Assessment of the developmental toxicity of acrylamide in albino rats and the possible protective effect of vitamin E: A morphological animal study

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SUMMARY

Acrylamide (ACR) is a known neurotoxin and carcinogen linked to frying and baking meals high in carbohydrates and is frequently present in soils and groundwater. It is used to create polymers for industry. Pregnant women are exposed to ACR through food, and their fetuses likely to be harmed. Vitamin E is significant lipid-soluble vitamin found in nature and essential for numerous physiological activities. Aim: to examine effects of exposure to acrylamide on skeletal development of rat embryos and the potential role of Vitamin E (Vit. E) in the reduction of these effects. Forty pregnant albino rats were divided into four groups. Control group was administrated distilled water orally. Low ACR group was administrated acrylamide (10 mg/kg/day) via gavage from GD 1 to GD20. High ACR group was administrated acrylamide (30 mg/kg/day) from GD 1 to GD 20. ACR vit. E group was administrated 100 mg/kg of vitamin E and 30 mg/kg of ACR orally. Alizarin red and Alcian blue stains were used to double-stain the skeletons, then examined by dissecting stereomicroscope.

Skeletal anomalies found were incomplete and un-ossified bones of the skull, sacral vertebrae, ribs, bones of forelimb and hind limb, unossified carpals, metacarpals, tarsals, metatarsals, and phalanges. These were obvious in ACR-treated groups compared to the control group and improved in ACR vitamin E group. Acrylamide caused skeletal congenital abnormalities, thus proving its teratogenicity, and vitamin E seems to protect against and reduce such abnormalities.

Key words: Acrylamide – Vitamin E – Developmental toxicity – Rats – Oxidative stress

INTRODUCTION

Acrylamide (ACR) is a highly water-soluble chemical compound that is utilized in a variety of commercial and research operations, including gel chromatography, sewage treatment, sewage supply for cities, and oil refining and mining (LoPachin et al.,2003; Radoiu et al., 2004).

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Recently, it has been found that during the thermal processing of carbohydrate-rich meals as bread and French fries acrylamide is produced via the Maillard reaction between asparagines and glucose (Huang et al., 2016; Hogervorst et al, 2016). And also tobacco smoke contains a significant amount of acrylamide (Lin et al., 2013).

Concern about ACR risks on human health has grown significantly among the public, and numerous investigations have shown that ACR is genotoxic, neurotoxic, and toxic to the developing fetus (Erkekoglu and Baydar, 2014; Pennisi et al., 2013). ACR has a deleterious effect on DNA, causing chromosomal defects such as Sister Chromatid exchange, aneuploidy, and micronucleus production (Lakshminarasaiah et al., 2011).

According to research on the carcinogenicity of ACR added to drinking water, rats have increased cancer rates like mammary glands, thyroid, uterus, and central nervous system cancers (Yalçın and Ayşegül, 2019).

Exposure to monomeric acrylamide can cause peripheral neuropathy and limb weakening (Kermanialghoraishi et al., 2010).

Exposure to acrylamide causes reproductive toxicity, which is shown by abnormalities in sperm morphology, viability and decreased fertility (Yalçın and Ayşegül, 2019).

ACR can affect embryos and newborns if their mothers have ACR exposure by any way, as it is a substance that is highly soluble in water and might enter infants' bodies through breast milk when they are breastfed (Mojska, 2022).

Infants have greater levels of ACR exposure than adults do. This is because newborns and fetuses have smaller bodies than adults. Fetuses and newborns may suffer lifelong, irreversible ACR-induced impairments (Hilbig et al., 2004).

Acrylamide exposure during pregnancy decreased the number of live fetuses, decreased the weights of embryos and placenta, as well as disrupted the expression patterns of vital genes for embryo development such as esx1, hand1, and hand2 (Yu et al., 2019).

Furthermore, acrylamide consumption during pregnancy was linked to unfavorable fetal growth shown by significant developmental markers, according to two significant European mother and baby cohort studies (Duarte-Salles et al., 2013; Pedersen et al., 2012).

