Received: 15 April 2023 | Revised: 5 August 2023 | Accepted: 7 August 2023

DOI: 10.1002/bdr2.2240

Birth Defects Research | Society for Birth Defects Research & Prevention | WILEY

RESEARCH ARTICLE

Protective effect of quercetin on fetal development and congenital skeletal anomalies against exposure of pregnant Wistar rats to crude oil vapor

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Funding information
Shahid Chamran University of Ahvaz, Grant/Award Number: VB1400.293

Abstract

Background: Epidemiological evidence indicates a relationship between maternal exposure to crude oil vapors (COV) during pregnancy and adverse pregnancy outcomes. Quercetin (QUE) is a plant flavonoid with purported antioxidant and anti-inflammatory effects, which has been shown to prevent birth defects. This study was aimed to investigate the protective role of QUE on fetal development and congenital skeletal anomalies caused by exposure of pregnant rats to COV.

Methods: Twenty-four pregnant Wistar rats were randomly categorized into four groups of control, COV, COV + QUE, and QUE (50 mg/kg). The inhalation method was used to expose pregnant rats to COV from day 0 to 20 of pregnancy, and QUE was administered orally during this period. On day 20 of gestation, the animals were anesthetized and a laparotomy was performed, and then the weight and crown rump length (CRL) of the fetuses were determined. Skeletal stereomicroscopic evaluations of fetuses were performed using Alcian blue/Alizarin red staining method, and the expression of osteogenesis-related genes (Runx2 and BMP-4) was evaluated using qPCR.

Results: This study showed that prenatal exposure to COV significantly reduced fetal weight and CRL, and expression of Runx2 and BMP-4 genes. Moreover, COV significantly increased the incidence of congenital skeletal anomalies such as cleft palate, spina bifida and non-ossification of the fetal bones. However, administration of QUE with exposure to COV improved fetal bone development and reduced congenital skeletal anomalies.

Conclusion: QUE can ameliorate the teratogenic effects of prenatal exposure to COV by increasing the expression of osteogenesis-related genes.

KEYWORDS

congenital skeletal anomalies, crude oil, developmental toxicity, fetus, quercetin

1 | INTRODUCTION

Crude oil vapors (COV) refer to the gaseous forms of hydrocarbons and other volatile compounds that are released from crude oil. Crude oil exposed to certain conditions such as high temperature or contact with air can evaporate and produce vapors. These vapors consist of a complex mixture of organic compounds, including

hydrocarbons of various sizes and structures (Sirotkin & Harrath, 2017). Depending on the location of the oil and gas production, the major chemical composition of petroleum and its hydrocarbons can be considerably different (Adipah, 2019). Crude oil contains various types of hydrocarbons including resin, asphaltene, and polycyclic aromatic hydrocarbons (PAHs), which are especially dangerous due to the multiple sources of pollution in the environment (Onojake et al., 2021). It is generally recognized that PAHs are the main toxic compounds in crude oil, which have toxic effects on organisms in various ways (Gao et al., 2019). PAHs in crude oil have a relatively high solubility in the water and air for easy migration and transport into cells, and the main route of exposure of living organisms to the toxic effects of PAHs is through inhalation in the environment (Abdel-Shafy & Mansour, 2016).

Studies have shown that exposure to gas and oil pollutants over a longer period of time has been associated with congenital heart defects (McKenzie et al., 2019). Disorders and complications associated with exposure to these pollutants include intrauterine growth restriction, birth defects, premature birth, fetal loss and death, childhood diseases, and several adult cancers (Wigle et al., 2008). Experimental studies on inhalation exposure of pregnant rats to petroleum pollutants from the 6th to 12th day of gestation indicated a decrease in fetal weight, obvious skeletal deformities, and weak mineralization, but no convincing evidence of teratogenicity was provided (Abd-Allah et al., 2023). The possible toxic mechanisms of petroleum pollutants are quite complex. However, it has been shown that gasoline derived from crude oil has an inherent potential to generate reactive oxygen species (ROS) (Uboh et al., 2012). Recently, it has been shown that the developmental toxicological mechanism of crude oil pollutants includes the production of ROS and increased oxidative stress, which causes apoptosis and cellular oxidative damage (Sadeghi et al., 2023). The developing fetus is highly sensitive to oxidative stress due to rapid cell division and immature antioxidant defense systems. Therefore, oxidative stress caused by exposure to petroleum pollutants can cause damage to cellular components such as proteins, lipids, and DNA, leading to cell death and a wide range of adverse effects on fetal development (Lu et al., 2022). Other studies have shown that oxidative stress caused by oil pollutants can affect the expression of bone synthesis-related genes, such as bone morphogenetic protein (BMP4) and Runt-related transcription factor 2 (Runx2) in the fetus. These genes are critical for skeletal development and their dysregulation can lead to congenital skeletal anomalies (Elfawy et al., 2021).

The use of flavonoids derived from medicinal plants, vegetables, and fruits has been well-established due to their biological potentials such as free-radical scavenging and anti-inflammatory activities (Parhi et al., 2020). Quercetin (QUE) is one of the essential natural flavonoids present in most of the fruits, vegetables, and in few plant leaves (Saakre et al., 2021). Various studies have shown that the antioxidant and anti-inflammatory activities of QUE are very effective function against oxidative stress, cell damage and apoptosis (Zhao et al., 2021). Furthermore, another study by Pérez-Pastén et al indicates that QUE has a protective effect against damage to fetal development (Pérez-Pastén et al., 2010). It has also been shown that the administration of QUE from day 5 to day 20 of gestation reduces fetal mortality and growth retardation in rats exposed to the inhalation of nano-enriched diesel exhaust particles, and the number of intrauterine absorbed embryos and fetal malformations (Ibrahim et al., 2020). Generally, oral administration of antioxidants is recommended to potentially treat and prevent the toxic effects of environmental pollutants on infertility and congenital anomalies (Dolati et al., 2021).

Therefore, this study (1) aimed to evaluate the relationship between the developmental toxicity of petroleum pollutants, specifically COV, on the fetal skeletal system, including expression of osteogenesis-related genes, and (2) determine the possible protective effect of QUE against COV-induced developmental toxicity.

2 | METHODS

2.1 | Animals and study design

This study was conducted using a 4-month-old male and female Wistar rats weighing 220–240 g. The rats were obtained from the Jundishapour Laboratory Animal Center and maintained in standard laboratory conditions (12:12 h light/dark cycle, temperature of $23 \pm 2°C$, relative humidity of $50 \pm 5\%$). Rats were fed ad libitum with the standard lab chow (Pars Dam, Tehran, Iran) and tap water. All experiments were carried out in accordance with the guidelines for the humane care and use of laboratory animals approved by the Ethics Committee of the Shahid Chamran University of Ahvaz (EE/1400.2.24.64232/scu.ac.ir). The female rats were mated with males overnight on a 3 to 1 basis. The following morning, female rats were examined for sperm in the vaginal smear, and sperm-positive vaginal smears were considered as day zero of pregnancy. Twenty-four pregnant rats were divided into four groups ($n = 6$) as follows:

1. Control group (normal saline (5 mL/kg) was administered to pregnant rats from day 0 to day 20 of gestation).

2. COV exposed group (pregnant rats were exposed to COV inhalation daily for 5 h from day 0 to 20 of gestation between 11 a.m. and 4 p.m.).
3. COV + QUE group (pregnant rats were exposed to COV inhalation for 5 h daily from 0 to 20 days of gestation between 11 a.m. and 4 p.m., and QUE was administered by oral gavage daily between 9 a.m. and 10 a.m.).
4. QUE-treated group (QUE (Sigma-Aldrich, CAS No: 117-39-5; 50 mg/kg, dissolved in 0.9% saline solution (1 mL/kg)) was administered by oral gavage daily to pregnant rats from day 0 to day 20 of gestation between 9 a.m. and 10 a.m.) (Costa et al., 2022; Sun et al., 2020).

