280 in 260/280 nm wavelength absorbance ratio. To assess RNA purity, the optical density ratio of 260/230 was photometrically checked, and samples with a ratio exceeding 1.8 were selected for cDNA synthesis. The synthesis of cDNA was carried out using 1 µg of total RNA following the instructions of the cDNA synthesis kit (Yekta Tajhiz, Iran). The GAPDH gene served as a calibrator for data analysis. The primers of BMP4, Runx2 and GAPDH genes were obtained from the NCBI database, and primer sets were designed using BatchPrimer3 software (BMC Bioinformatics, USA) and analyzed using nBLAST web browser (<https://www.ncbi.nlm.nih.gov/geo/query/blast.html>) to check the target template specificity (Table 1).

Table 1: List of primers employed for quantitative real-time RT-PCR in rat target genes.

Gene	Purpose	Forward primer (5'→3')	Reverse primer (3'→5')	Product (bp)	GenBank accession number
BMP-4	qPCR	GGAGTTTCCATCACGAA GAACATC	GAGATCACCTCATTCTCTG GGAT	124	NM_012827.2
Runx-2	qPCR	ACTCTGCCGAGCTACGA AAT	AAGTGAAACTCTTGCTCG TC	105	XM_006244550.3
GAPDH	qPCR	AGTTCAACGGCACAGTC AAG	TACTCAGCACCAGCATCAC C	119	XM_017593963.1

Quantitative real-time RT-PCR

Real-time PCR reactions were carried out following the YTA SYBR Green qPCR Master Mix 2X kit protocol (Yekta Tazehiz, Iran). Each reaction, with a volume of 12.5 µl, comprised 6.25 µl of Syber Green qPCR Master Mix 2x, 0.25 µl of each primer at a final concentration of 200 nM, 3 µl of cDNA with a final concentration of 200 nM,

and 2.75 µl of ddH₂O. The temperature cycle involved an initial denaturation at 94°C for 5 min, followed by 45 cycles of denaturation at 94°C for 15s. and annealing/extension at 60°C for 30 s. Result analysis was performed using LightCycler SW1.1 software, and calculations were based on the $2^{-\Delta\Delta C_t}$ formula.

Statistical analysis

Statistical analyses were conducted using the GraphPad Prism software (Graph Pad Software Inc. San Diego, California, USA). The findings regarding fetal lengths, weights, and the expression of BMP4 and Runx2 genes were presented as mean \pm SD. A significance threshold of $P < 0.05$ was applied for statistical significance.

Results

Morphometry of fetal growth

The results of the cesarean section are depicted in Fig 1. The fetal weight and CRL in the FA group exhibited a significant

decrease compared to the control and other groups ($p < 0.05$). Co-administration of FO with FA significantly increased the weight and CRL of fetuses as compared to the FA group ($p < 0.05$). The reduction in the weight and CRL of fetuses in the FA + FO group was significant compared to the control ($p < 0.05$; Figure 1). No evident congenital anomalies were observed in the fetuses from the control, sham, FO, and FA + FO groups. In contrast, the FA group exhibited various apparent congenital anomalies, including subcutaneous hemorrhage at the neck and thorax, as well as the presence of undeveloped fetuses.

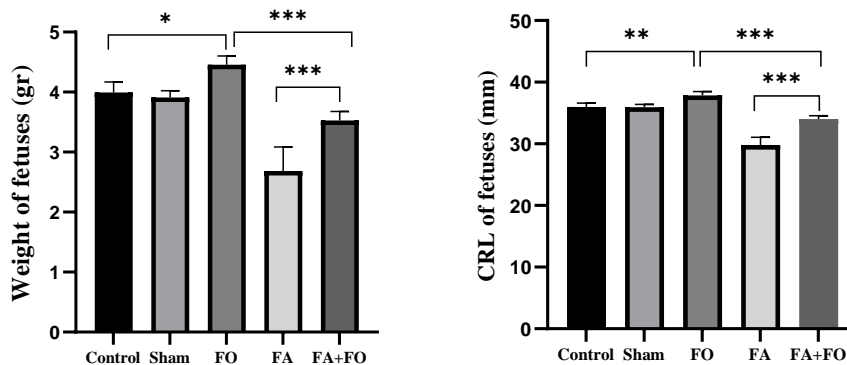


Figure 1: Mean \pm standard deviation of fetal weight and crown-rump length (CRL) in different groups. FO: Fish Oil; FA: Formaldehyde. * $p < 0.05$, ** $p < 0.01$, * $p < 0.001$ indicates significant difference between groups.**