In addition to cell damage or death, oxidative stress and damage to important molecules like DNA and proteins constitute the most commonly accepted mechanism of the negative consequences of ACR (Mannaa et al., 2006; Mogda et al., 2008).

Free radicals are made harmless by antioxidant systems when physiologic circumstances are normal because of the balance that exists between them. Oxidative stress leads to oxidative tissue damage when this equilibrium is tipped in favor of oxidants. Vitamin E guards the cells from oxidative stress by preventing the formation of oxidative stress by free radicals in the cell's fat phase by converting free radicals into less reactive molecules (Reiter et al., 2007; Naito et al., 2005).

Vitamin E can reach the embryos and the brain as the placenta may be readily crossed by vitamin E and the blood-brain barrier is broken down by vitamin E, which enables vitamin E to have a strong fetus-protective and neuro-protective effects (Hidiroglou et al., 2001; Sung et al., 2004).

Furthermore, skeletal development is very important for embryological functions and future growth. The toxicological impact of ACR on gestation durations for skeletal development has, however, only been covered in a few research works. To fill this knowledge gap, we seek to understand the impacts of ACR toxicity on skeletal development and evaluate the preventive benefits of vitamin E against the potential harm that ACR might do to the developing fetal skeleton.

The study aimed to assess the harmful effects of ACR on rat fetuses and to measure the role of vitamin E to protect against theses harms.

MATERIALS AND METHODS

Chemicals

ACR (C3H5NO; 99.9% purity) obtained from sigma chemical company. Vitamin E was supplied by Pharco pharmaceuticals Company, Egypt. Gelatin capsules 400mg.

Stains

Alcian blue stain (Biodiagnostic Company, Egypt).

Alizarin Red stain was prepared: 0.005 mg Alizarin red stain (Sigma chemical company), 100 ml of 1% potassium hydroxide (KOH) solution.

Equipment

Olympus SZ dissecting stereo microscope. Olympus corporation- Japan. (Embryology lab, Anatomy department, Faculty of Medicine).

Experimental animals

Pure strain Albino rats, 180-220 g average weight and approximately 6-8 weeks of age were obtained from the Animal house, Faculty of Medicine, Alexandria University. Rats were left to adapt for two weeks.

Rats were maintained under standard laboratory conditions of temperature, humidity and 12 hours light/dark cycle and had free access to food and water. Diet was obtained from Tanta Oil and Soap Company, Egypt. Diet components: Bran, cotton seed meal, yellow corn, molasses, limestone powder, table salt.

Female rats were inserted in cages containing a male rat (one male/five females). Mating was confirmed by appearance of a vaginal plug, which was considered day 1 of gestation.

The research protocol approved by Ethics Committee of Faculty of Medicine, Alexandria University [IRB No: 00012098 (expires 6-10-2025) - FWA No: 00018699 (expires 21-1-2026)]. Serial number 0305642 and followed the guidelines for use of animals with adherence to ARRIVE guidelines.

Experiment design

40 pregnant female rats were divided blindly into four groups of ten rats each:

Group 1: (Control group) orally administered distilled water.

Group 2: (ACR low dose) given oral acrylamide dissolved in distilled water 10 mg/kg/day using gavage from GD 1 to GD 20.

Group 3: (ACR high dose) given oral acrylamide dissolved in distilled water 30 mg/kg/day using gavage from GD 1 to GD 20. (Duan et al., 2015).

Group 4: (ACR vit. E group) given oral acrylamide dissolved in distilled 30 mg/kg/day and 100 mg/kg/day of vitamin E using gavage from GD 1 to GD 20.

Pregnant rats were followed daily from day 1 gestation for any signs of toxicity. Pregnant females were sacrificed on gestational day 20 by cervical dislocation.

After caesarean section, the uterine horns were exposed, dissected and examined for the number of implantation sites and fetuses (live or dead). Body weight and length measurements were taken on each embryo.