2.2 | Exposure of pregnant rats to COV

In order to expose rats to COV by inhalation method, a vivarium (COV chamber) was designed for this study and used as described previously (Khazaeel et al., 2022). Briefly, this COV chamber had a COV inlet, an outlet valve, and a temperature and humidity regulator for the COV chamber. Pregnant rats were exposed to COV daily for 5 h from 0 to 20 days of gestation in the COV chamber and allowed to move around freely. The selected duration of exposure of rats to crude oil vapor was chosen according to the environmental conditions of most oil extraction areas in Khuzestan province, Iran. Therefore, in cities and oil extraction areas, the temperature of oil extraction areas reaches 60°C and the minimum time people are exposed to these pollutants is about 5 h. Crude oil used in this study was obtained from National Iranian South Oil Company (NISOC). The temperature of crude oil in this study was set at 60°C to simulate the natural conditions of oil extraction areas, and the exposure dose was adjusted to 25 μg/m^3 (Archibong et al., 2002). The compounds in the crude oil were analyzed by the GC Mass method by Mahamax Company (Iran), and the results of crude oil analysis used in this study are shown in Table S1.

2.3 | Fetus preparation

Initial and final body weights of pregnant rats were monitored and the difference between initial and final body weight of respective groups were taken as body weight gains. The pregnant rats were anesthetized by intraperitoneal injection of ketamine–xylazine combination on the 20th day of gestation, and then laparotomy was performed. After uterus dissection and rat embryos excision from the amniotic sac, the number of viable and absorbed fetuses 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 digital caliper, respectively. The fetuses were carefully examined for external apparent anomalies. Then, the fetuses available in the right horn were eviscerated and fixed in alcohol 96% for staining and macroscopic evaluations, and the fetuses located in the left uterine horn were used for gene expression assessment.

2.4 | Cartilage and bone staining

Staining of the fetal skeleton is typically accomplished by staining the whole fetus with Alizarin Red S (bone) and Alcian Blue (cartilage). For investigating of congenital skeletal anomalies, the fetuses were completely skinned and rinsed in acetone for 1 day then 96% ethanol for 7 to 10 days. The specimens were placed in a mixture of 0.14% Alcian Blue and 0.12% Alizarin Red S in ethanol and glacial acetic acid. Fetuses were then macerated in 2.00% KOH, cleared and hardened in 1:1 glycerin and distilled water, and stored in pure glycerin (Khazaeel et al., 2021). The incidence of congenital skeletal anomalies was carefully examined using a stereo-microscope (Model SMZ800; Nikon, Tokyo, Japan) and compared between groups. Congenital skeletal anomalies that were recorded included cleft palate (CP), spina bifida (SB), fused ribs (FR), non-ossification of the sternum (NO-St), non-ossification of the last rib (NO-LR), delayed ossification of the forelimb (DO-FL) and delayed ossification of the hindlimb (DO-HL).

2.5 | Expression of osteogenesis-related genes (BMP-4 and Runx-2)

2.5.1 | Total RNA isolation and cDNA synthesis

The fetuses located in the left uterine horn were used to assess the expression of BMP-4 and Runx-2 genes. In this experiment, total RNA was isolated from femurs of 20-day-old fetuses using RNX™ reagent according to the manufacturer's instructions (SinaClon, Iran). RNA concentration was measured using a nanodrop device (Eppendorf BioPhotometer D30, Germany) at 260 and 280 nm wavelengths. To determine the purity of RNA, the optical density ratio of 260/230 was checked photometrically, and samples with a higher ratio of 1.8 were used for cDNA synthesis. The cDNA synthesis was

performed using 1 μg of total RNA according to the instructions of the cDNA synthesis kit (Yekta Tajhiz, Iran). The primers of BMP-4 and Runx-2 and GAPDH genes were designed using BatchPrimer3 software (BMC Bioinformatics, USA). The GAPDH gene was used as a calibrator for the analysis of data (Table 1).

2.5.2 | Quantitative real-time RT-PCR

Real-time PCR reactions were conducted according to the protocol of the YTA SYBR Green qPCR Master Mix 2X kit (Yekta Tazehiz, Iran). The reactions in a volume of 12.5 μL included 6.25 μL of Syber Green qPCR Master Mix 2x, 0.25 μL of each primer with a final concentration of 200 nM, 3 μL of cDNA with a final concentration of 200 nM, and 2.75 μL of ddH_2O. The temperature cycle consisted of 94°C for 5 min as the first denaturation, 45 cycles including 94°C for 15 s, and 60°C for 30 s. Analysis of the results was evaluated using LightCycler SW1.1 software and calculated based on formula $2^{-\Delta\Delta Ct}$.

2.6 | Statistical analysis

Statistical analyses were performed using the SPSS program (Windows version 24; Chicago, USA). The results of lengths and weights of the fetuses and expression of BMP-4 and Runx-2 genes were displayed as mean ± SD. A *p*-value below .05 was considered statistically significant.

3 | RESULTS

3.1 | Maternal body weight gain

The results of maternal body weight gain in different groups showed that the exposure of pregnant rats to COV caused a significant decrease in body weight gain compared to the control group ($p < .05$). The administration of QUE along with exposure of pregnant rats to the COV caused a significant increase in maternal body weight gain compared to the COV group ($p < .05$). There was no significant difference in maternal body weight gain between the COV + QUE and the control group ($p > .05$). However, maternal body weight gain in the COV + QUE group showed a significant decrease compared to the QUE group ($p < .05$; Figure 1).