Live and resorbed fetuses

The injection of formaldehyde into pregnant rats did not result in any mortality among the pregnant rats throughout the study duration. The total number of fetuses obtained from six mothers in the control, sham, FO, FA, and FA + FO groups was 75, 73, 78, 38, and 68, respectively. Examination of the percentage of these values revealed a decrease in the percentage

of live fetuses in the FA group (65.78%) compared to the control group (93.33%). Additionally, the percentage of resorbed fetuses in the FA group (34.21%) increased in comparison to the control group (6.66%). While the percentage of these values in the FA + FO group was 88.23% and 11.76%, respectively, which indicated the positive effect of FO on improving the number of live and resorbed fetuses (Table 2).

Table 2: Total number (N) of live and resorbed fetuses, as well as the incidence (%) of fetal congenital anomalies in different groups

Variables	Groups				
	Control	Sham	FO	FA	FA + FO
NF	75	73	78	38	68
LF (%)	93.33	91.78	96.15	65.78 ***	88.23 *
RF (%)	6.66	8.21	3.84	34.21 ***	11.76 *
CP (%)	-	-	-	36.84 ***	10.29 **
SB (%)	-	-	-	21.05 ***	4.41 *
FR (%)	-	-	-	39.47 ***	7.35 **
NO-St (%)	5.33	5.47	2.56	44.73 ***	11.76 *
DO-FL (%)	-	-	-	31.57 ***	5.88 **
DO-HL (%)	-	-	-	34.21 ***	8.82 **

*p< 0.05, **p< 0.01, ***p< 0.001 indicates significant changes between groups. FO: Fish Oil; FA: Formaldehyde. NF: number of fetuses, LF: live fetuses, RF: resorbed fetuses, CP: cleft palate, SB: spina bifida, FR: fused ribs, NO-St: non-ossification of the sternum, DO-FL: delayed ossification of the forelimb, DO-HL: delayed ossification of the hindlimb.

Congenital skeletal anomalies

As presented in Table 2, congenital skeletal anomalies such as SB, CP, FR, DO-FL, and DO-HL were not detected in the control, sham, and FO groups. In contrast, the FA group exhibited a notable prevalence of congenital skeletal anomalies, including CP, SB, FR, NO-St, DO-FL, and DO-HL

was observed. Administration of FO along with FA injection to pregnant rats reduced congenital skeletal anomalies compared to the FA group. However, the percentage of congenital skeletal anomalies of SB, CP, FR, NO-St, DO-FL, and DO-HL in the FA + FO group increased compared to the control group (Table 2, Figures 2 and 3).