For skeletal examination, double staining technique was done using Alcian blue and Alizarin red to stain cartilages and bones. Embryos were processed through the following steps (Aliesfehani, 2015; Sadeghi, 2014):

Preservation and fixation: skinning and eviscerating were done by clearing the thoracic and abdominal cavities. The samples were immersed in absolute ethanol for seven days.

Cartilage staining: fetuses were put in a solution of 0.01% Alcian blue until uptake of the dye for 3 days.

Rehydration: fetuses were immersed in a bath of 95% ethyl alcohol for two hours. Then they were immersed in baths with successively decreasing concentrations of ethyl alcohol 75%, 40%, and 15%, two hours for each concentration.

Clearing: the samples were immersed in 1% KOH until complete clearance of the skeleton.

Bone staining: The samples were put in 0.001% Alizarin red three days to stain bones.

Washing: to remove excess stains, the specimens were washed by 1% KOH three times, several hours each time.

Clearing and dehydration: the samples immersed in ascending concentrations of glycerol in 1% KOH, “1:3, 1:1, and 3:1”, 24 hours for each concentration. Each skeleton was examined by stereomicroscope and photographed to document the findings.

Statistical analysis of the data

Data were introduced to the computer and analyzed using IBM SPSS software package version 20.0. (Armonk, NY: IBM Corp). Categorical data were represented as numbers and percentages. Chi-square test was applied to compare between two groups. Alternatively, Monte Carlo correction test was applied when more than 20% of the cells had expected count less than 5.

Continuous data were tested for normality by the Shapiro-Wilk test. Quantitative data were expressed as range (minimum and maximum), mean and standard deviation for normally distributed quantitative variables. One way ANOVA test was used for comparing the four studied groups and followed by Post Hoc test (Tukey) for pairwise comparison. Significance of the obtained results was judged at the 5% level.

RESULTS

Mortality rate: maternal mortality was 50% in the ACR high dose group. No mortalities were found in ACR low dose group, ACR vit. E group or the control group (Fig. 1).

The parameters of fetal growth

Parameters of weight and infant length in the different groups were measured and recorded. Results were expressed as mean ± standard deviation. Growth retardation was indicated by the decrease of both fetal weight and length. The average fetal weight and length in ACR high dose and ACR low dose groups were significantly less than those of the control group. The mean weight and length in groups that received ACR plus Vit. E were significantly higher than the group that received only ACR. (Table 1) (Fig. 2).

Morphological examination of fetuses

Eye abnormalities

Anophthalmia (absence of the eye bulge): eighty rat fetuses were obtained from ten rats of control group. No anophthalmia found in control animals or ACR low dose group. ACR high dose group anophthalmia was reported in 6 out of 38 (15.8%), and in ACR vit. E group; it was in 4 out of 75 (5.3%) (Figs. 3a, b and Fig. 6). There was a significant statistical difference between the control and ACR high dose groups and between ACR high dose and ACR vit. E groups. (Table 2.).