3.2 | Fetal growth parameters

The findings of the cesarean section are shown in Figures 1 and 2. There were no external apparent congenital anomalies in the fetuses obtained from different experimental groups. The mean fetal weight and CRL in the COV group showed a significant decrease compared to the control group and other groups ($p < .05$). Administration of QUE along with exposure of pregnant rats to COV caused a significant increase in the mean weight and CRL of fetuses compared to COV-exposed rats ($p < .05$). However, the reduction of mean weight and CRL of fetuses in the COV + QUE group

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	GGAGTTTCCATCACGAAGAACATC	GAGATCACCTCATTCTCTGGGAT	126	NM_012827.2
Runx-2	qPCR	ACTCTGCCGAGCTACGAAAT	AAGTGAAACTCTTGCCTCGTC	105	XM_006244550.3
GAPDH	qPCR	AGTTCAACGGCACAGTCAAG	TACTCAGCACCAGCATCACC	119	XM_017593963.1

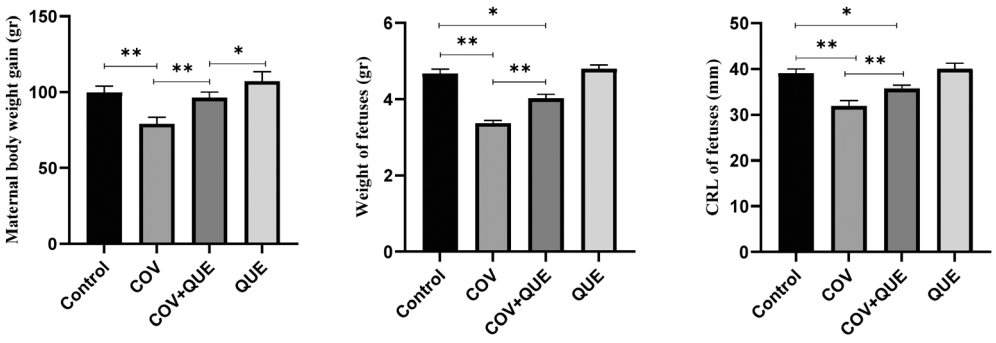

FIGURE 1 Mean ± standard deviation of maternal body weight gain, fetal weight and crown-rump length (CRL) in different groups. COV, crude oil vapor; QUE, quercetin. *$p < .05$, **$p < .01$ indicates significant changes between groups.

was significant compared to the control group ($p < .05$; Figures 1 and 2).

3.3 | Live and resorbed fetuses

Exposure of pregnant rats to COV did not show any mortality in pregnant rats during the study period. The total number of fetuses obtained from each of the six mothers in control, COV, COV + QUE, and QUE groups were 52, 43, 49, and 53, respectively. The evaluation of the percentage of live and resorbed fetuses in different treatment groups indicated that the percentage of live fetuses in the COV group (67.44%) was reduced compared to the control (94.23%). Also, the percentage of resorbed fetuses in the COV group (32.55%) was increased compared to the control (5.76%). The percentage of live (89.79%) and resorbed (16.32%) fetuses in the COV + QUE group indicated QUE improved the number of live and resorbed fetuses (Table 2).

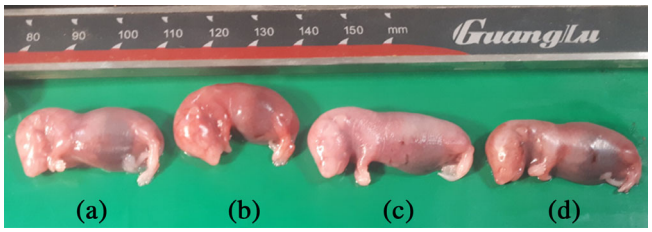

FIGURE 2 Lateral view of 20-day Wistar rat fetuses in different experimental groups. (a) Control group; (b) COV group; (c) COV + QUE group; (d) QUE group.

TABLE 2 Total number (*N*) of live and resorbed fetuses, and incidence (%) of fetal congenital anomalies in different groups.

	Groups			
Variables	**Control**	**COV**	**COV + QUE**	**QUE**
NF	52	43	49	53
LF (%)	94.23	67.44*	89.79*#	96.22
RF (%)	5.76	32.55*	16.32*#	3.77
CP (%)	-	31.81*	11.53*#	-
SB (%)	-	27.27*	7.69*#	-
FR (%)	3.84	40.90*	15.38*#	-
NO-St (%)	7.69	47.82*	19.23*#	3.70
NO-LR (%)	-	13.63*	-	-
DO-FL (%)	-	31.81*	7.69*#	-
DO-HL (%)	-	27.27*	3.84*#	-

Abbreviations: CP, cleft palate; DO-FL, delayed ossification of the forelimb; DO-HL, delayed ossification of the hindlimb; FR, fused ribs; LF, live fetuses; NF, number of fetuses; NO-LR, non-ossification of the last rib; NO-St, non-ossification of the sternum; RF, resorbed fetuses; SB, spina bifida.
*Significant (#$p < .05$) changes compared with the control group and # indicates significant (#$p < .05$) changes compared with COV group.

3.4 | Fetal skeletal anomalies

As presented in Table 2, the high incidence of congenital skeletal anomalies including CP, SB, FR, NO-St, NO-LR, DO-FL, and DO-HL was observed in the COV-exposed group. Administration of QUE along with exposure of pregnant rats to COV decreased these values compared to COV-exposed rats. However, the percentage of CP, SB, FR, NO-St, DO-FL, and DO-HL anomalies in the COV + QUE group increased compared to the control group. Also, the NO-LR abnormality was not observed in the COV + QUE group fetuses (Table 2, Figures 3–5).

3.5 | Alteration of BMP-4 and Runx-2 expression

The results of examining the expression of osteogenesis-related genes in different groups are shown in Figure 6. The expression of BMP-4 and Runx-2 genes was significantly decreased in the fetuses of the COV group

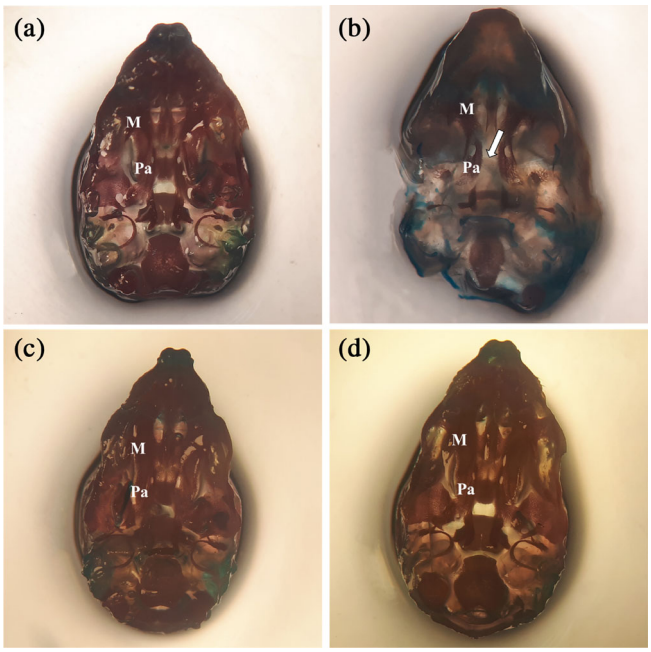

FIGURE 3 The ventral view of the rat fetal skull on day 20 of gestation stained with Alizarin red S and Alcian blue. (a) Normal palatine bone in the control group; (b) cleft palate caused by exposure to COV (white arrow); (c) normal palatine bone in COV + QUE group. (d) Normal palatine bone in QUE group. M, maxilla; Pa, palatine.