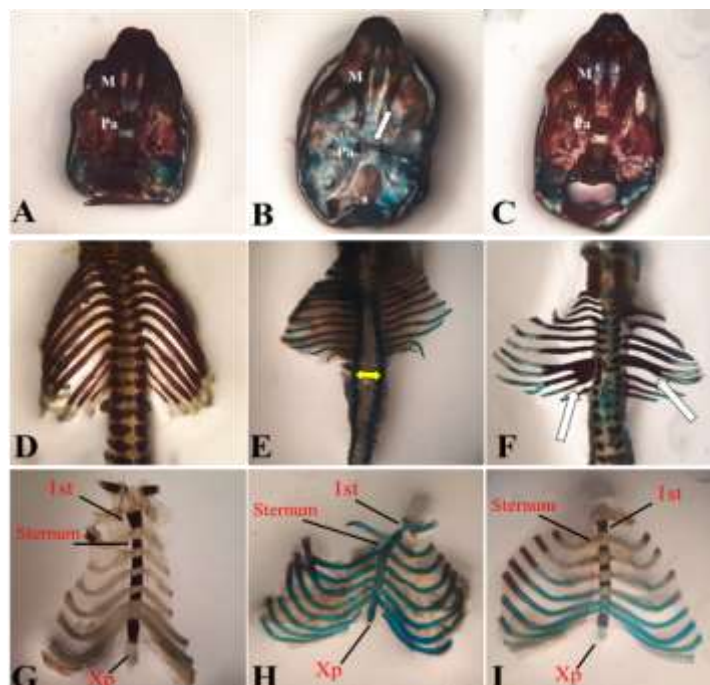


Figure 2: The ventral view of the fetal skull and the dorsal view of the vertebral column of Wistar rat fetus stained with Alizarin red S and Alcian blue. A) Normal palatine bone in the control group; B) Formaldehyde-induced cleft palate (white arrow); C) Normal palatine bone in formaldehyde + fish oil group. D) Normal vertebral column and ribs (control group); E) Spina bifida (yellow arrow) induced by formaldehyde. F) Fused ribs (white arrow) induced by formaldehyde. G) Normal sternum in the control group; H) Sternum with non-ossification of all sternebrae in the formaldehyde group; I) Normal sternum in the formaldehyde + fish oil group, (M: Maxilla; Pa: Palatine; Xp: Xiphoid process; 1st: First sternebra).

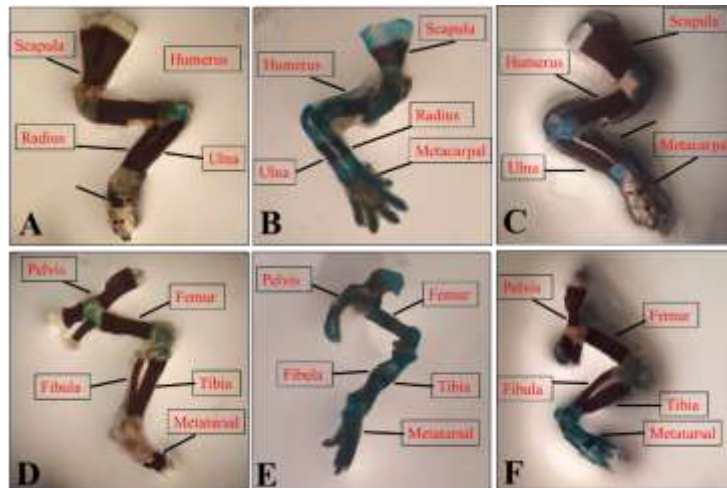


Figure 3: Lateral view of forelimbs and hindlimbs of Wistar rat fetus, stained with Alizarin red S and Alcian blue. A) Normal forelimb in the control group; B) Delay ossification of forelimb in the formaldehyde group; C) Normal forelimb in formaldehyde + fish oil group. D) Normal hindlimb in the control group; E) Delay ossification of hindlimb in the formaldehyde group; F) Normal hindlimb in formaldehyde + fish oil group.

Expression of osteogenesis-related genes

The outcomes of analyzing the expression of BMP-4 and Runx2 genes in various groups are depicted in Fig 4. The expression of BMP-4 and Runx2 genes in fetuses from the FA group exhibited a significant decrease compared to the control

group ($P < 0.05$). Co-administration of FO with FA significantly elevated the expression of BMP-4 and Runx2 genes in comparison to the FO group ($P < 0.05$). No significant difference in these values was observed between the FA + FO and control groups (Figure 4; $P > 0.05$).

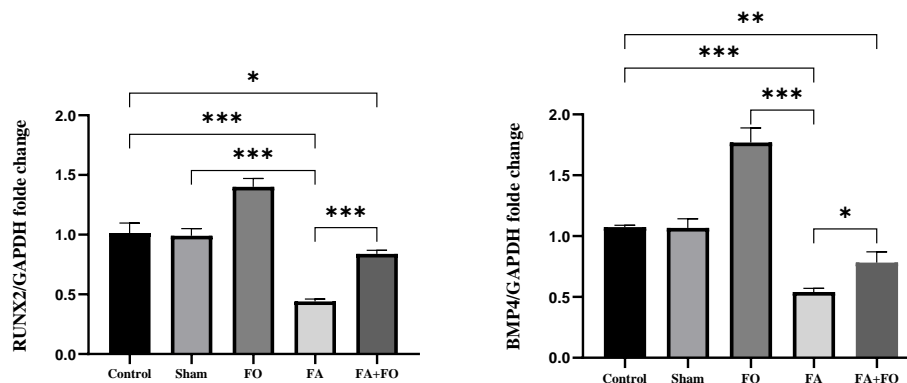


Figure 4: The expression of BMP4 and Runx2 genes in control and treatment groups. FO: Fish Oil; FA: Formaldehyde. * $p < 0.05$, ** $p < 0.01$, * $p < 0.001$ indicates significant difference between groups.**