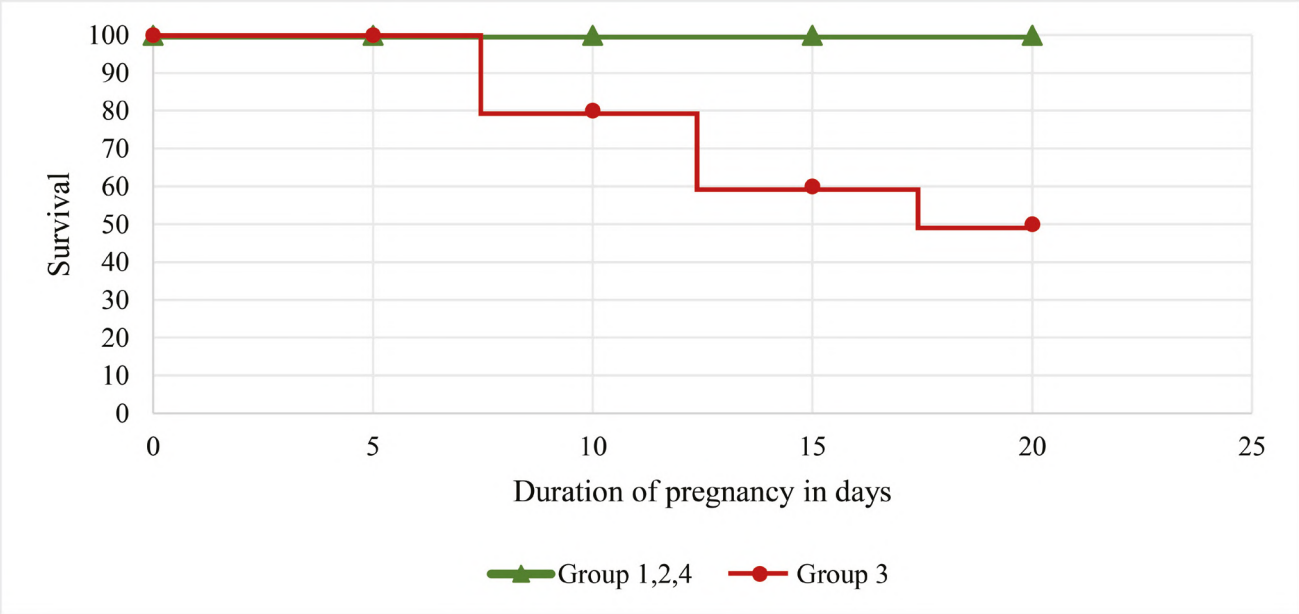

Fig. 1.- Kaplan-Meier curve representing the mortality result. It shows 100% survival in control, low dose acrylamide and vitamin E groups (groups 1, 2 and 4); the survival of ACR high dose group declined after the seventh day of pregnancy till it reached 50 % near end of pregnancy duration indicating death of half of the pregnant rats receiving acrylamide high dose. The groups 1, 2 and 4 are having the same survival so they represented by one line; the green line.

Table 1. Comparison between the different studied groups according to fetal weight (g) and length (mm).

	Group 1	Group 2	Group 3	Group 4	F	p
Fetal weight (g)						
Min. – Max.	4.50 – 6.0	3.0 – 4.0	1.90 – 3.0	4.0 – 6.0	47.808*	<0.001*
Mean ± SD.	5.40 ± 0.58	3.57# ± 0.45	2.39#@ ± 0.38	4.97@$ ± 0.65		
Fetal length (mm)						
Min. – Max.	35.0 – 39.0	24.0 – 29.0	20.0 – 25.0	30.0 – 35.0	99.881*	<0.001*
Mean ± SD.	37.29 ± 1.50	26.0# ± 2.0	22.29#@ ± 1.80	33.0#@$ ± 1.83		

7 Rat fetuses in each group SD: **Standard deviation**
F: **F for One way ANOVA test**, Pairwise comparison bet. each 2 groups was done using **Post Hoc Test (Tukey)**
p: p value for comparing between the different studied groups
*: Statistically significant at $p \leq 0.05$

#: Significant with **Group 1**
@: Significant with **Group 2**
$: Significant with **Group 3**