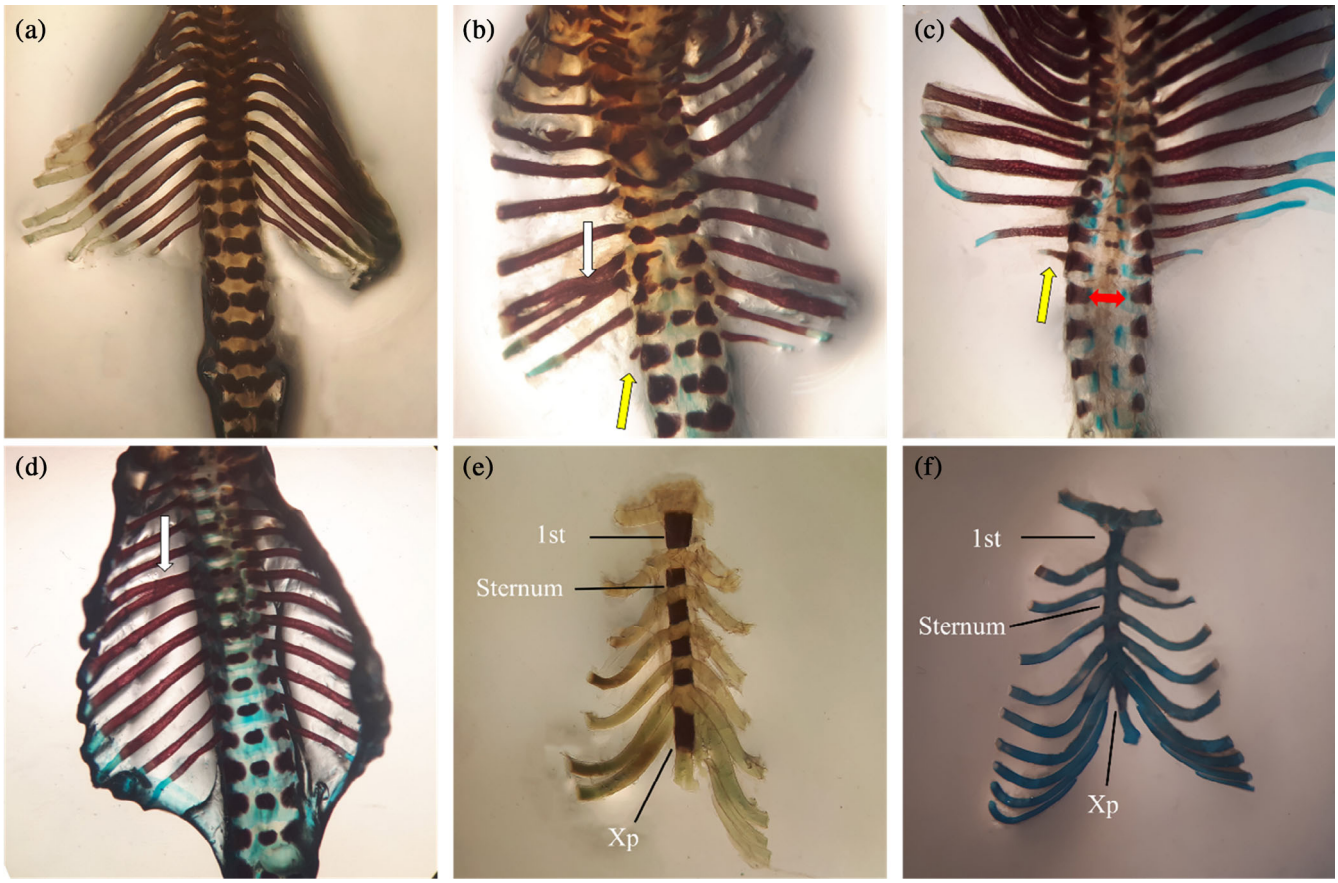

FIGURE 4 (a–d) Dorsal view of vertebral column of fetal Wistar rats on day 20 of gestation stained with Alizarin red S and Alcian blue. (a) Normal vertebral column and ribs (control group); (b–d) Fused ribs (white arrow), underdeveloped ribs (yellow arrows), and spina bifida (red arrow) induced by COV. (e, f) Dorsal view of sternum of 20-day Wistar rat fetuses, stained with Alizarin red S and Alcian blue. (e) Normal sternum; (f) sternum with non-ossification of all sternebrae in the COV group. 1st, first sternebra; Xp, xiphoid process.

compared to the controls ($p < .05$). The administration of QUE along with the exposure of pregnant rats to COV caused a significant increase in the expression of BMP-4 and Runx-2 genes compared to the COV group ($p < .05$). No significant difference was found in these values between the COV + QUE and control groups ($p > .05$). Also, the expression of the BMP-4 gene in the fetuses of the QUE group significantly increased compared to the control group (Figure 6; $p < .05$).

4 | DISCUSSION

In the present study, we documented the teratogenic effects of prenatal exposure of rats to COVs on fetal development and congenital skeletal anomalies. Also, we determined the potential protective role of quercetin in preventing fetal growth and skeletal changes induced by COVs. The results of our study suggest that prenatal exposure to COV by inhalation method impaired skeletal development in rat fetuses, as shown by decreased expression of osteogenesis-related genes, ultimately resulting in shortened body length, reduced skeletal area, and increased congenital skeletal anomalies. Moreover, oral administration of QUE to COV-exposed pregnant rats led to improved fetal growth and reduced congenital skeletal anomalies by increasing the expression of osteogenesis-related genes in comparison with COV-exposed fetuses.

Recent studies have shown the relationship between maternal toxicity with fetal weight loss and delayed ossification in fetuses (Kim et al., 2023). Exposure of pregnant mothers to toxic substances or harmful environmental factors can negatively affect the developing fetus. Fetal weight loss occurs when exposure to toxins interferes with the normal growth and development of the fetus during pregnancy, which can lead to low birth weight of the fetus (Gómez-Roig et al., 2021). The formation of bone tissue, or ossification, occurs in two main ways: intramembranous ossification and endochondral ossification. Intramembranous ossification occurs when mesenchymal cells differentiate into