Discussion

Formaldehyde is a highly useful chemical compound that has a wide range of industrial and medical applications. However, exposure to formaldehyde at high

levels is known to carry a series of health risks to individuals (Zhang et al, 2020). There have been limited studies on the effect of formaldehyde on the

morphometric and skeletal structure of the fetus in pregnant rats, but no study has been done on the effect of fish oil and its protective effects against the destructive properties of formaldehyde. Therefore, the present study determined the protective role of fish oil against developmental toxicity caused by treatment with formaldehyde on fetal morphometry and skeletal system. The findings of this study suggest that formaldehyde injecting into pregnant rats throughout days 0 to 20 of pregnancy disrupts the skeletal development of rat fetuses. This disruption is evident in the diminished expression of osteogenesis-related genes, ultimately leading to shortened body length, reduced skeletal area, and an increase in congenital skeletal anomalies. Furthermore, the oral administration of fish oil to pregnant rats treated with formaldehyde resulted in enhanced fetal growth and reduced congenital skeletal anomalies by upregulating the expression of osteogenesis-related genes in rat fetuses.

In prenatal developmental toxicity studies, the evaluation of the fetus is a critical aspect of the study. This evaluation includes the assessment of external, visceral, and skeletal endpoints. External endpoints include examination of the fetal body for the presence of any external apparent anomalies. This can include an examination of the fetal head, eyes, limbs, and other areas for any visible signs of defects (Hougaard et al, 2015). Skeletal endpoints involve the examination of the fetal bones and joints for any anomalies or malformations that may be present. It may include an evaluation of the skull, spine, limbs, and other areas for any structural or functional deficiencies (Rodríguez and Mandalunis, 2018). Prenatal developmental toxicity studies are typically conducted in laboratory animals such as rats to determine the harmful effects a chemical may have on fetal development. These studies help identify any morphological, physiological, and functional abnormality in the fetus, as

well as anomalies in the external, visceral, skeletal, or reproductive systems. Chemical substances have the potential to cross the placenta and enter the developing fetus, which may interfere with the normal growth and development of the fetus (Scialli et al, 2018). It has recently been shown that maternal exposure to environmental chemical pollutants is the second leading cause of infant mortality in developing countries (Basal et al, 2020).

The developing fetus is highly vulnerable to the harmful effects of environmental toxins, and exposure to formaldehyde during pregnancy can have adverse effects on fetal growth and development (Webb et al, 2014). Formaldehyde can easily penetrate the placental barrier and cause fetal defects in humans and various animal species (Pidoux et al, 2015; Franklin et al, 2019). Studies have shown that formaldehyde exposure during pregnancy can lead to a range of developmental anomalies and fetal defects, including craniofacial defects, neural tube defects, and limb anomalies (Tung and Winn, 2011). Furthermore, individuals may vary in their susceptibility to the harmful effects of formaldehyde exposure, with some populations, such as pregnant women, children, and the elderly, being more vulnerable than others (Herrero et al, 2022). The findings of the current study showed that formaldehyde decreases fetal weight and CRL index in rat fetuses. These changes were probably associated with skeletal degeneration. A developmental toxicity study in FA-exposed rats showed that exposure to FA for 6 h/day from days 6 to 20 of pregnancy had reduced fetal body weight. However, they did not observe a significant change in the average implantation site, average embryo loss, average absorption sites, average live embryo and embryo sex ratio in FA-exposed rats (Nielsen et al, 2013). Another study was conducted on rats to determine the developmental toxicity of formaldehyde. The rats were exposed to 2,

5 or 10 ppm formaldehyde for six hours a day from the 6th to the 15th day of pregnancy. The results of the study showed that exposure to formaldehyde had no adverse effects on live fetuses, dead fetuses and resorptions, fetal weight, mortality before and after implantation. However, this study showed that the incidence of reduced ossification of the pubic and ischial bones was significantly higher in the 5 and 10 ppm treated groups compared to the control group (Martin, 1990). In addition, embryo culture studies have shown that formaldehyde can cause severe embryotoxicity, including death (Hansen et al, 2005). However, the absence of these effects in trials using doses up to 40 ppm suggests that FA does not enter the foetus through inhalation (Nielsen et al, 2013).