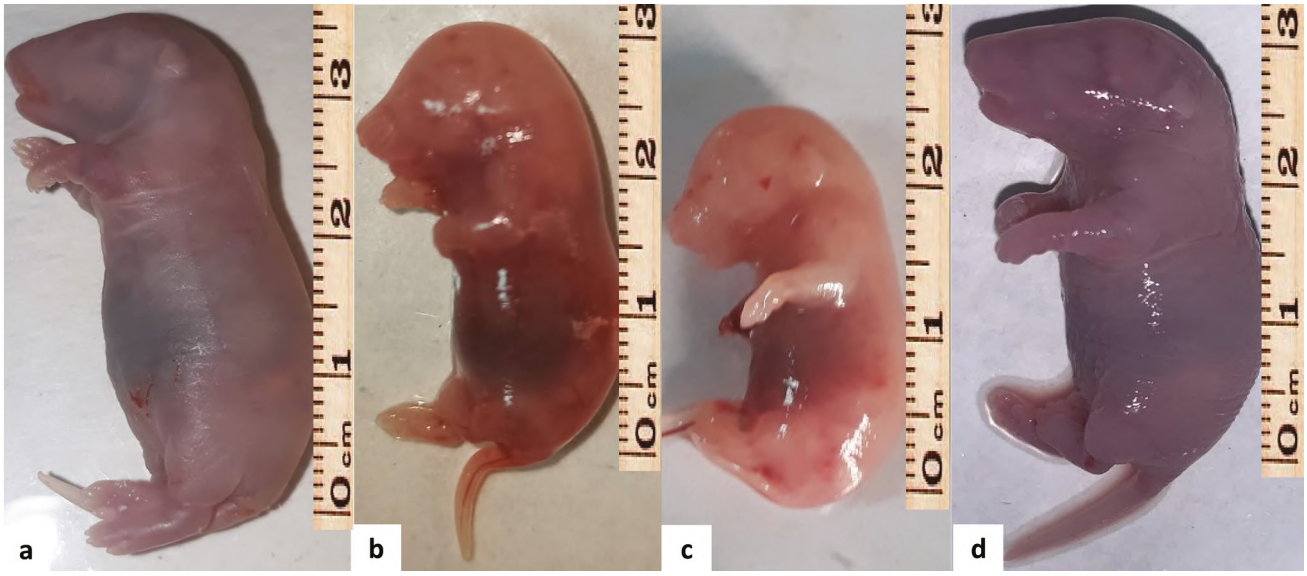

Fig. 2.- 20 GD rats' embryos from different groups lateral view showing: **a**) Normal length in the control group. **b, c**) Decrease the embryos' length in the two ACR treated groups low and high doses respectively. **d**) the embryo length in ACR vit. E group was greater than the two ACR treated groups. A ruler scale bar is inserted to indicate the length of each embryo.

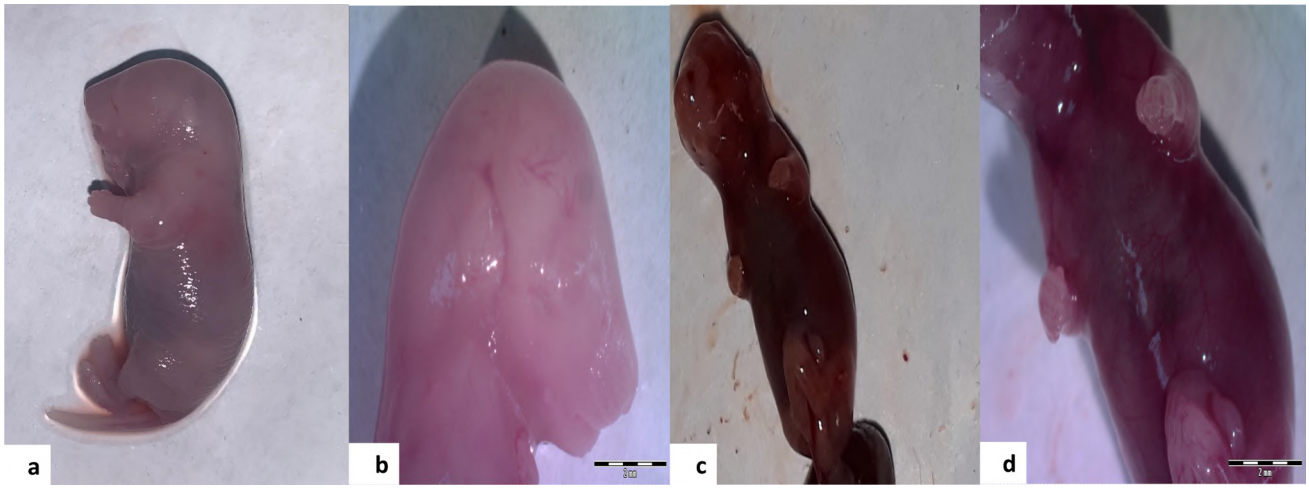

Fig. 3.- 20 GD rats' embryos from ACR high dose group showing: **a, b**) anophthalmia. **c, d**) subcutaneous hemorrhage in head, neck regions and intra-abdominal hemorrhage.