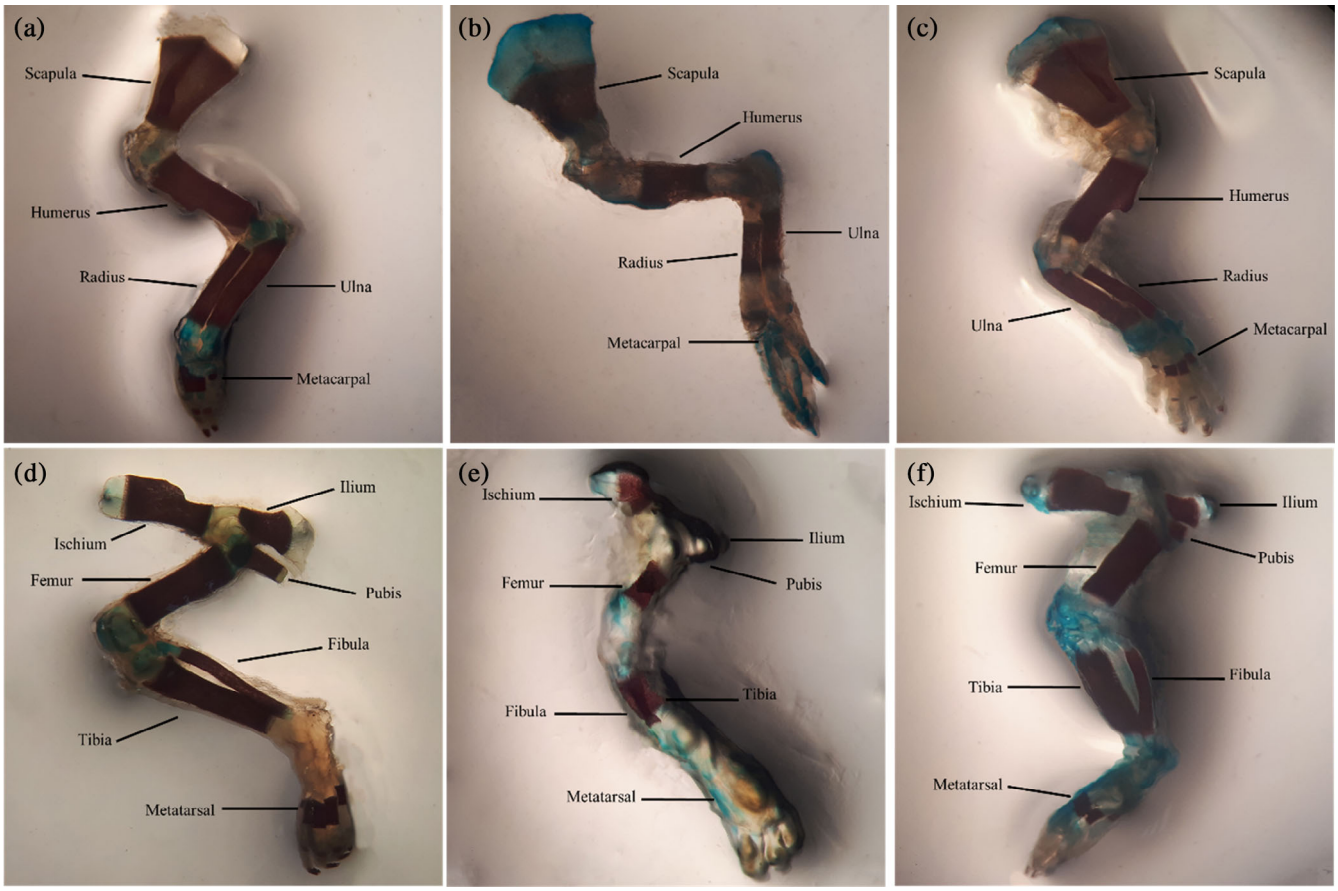

FIGURE 5 Lateral view of forelimbs and hindlimbs of 20-day-old Wistar rat fetus, stained with Alizarin red S and Alcian blue. (a) Normal forelimb (control group); (b) delay ossification of forelimb in the COV group; (c) normal forelimb in COV + QUE group; (d) normal hindlimb (control group); (e) delay ossification of hindlimb in the COV group; (f) normal hindlimb in COV + QUE group.

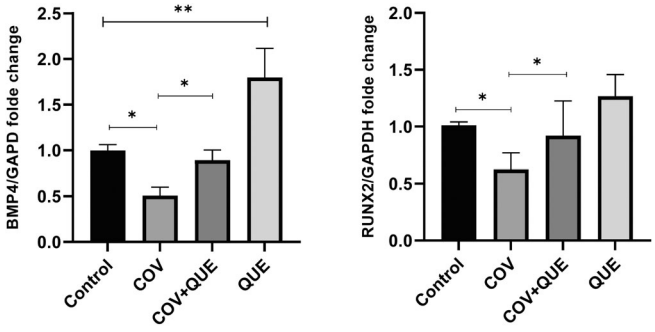

FIGURE 6 Effect of COV and QUE on the expression of BMP-4 and Runx-2 genes in control and treatment groups. COV, crude oil vapor; QUE, quercetin. *$p < .05$, **$p < .01$ indicates significant changes between groups.

osteoblasts, which then deposit bone matrix to form flat bones such as those of the skull. On the other hand, endochondral ossification occurs when a cartilage template is first formed and then is gradually replaced with bone tissue. This process occurs in long bones, such as those in the arms and legs (Ghimire et al., 2021). As embryonic development progresses, the limbs continue to grow and develop. The long bones of the limbs first form as cartilage models, and then the cartilage is progressively replaced with bone. Secondary ossification centers form at the ends of the long bones, and ultimately the bones grow in length and thickness through processes of bone deposition and resorption (Blumer, 2021). Due to the effect of Runx-2 and BMP-4 expression on the differentiation of mesenchymal progenitor cells into osteoblasts, the dynamic balance is required between differentiation of pluripotent mesenchymal stem cells (MSCs) through intramembranous ossification into osteoblasts or MSCs differentiate into chondrocytes through an endochondral ossification process (Fakhry et al., 2013). However, this balance may be disrupted by exposure to petroleum products and cause morphological anomalies in vertebrate embryos (Xu et al., 2020). In the present study, the exposure of pregnant rats to COV decreased the expression of osteogenesis-related genes.

It is generally known that PAHs are the main toxic components in crude oil (Meador & Nahrgang, 2019). The specific concentration of PAHs in a given sample of

crude oil depends on factors such as the type of oil, the place of extraction, and the refining process used. As shown in Table 1, the results of crude oil pollutant analysis in this study showed the presence of a wide range of PAHs in crude oil extracted from the Khuzestan region of Iran. Inhalation is the main route of exposure of humans and organisms to polycyclic aromatic hydrocarbons in the environment (Abdel-Shafy & Mansour, 2016). It has recently been shown that maternal exposure to PAHs is associated with a 6.8% reduction in body weight and a 3% reduction in head circumference at birth, and reduced white matter surface of the left hemisphere in childhood (Yi et al., 2022). It is well known that PAHs easily penetrate the placental barrier and cause fetal defects in humans and various animal species (Dehghani et al., 2022; Webb et al., 2018). PAHs cause toxic effects on humans and organisms through different mechanisms. The main proposed mechanism for the toxicity of PAHs is their interference with the function and enzyme systems of cell membranes (Rengarajan et al., 2015). Recently, it has been shown that PAHs cause reproductive dysfunction by decreasing the activity of antioxidant enzymes and increasing the level of lipid peroxidation (Zhang et al., 2022). The mechanism of action of polycyclic aromatic hydrocarbons is the activation of aryl hydrocarbon receptors (AhR), which leads to changes in cellular DNA and increased oxidative stress (O'Driscoll et al., 2018). There is evidence that the toxic consequences of AhR activation by PAHs lead to oxidative stress due to the metabolic process of the ligand and the induction of CYP1 enzymes (Dietrich, 2016). Depending on the dose and time of pregnancy, environmental factors and chemicals can cause congenital skeletal anomalies and reproductive effects by disrupting the balance between ROS production and antioxidant defenses (Laforgia et al., 2018). An animal study exposing pregnant rats to inhaled petroleum pollutants at 313 ppm for 24 h/day on days 9–14 of gestation reported decreased fetal weight and increased skeletal variants, but did not provide conclusive evidence of teratogenicity (Hudak & Ungváry, 1978).