One of the mechanisms of the toxic effects of formaldehyde is the production of reactive oxygen species. It has been shown that formaldehyde can stimulate the production of ROS in cells, which can lead to oxidative stress and cellular damage (Zerin et al, 2015). Indeed, exposure to formaldehyde and the resulting oxidative stress are cytotoxic, which can lead to developmental toxicity in the fetus. Formaldehyde can also disrupt fetal DNA synthesis and repair, leading to genetic damage that can manifest as developmental anomalies or other health problems later in life (Zhang et al, 2021). In men, oxidative stress caused by environmental chemical agents may cause suppression of spermatogenesis, decreased testosterone levels, and apoptosis in the testis (Khazaeel et al, 2022). In females, congenital anomalies and reproductive effects can occur due to environmental factors and chemicals, depending on the dosage and timing during pregnancy. This is often attributed to the disruption of the equilibrium between the production of reactive oxygen species and the body's antioxidant defenses (Laforgia et al, 2018). The expression of Runx2 and BMP4 genes, which play important roles in the regulation

of bone development and growth, can be affected by oxidative stress (Sadeghi et al, 2023; Rai et al, 2022). The synchronized functioning of BMP-4 and Runx2 is crucial for the process of ossification in the developing fetus. BMP-4, a protein with a pivotal role, stimulates the differentiation of cells into osteoblast cells, responsible for bone matrix production. Meanwhile, Runx2 regulates gene expression necessary for bone formation. A decline in Runx2 expression hinders cell differentiation into osteoblasts, thereby impairing bone formation (Rahman et al, 2015). The present study reveals that prenatal exposure to formaldehyde (FA) has the potential to diminish the expression of BMP-4 and Runx2 genes in the fetus, resulting in abnormal skeletal growth and skeletal malformations. Other studies have shown that oxidative stress can downregulate the expression of Runx2 and BMP4 genes, leading to decreased bone formation and growth (Lee et al, 2021). Oxidative stress inhibits Runx2 and BMP4 signaling pathways and DNA epigenetic changes lead to the inactivation of transcription factors that regulate the expression of Runx2 and BMP4 genes (Zhu et al, 2018).

Omega-3 fatty acids are a type of polyunsaturated fatty acid that cannot be synthesized by the body and must be obtained through the diet. Fish oil contains high levels of omega-3 fatty acids, specifically eicosapentaenoic acid (EPA) and docosahexaenoic acid, which are essential fatty acids required by the human body for normal growth and development (Ghotbeddin et al, 2022). Studies have shown that omega-3 fatty acids, including EPA and DHA, increase the expression of genes that code for antioxidant enzymes such as glutathione peroxidase (GPx), catalase, and superoxide dismutase (SOD) in fetal cells and tissues (Garrel et al, 2012; Shabani et al, 2019). These antioxidant genes encode enzymes and other proteins that reduce oxidative stress and protect cells from ROS-induced

damage. In addition to increasing the expression of antioxidant genes, it has been reported that omega-3 fatty acids enhance the osteogenic differentiation of mesenchymal stem cells (MSCs) through signaling activation (Levental et al, 2017). The underlying mechanisms involved in the osteogenic effects of omega-3 fatty acids are thought to involve several signaling pathways, including the Wnt/beta-catenin and PI3K/Akt pathways. These pathways are involved in regulating osteoblast differentiation, proliferation, and mineralization, and can be activated by omega-3 fatty acids to promote bone formation (Sadeghi et al, 2023; Sharma and Mandal, 2020). This study showed that fish oil has beneficial effects on the congenital skeletal anomalies caused by prenatal exposure to formaldehyde in rat fetuses. Following the administration of fish oil to formaldehyde-treated rats, the weight, CRL and number of viable fetuses were significantly increased compared to formaldehyde-treated rats. Moreover, the administration of fish oil to formaldehyde-exposed fetuses significantly reduced the number of congenital anomalies. In line with our findings, recently Shrestha et al showed that the administration of Omega-6 and Omega-3 fatty acids in rat fetuses improves the growth, development and the overall fetal health and prevent preterm labor in rats (Shrestha et al, 2020). The study by Zararsiz et al demonstrated that omega-3 fatty acids reduced formaldehyde-induced neurotoxic effects, maintained the levels of SOD, GSH-Px and MDA enzymes, and reduced formaldehyde-induced cell damage, indicating the potential of their neuroprotection against formaldehyde toxicity (Zararsiz et al, 2006). Research indicates that antioxidants