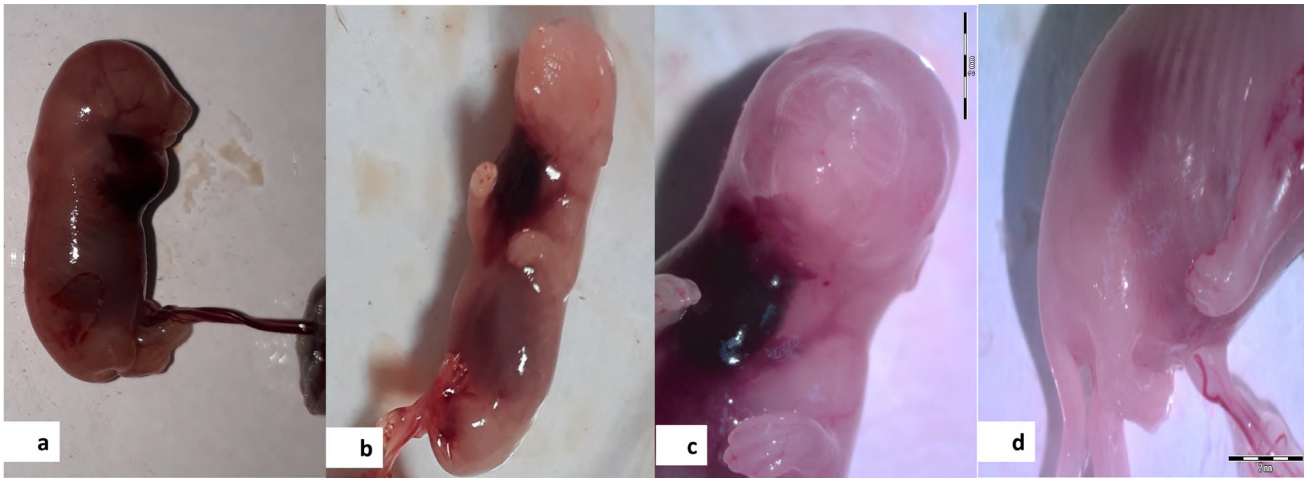

Fig. 4.- 20 GD rats' embryos from different groups showing: **a**) large area of subcutaneous hemorrhage in neck, forelimbs and anophthalmia. **b, c**) subcutaneous hemorrhage in neck region. **d**) subcutaneous hemorrhage over side of abdomen and right forelimb.