Our findings indicated that exposure to COV is associated with reduced fetal weight and CRL index, which were associated with fetal skeletal changes. Coordinated activity of BMP-4 and Runx-2 is required for ossification in the developing fetus. BMP-4 is a protein that plays an important role in stimulating the differentiation of cells into osteoblast cells, which are responsible for the production of bone matrix. Runx-2, regulates the expression of genes necessary for bone formation, and with a decrease in Runx-2 expression, cells are unable to differentiate into osteoblasts, and bone formation is impaired (Rahman et al., 2015). As we identified in the current study, prenatal exposure to COV reduced the expression of BMP-4 and Runx-2 genes in fetuses, leading to abnormal skeletal development and skeletal anomalies. Other studies have shown that exposure to petroleum pollutants can alter the expression of BMP-4 and Runx-2 genes by causing oxidative stress, inflammation, and DNA damage (Seemann et al., 2015). Petroleum pollutants can also directly interact with DNA, leading to DNA damage and altered gene expression. Studies have shown that exposure to petroleum pollutants can induce mutations and structural changes in DNA, which can affect the expression of BMP-4 and Runx-2 genes (Kuppusamy et al., 2020).

There is strong evidence that the production of ROS is often the mechanism by which PAHs affect fetal and newborn health (Kuang et al., 2020). Various mechanisms of PAH toxicity have been proposed at the molecular level, and it seems that modulation of antioxidant enzymes, and damage to cell membrane lipids, DNA, and proteins are the most important effects of exposure to PAHs (Yun et al., 2019). Recently, it has been found that QUE can act as an aryl hydrocarbon receptor (AhR) ligand that regulates cytochrome P450 (CYP) 1A enzyme expression (Pinto et al., 2023). The AhR is a nuclear receptor that is activated by certain environmental pollutants and natural compounds, including flavonoids like QUE. AhR activation regulates the expression of genes involved in processes such as xenobiotic metabolism, immunity, and inflammation (Shivanna et al., 2022). QUE increases the expression of antioxidant genes and reduces the production of ROS. In addition, QUE can prevent the activation of other oxidative stress pathways, including the NF-kB pathway, as well as signal transduction pathways and ROS caused by environmental factors (Xu et al., 2019). Our recent study showed that the oral administration of QUE can protect the male reproductive system against COV-induced toxicity by reducing cellular oxidative stress and the expression of apoptotic genes (Khazaeel et al., 2022). The potential role of QUE in the treatment of diseases associated with bone loss has been accompanied by encouraging results. QUE has been reported to enhance osteogenic differentiation and antioxidant responses of mouse bone MSCs through the activation of AMPK/SIRT1 signaling (Wang et al., 2021). The present study showed that COV exposure leads to apparent maternal toxicity, as fetal changes could be secondary to maternal toxicity and the observed fetal changes occurred in the presence of maternal toxicity. Also, this study indicated that QUE reduces congenital skeletal malformations caused by COV exposure during pregnancy in rat fetuses. Administration of quercetin to COV-exposed rats significantly increased fetal weight, CRL, and the number of live embryos compared

to COV-exposed rats. Moreover, the administration of QUE to COV-exposed rats significantly reduced the number of congenital skeletal anomalies. Our findings provide evidence that QUE may partially protect fetal development and congenital skeletal anomalies against COV-induced oxidative stress.

5 | CONCLUSIONS

In summary, this study suggests that exposure to COV increased congenital skeletal anomalies and subsequently impaired fetal development in rat fetuses. We demonstrated the toxicological effect that exposure to COV negatively interferes the expression of osteogenesis-related genes by suppressing differentiation and maturation of osteoblasts and cartilage matrix, ultimately causing congenital skeletal anomalies in rat fetuses. Pregnant rats exposed to COV also had evidence of maternal toxicity, and fetal changes occurred in COV-exposed groups in the presence of maternal toxicity. Moreover, the results of this study indicate that the administration of QUE improved the teratogenic effects of prenatal exposure to COV by increasing the expression of osteogenesis-related genes and preventing COV-induced oxidative stress.

AUTHOR CONTRIBUTIONS

Kaveh Khazaeel, Reza Ranjbar, Mohammad Reza Tabandeh, and Jala Amir Salman Alahmed conceptualized and designed the study and contributed to drafting the manuscript. Haifa Ali Hussein conducted the analysis and drafted the initial manuscript. All authors contributed to data interpretation, reviewed the manuscript, and approved the final version for submission.

ACKNOWLEDGMENTS

The authors would like to thank the research council of Shahid Chamran University of Ahvaz for financial funding of this study.

FUNDING INFORMATION

This study was supported by a grant (no. SCU, VB1400.293) from the Shahid Chamran University of Ahvaz, Iran.

CONFLICT OF INTEREST STATEMENT

The authors report no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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REFERENCES

Abd-Allah, E. R., Fouad, N. Y., Ghareeb, A. E. W. E., & Eldebss, T. M. (2023). Chloroacetonitrile reduces rat prenatal bone length and induces oxidative stress, apoptosis, and DNA damage in rat fetal liver. *Birth Defects Research*, *115*(6), 614–632. https://doi.org/10.1002/bdr2.2155

Abdel-Shafy, H. I., & Mansour, M. S. (2016). A review on polycyclic aromatic hydrocarbons: Source, environmental impact, effect on human health and remediation. *Egyptian Journal of Petroleum*, *25*(1), 107–123. https://doi.org/10.1016/j.ejpe.2015.03.011

Adipah, S. (2019). Introduction of petroleum hydrocarbons contaminants and its human effects. *Journal of Environmental Science and Public Health*, *3*(1), 1–9. https://doi.org/10.26502/jesph.96120043

Archibong, A. E., Inyang, F., Ramesh, A., Greenwood, M., Nayyar, T., Kopsombut, P., Hood, D. B., & Nyanda, A. M. (2002). Alteration of pregnancy related hormones and fetal survival in F-344 rats exposed by inhalation to benzo (a) pyrene. *Reproductive Toxicology*, *16*(6), 801–808. https://doi.org/10.1016/S0890-6238(02)00058-8

Blumer, M. J. (2021). Bone tissue and histological and molecular events during development of the long bones. *Annals of Anatomy-Anatomischer Anzeiger*, *235*, 151704. https://doi.org/10.1016/j.aanat.2021.151704

Costa, P. C. T. D., de Souza, E. L., Lacerda, D. C., Cruz Neto, J. P. R., Sales, L. C. S. D., Silva Luis, C. C., & de Brito Alves, J. L. (2022). Evidence for quercetin as a dietary supplement for the treatment of cardio-metabolic diseases in pregnancy: A review in rodent models. *Food*, *11*(18), 2772. https://doi.org/10.3390/foods11182772

Dehghani, S., Fararouei, M., Rafiee, A., Hoepner, L., Oskoei, V., & Hoseini, M. (2022). Prenatal exposure to polycyclic aromatic hydrocarbons and effects on neonatal anthropometric indices and thyroid-stimulating hormone in a middle eastern population. *Chemosphere*, *286*, 131605. https://doi.org/10.1016/j.chemosphere.2021.131605

Dietrich, C. (2016). Antioxidant functions of the aryl hydrocarbon receptor. *Stem Cells International*, *2016*, 7943495. https://doi.org/10.1155/2016/7943495