like vitamin E can counteract formaldehyde's toxicity. Antioxidants scavenge reactive oxygen species generated by formaldehyde, mitigating oxidative stress, apoptosis and cellular damage. This suggests a potential protective role for antioxidants against formaldehyde-induced toxicity, expanding therapeutic avenues for mitigating environmental toxin exposure (Wu et al, 2017; Bakar et al, 2015). In addition, other studies have shown that in rat fetuses, the administration of fatty acids increases the weight, length and head circumference of the fetus, as well as the gestational age and fetal viability. Also, these studies have shown that Omega-3 fatty acids during pregnancy is associated with reducing the risk of fetal congenital anomalies (Akerlele and Cheema, 2016). This shows that adequate consumption of fatty acids during pregnancy is important for the optimal growth and development of the fetus.

In conclusion, the findings of the current study demonstrated the effects of fish oil administration on the elimination of formaldehyde-induced teratogenicity for the first time. Prenatal exposure of rat fetuses to formaldehyde disrupts skeletal development and an increase in congenital anomalies including spina bifida, cleft palate, fused ribs, non-ossification of the sternum, delayed ossification of the forelimb and hindlimb in rats. These congenital skeletal anomalies were associated with a decrease in the expression of Runx2 and BMP4. Moreover, the study highlights that the antioxidant properties of fish oil alleviate the teratogenic effects of prenatal formaldehyde exposure by enhancing the expression of osteogenesis-related genes and preventing formaldehyde-induced oxidative stress.

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Conflict of interest

The authors declared no conflict of interest.

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اثرات مکمل روغن ماهی بر ناهنجاری‌های اسکلتی مادرزادی ناشی از فرمالدئید در موش‌های صحرایی ویستار

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چکیده

نشان داده شده است که فرمالدئید به عنوان یک ترکیب آلی رایج باعث ناهنجاری‌های رشدی و نقص جنین می‌شود. روغن ماهی (FO) به دلیل دارا بودن اسید چرب دوکوزاهگزانوئیک برای رشد و نمو طبیعی جنین توصیه می‌شود. هدف از این مطالعه بررسی پتانسیل روغن ماهی در محافظت از رشد جنین و جلوگیری از ناهنجاری‌های اسکلتی ناشی از تیمار موش‌های صحرایی بارداری بود. ۳۰ موش صحرایی بارداری ویستار به طور تصادفی در ۵ گروه کنترل، شم (سرم فیزیولوژی؛ گاواژ و تزریق داخل صفاقی)، روغن ماهی (۰/۵ میلی‌گرم/کیلوگرم وزن بدن؛ گاواژ)، فرمالدئید (۱۰ میلی‌گرم/کیلوگرم وزن بدن؛ تزریق داخل صفاقی)، فرمالدئید + روغن ماهی دسته‌بندی شدند. دوره تیمار از روز صفر تا ۲۰ بارداری بود. در روز بیستم بارداری، حیوانات تحت بیهوشی قرار گرفتند و پس از آن لاپاراتومی برای تعیین وزن و طول جنین (CRL) انجام شد. ارزیابی‌های استریومیکروسکوپی اسکلتی جنین‌ها با استفاده از روش رنگ‌آمیزی آلزارین قرمز/آلیسین آبی انجام شد. علاوه بر این، بیان Runx2 و BMP4 از طریق qPCR ارزیابی شد. یافته‌ها نشان داد که قرار گرفتن در معرض فرمالدئید قبل از تولد به طور قابل توجهی وزن جنین و CRL و همچنین بیان ژن‌های Runx2 و BMP4 را کاهش می‌دهد. علاوه بر این، فرمالدئید وقوع ناهنجاری‌های اسکلتی مادرزادی، از جمله شکاف کام، اسپینا بیفیدا و عدم استخوان‌سازی استخوان‌های جنین را افزایش داد. با این وجود، تجویز همزمان روغن ماهی و فرمالدئید در موش‌های صحرایی بارداری، رشد استخوان جنین را بهبود بخشید و ناهنجاری‌های اسکلتی را کاهش داد. روغن ماهی پتانسیل کاهش اثرات تراژژنیک مواجهه با فرمالدئید را با افزایش بیان ژن‌های مرتبط با استخوان‌زایی نشان داد.

کلمات کلیدی: ناهنجاری‌های مادرزادی اسکلتی، فرمالدئید، روغن ماهی، جنین، موش صحرایی

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