Dolati, P., Zamiri, M. J., Akhlaghi, A., Khodabandeh, Z., Mehrabani, D., Atashi, H., & Jamhiri, I. (2021). Reproductive and embryological toxicity of lead acetate in male mice and their offspring and mitigation effects of quercetin. *Journal of Trace Elements in Medicine and Biology*, *67*, 126793. https://doi.org/10.1016/j.jtemb.2021.126793

Elfawy, H. A., Anupriya, S., Mohanty, S., Patel, P., Ghosal, S., Panda, P. K., Das, B., Verma, S. K., & Patnaik, S. (2021). Molecular toxicity of benzo (a) pyrene mediated by elicited oxidative stress infer skeletal deformities and apoptosis in embryonic zebrafish. *Science of the Total Environment*, *789*, 147989. https://doi.org/10.1016/j.scitotenv.2021.147989

Fakhry, M., Hamade, E., Badran, B., Buchet, R., & Magne, D. (2013). Molecular mechanisms of mesenchymal stem cell differentiation towards osteoblasts. *World Journal of Stem Cells*, *5*(4), 136–148. https://doi.org/10.4252/wjsc.v5.i4.136

Gao, Y., Xiong, D., Qi, Z., Li, X., Ju, Z., & Zhuang, X. (2019). Distribution of polycyclic aromatic hydrocarbons in sunken oils in the presence of chemical dispersant and sediment. *Journal of Marine Science and Engineering*, *7*(9), 282. https://doi.org/10.3390/jmse7090282

Ghimire, S., Miramini, S., Edwards, G., Rotne, R., Xu, J., Ebeling, P., & Zhang, L. (2021). The investigation of bone fracture healing under intramembranous and endochondral ossification. *Bone Reports*, *14*, 100740. https://doi.org/10.1016/j.bonr.2020.100740

Gómez-Roig, M. D., Pascal, R., Cahuana, M. J., García-Algar, O., Sebastiani, G., Andreu-Fernández, V., Martínez, L., Rodríguez, G., Iglesia, I., Ortiz-Arrabal, O., Mesa, M. D., Cabero, M. J., Guerra, L., Llurba, E., Domínguez, C., Zanini, M. J., Foraster, M., Larqué, E., Cabañas, F., … Vento, M. (2021). Environmental exposure during pregnancy: Influence on prenatal development and early life: A comprehensive review. *Fetal Diagnosis and Therapy*, *48*(4), 245–257. https://doi.org/10.1159/000514884

Hudak, A., & Ungváry, G. (1978). Embryotoxic effects of benzene and its methyl derivatives: Toluene, xylene. *Toxicology*, *11*, 55–63. https://doi.org/10.1016/S0300-483X(78)90439-0

Ibrahim, K. A., Eleyan, M., Abd El-Rahman, H. A., Khwanes, S. A., & Mohamed, R. A. (2020). Quercetin attenuates the oxidative injury–mediated upregulation of apoptotic gene expression and catecholaminergic neurotransmitters of the fetal rats' brain following prenatal exposure to fenitrothion insecticide. *Neurotoxicity Research*, *37*, 871–882. https://doi.org/10.1007/s12640-020-00172-6

Khazaeel, K., Daaj, S. A. Z., Sadeghi, A., Tabandeh, M. R., & Basir, Z. (2022). Potential protective effect of quercetin on the male reproductive system against exposure of Wistar rats to crude oil vapor: Genetic, biochemical, and histopathological evidence. *Reproductive Toxicology*, *113*, 10–17. https://doi.org/10.1016/j.reprotox.2022.08.001

Khazaeel, K., Khaksary-Mahabady, M., Jamshidian, J., & Zolfaghari, N. (2021). Comparative effect of bromelain and vitamin E on bisphenol A-induced skeletal anomalies in the rat fetus. *Journal of Advanced Biomedical Sciences*, *11*(2), 3877–3885. https://doi.org/10.18502/jabs.v11i2.8780

Kim, W. I., Pak, S. W., Lee, S. J., Moon, C., Shin, I. S., Lee, I. C., & Kim, J. C. (2023). Effects of melamine and cyanuric acid on placental and fetal development in rats. *Food and Chemical Toxicology*, *177*, 113862. https://doi.org/10.1016/j.fct.2023.113862

Kuang, H., Liu, J., Zeng, Y., Zhou, W., Wu, P., Tan, J., Li, Y., Pang, Q., Jiang, W., & Fan, R. (2020). Co-exposure to polycyclic aromatic hydrocarbons, benzene and toluene may impair lung function by increasing oxidative damage and airway inflammation in asthmatic children. *Environmental Pollution*, *266*, 115220. https://doi.org/10.1016/j.envpol.2020.115220

Kuppusamy, S., Maddela, N. R., Megharaj, M., & Venkateswarlu, K. (2020). Impact of total petroleum hydrocarbons on human health. In *Total petroleum hydrocarbons: Environmental fate, toxicity, and remediation* (pp. 139–165). Springer. https://doi.org/10.1007/978-3-030-24035-6_6

Laforgia, N., Di Mauro, A., Favia Guarnieri, G., Varvara, D., De Cosmo, L., Panza, R., Capozza, M., Baldassarre, M. E., & Resta, N. (2018). The role of oxidative stress in the pathomechanism of congenital malformations. *Oxidative Medicine and Cellular Longevity*, *2018*, 1–12. https://doi.org/10.1155/2018/7404082

Lu, J., Wang, W., Xu, W., Zhang, C., Zhang, C., Tao, L., Li, Z., & Zhang, Y. (2022). Induction of developmental toxicity and cardiotoxicity in zebrafish embryos by emamectin benzoate through oxidative stress. *Science of the Total Environment*, *825*, 154040. https://doi.org/10.1016/j.scitotenv.2022.154040

McKenzie, L. M., Allshouse, W., & Daniels, S. (2019). Congenital heart defects and intensity of oil and gas well site activities in early pregnancy. *Environment International*, *132*, 104949. https://doi.org/10.1016/j.envint.2019.104949

Meador, J. P., & Nahrgang, J. (2019). Characterizing crude oil toxicity to early-life stage fish based on a complex mixture: Are we making unsupported assumptions? *Environmental Science & Technology*, *53*(19), 11080–11092. https://doi.org/10.1021/acs.est.9b02889

O'Driscoll, C. A., Gallo, M. E., Hoffmann, E. J., Fechner, J. H., Schauer, J. J., Bradfield, C. A., & Mezrich, J. D. (2018). Polycyclic aromatic hydrocarbons (PAHs) present in ambient urban dust drive proinflammatory T cell and dendritic cell responses via the aryl hydrocarbon receptor (AHR) in vitro. *PLoS One*, *13*(12), e0209690. https://doi.org/10.1371/journal.pone.0209690

Onojake, M. C., Eromosele, G. O., & Osuji, L. C. (2021). Profiling of polycyclic aromatic hydrocarbons and diagnostic ratios of kpite oil spill impacted site in Rivers state, Nigeria. *Pollution*, *7*(1), 17–24. https://doi.org/10.22059/POLL.2020.303392.827

Parhi, B., Bharatiya, D., & Swain, S. K. (2020). Application of quercetin flavonoid based hybrid nanocomposites: A review. *Saudi Pharmaceutical Journal*, *28*(12), 1719–1732. https://doi.org/10.1016/j.jsps.2020.10.017

Pérez-Pastén, R., Martínez-Galero, E., & Chamorro-Cevallos, G. (2010). Quercetin and naringenin reduce abnormal development of mouse embryos produced by hydroxyurea. *Journal of Pharmacy and Pharmacology*, *62*(8), 1003–1009. https://doi.org/10.1111/j.2042-7158.2010.01118.x

Pinto, C. G., Ávila-Gálvez, M. Á., Lian, Y., Moura-Alves, P., & Dos Santos, C. N. (2023). Targeting the aryl hydrocarbon receptor by gut phenolic metabolites: A strategy towards gut inflammation. *Redox Biology*, *61*, 102622. https://doi.org/10.1016/j.redox.2023.102622

Rahman, M. S., Akhtar, N., Jamil, H. M., Banik, R. S., & Asaduzzaman, S. M. (2015). TGF-β/BMP signaling and other molecular events: Regulation of osteoblastogenesis and bone formation. *Bone Research*, *3*(1), 1–20. https://doi.org/10.1038/boneres.2015.5

Rengarajan, T., Rajendran, P., Nandakumar, N., Lokeshkumar, B., Rajendran, P., & Nishigaki, I. (2015). Exposure to polycyclic aromatic hydrocarbons with special focus on cancer. *Asian Pacific Journal of Tropical Biomedicine*, *5*(3), 182–189. https://doi.org/10.1016/S2221-1691(15)30003-4

Saakre, M., Mathew, D., & Ravisankar, V. (2021). Perspectives on plant flavonoid quercetin-based drugs for novel SARS-CoV-2. *Beni-Suef University Journal of Basic and Applied Sciences*, *10*(1), 1–13. https://doi.org/10.1186/s43088-021-00107-w

Sadeghi, A., Ghahari, L., Yousefpour, M., Khazaeel, K., & Zareian, P. (2023). Inhalation exposure to crude oil vapor induces behavioural deficits by increasing oxidative stress and histopathological changes in rat hippocampus: Quercetin

therapeutic approach. *Journal of Chemical Neuroanatomy*, *131*, 102290. https://doi.org/10.1016/j.jchemneu.2023.102290

Seemann, F., Peterson, D. R., Witten, P. E., Guo, B. S., Shanthanagouda, A. H., Rui, R. Y., & Au, D. W. (2015). Insight into the transgenerational effect of benzo [a] pyrene on bone formation in a teleost fish (Oryzias latipes). *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, *178*, 60–67. https://doi.org/10.1016/j.cbpc.2015.10.001

Shivanna, B., Chu, C., & Moorthy, B. (2022). The aryl hydrocarbon receptor (AHR): A novel therapeutic target for pulmonary diseases? *International Journal of Molecular Sciences*, *23*(3), 1516. https://doi.org/10.3390/ijms23031516

Sirotkin, A. V., & Harrath, A. H. (2017). Influence of oil-related environmental pollutants on female reproduction. *Reproductive Toxicology*, *71*, 142–145. https://doi.org/10.1016/j.reprotox.2017.05.007

Sun, X., Zhang, S., & Song, H. (2020). Quercetin attenuates reduced uterine perfusion pressure-induced hypertension in pregnant rats through regulation of endothelin-1 and endothelin-1 type A receptor. *Lipids in Health and Disease*, *19*(1), 1–9. https://doi.org/10.1186/s12944-020-01357-w

Uboh, F. E., Ebong, P. E., Akpan, H. D., & Usoh, I. F. (2012). Hepatoprotective effect of vitamins C and E against gasoline vapor-induced liver injury in male rats. *Turkish Journal of Biology*, *36*(2), 217–223. https://doi.org/10.3906/biy-1004-111

Wang, N., Wang, L., Yang, J., Wang, Z., & Cheng, L. (2021). Quercetin promotes osteogenic differentiation and antioxidant responses of mouse bone mesenchymal stem cells through activation of the AMPK/SIRT1 signaling pathway. *Phytotherapy Research*, *35*(5), 2639–2650. https://doi.org/10.1002/ptr.7010

Webb, E., Moon, J., Dyrszka, L., Rodriguez, B., Cox, C., Patisaul, H., Bushkin, S., & London, E. (2018). Neurodevelopmental and neurological effects of chemicals associated with unconventional oil and natural gas operations and their potential effects on infants and children. *Reviews on Environmental Health*, *33*(1), 3–29. https://doi.org/10.1515/reveh-2017-0008

Wigle, D. T., Arbuckle, T. E., Turner, M. C., Bérubé, A., Yang, Q., Liu, S., & Krewski, D. (2008). Epidemiologic evidence of relationships between reproductive and child health outcomes and environmental chemical contaminants. *Journal of Toxicology and Environmental Health, Part B*, *11*(5–6), 373–517. https://doi.org/10.1080/10937400801921320

Xu, D., Hu, M. J., Wang, Y. Q., & Cui, Y. L. (2019). Antioxidant activities of quercetin and its complexes for medicinal application. *Molecules*, *24*(6), 1123. https://doi.org/10.3390/molecules24061123

Xu, X., Tang, Y., Lang, Y., Liu, Y., Cheng, W., Xu, H., & Liu, Y. (2020). Oral exposure to ZnO nanoparticles disrupt the structure of bone in young rats via the OPG/RANK/RANKL/IGF-1 pathway. *International Journal of Nanomedicine*, *15*, 9657–9668. https://doi.org/10.2147/IJN.S275553

Yi, C., Wang, Q., Qu, Y., Niu, J., Oliver, B. G., & Chen, H. (2022). In-utero exposure to air pollution and early-life neural development and cognition. *Ecotoxicology and Environmental Safety*, *238*, 113589. https://doi.org/10.1016/j.ecoenv.2022.113589

Yun, Y., Liang, L., Wei, Y., Luo, Z., Yuan, F., Li, G., & Sang, N. (2019). Exposure to nitro-PAHs interfere with germination and early growth of Hordeum vulgare via oxidative stress. *Ecotoxicology and Environmental Safety*, *180*, 756–761. https://doi.org/10.1016/j.ecoenv.2019.05.032

Zhang, L., Ji, X., Ding, F., Wu, X., Tang, N., & Wu, Q. (2022). Apoptosis and blood-testis barrier disruption during male reproductive dysfunction induced by PAHs of different molecular weights. *Environmental Pollution*, *300*, 118959. https://doi.org/10.1016/j.envpol.2022.118959

Zhao, L., Wang, H., & Du, X. (2021). The therapeutic use of quercetin in ophthalmology: Recent applications. *Biomedicine & Pharmacotherapy*, *137*, 111371. https://doi.org/10.1016/j.biopha.2021.111371

SUPPORTING INFORMATION

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How to cite this article: Hussein, H. A., Khazaeel, K., Ranjbar, R., Tabandeh, M. R., & Alahmed, J. A. S. (2023). Protective effect of quercetin on fetal development and congenital skeletal anomalies against exposure of pregnant Wistar rats to crude oil vapor. *Birth Defects Research*, 1–11. https://doi.org/10.1002/bdr2.2240