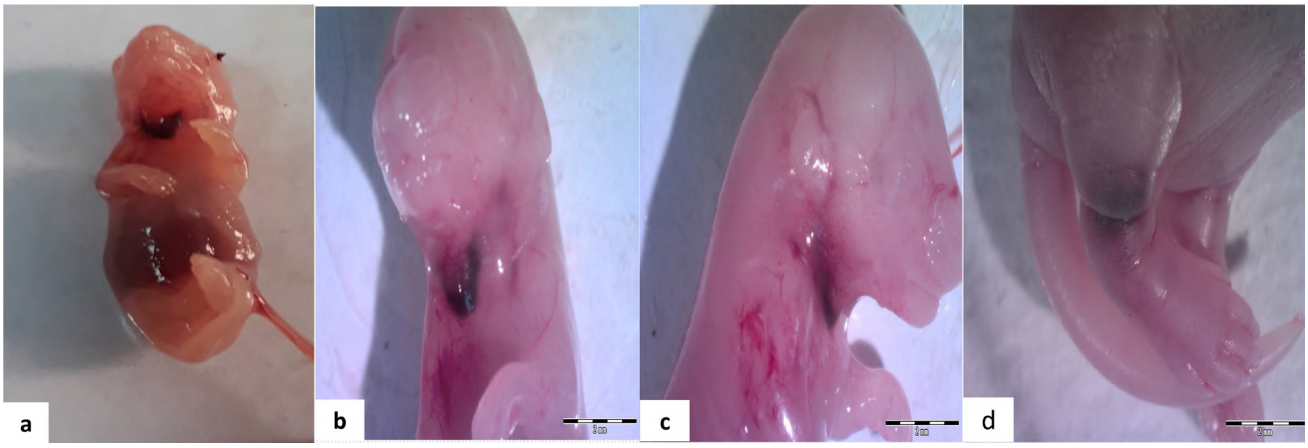

Fig. 5.- 20 GD rats' embryos from different groups showing: **a, b, c**) small areas of subcutaneous hemorrhage in neck region. **d**) subcutaneous hemorrhage over hind limb.

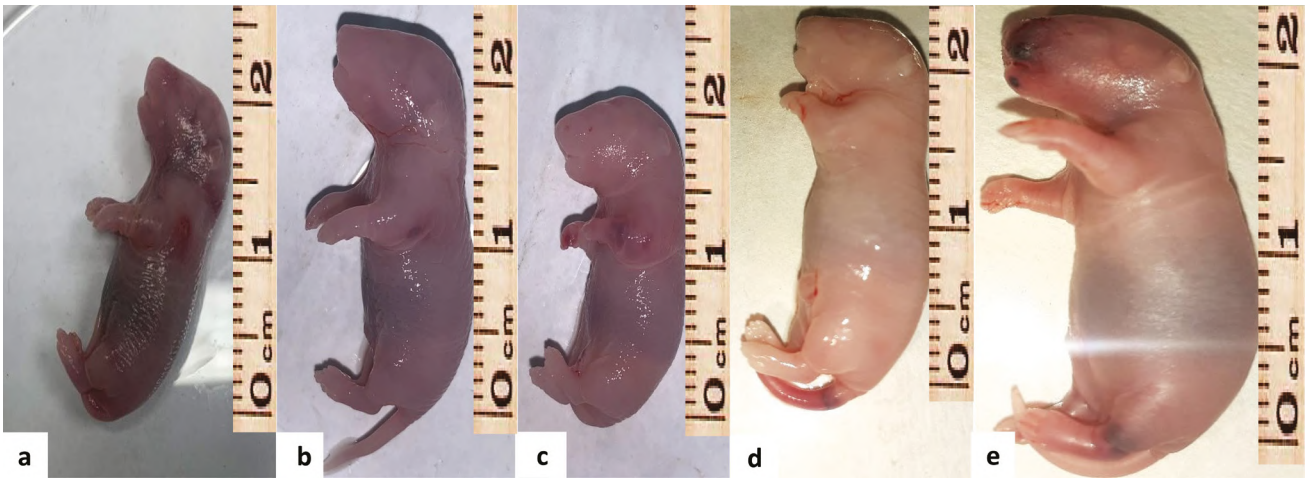

Fig. 6.- 20 GD rats' embryos from ACR high dose group showing: **a, b, c**) areas of subcutaneous hemorrhage in neck, left shoulder and forelimb regions and anophthalmia. **d, e**) subcutaneous hemorrhage in tail, right hind limb, face regions and anophthalmia in ACR high dose group. A ruler scale bar is inserted to indicate the length of each embryo.

Table 2. Comparison between the different studied groups according to incidence of morphologic anomalies.

Number of examined fetuses	Group 1 (n = 80)	Group 2 (n = 72)	Group 3 (n = 38)	Group 4 (n = 75)	χ^2	p
1.Anophthalmia	0 (0.0%)	0 (0.0%)	$6^{\#@}$ (15.8%)	4 (5.3%)	16.758^{*}	$^{MC}p < 0.001^{*}$
2.Intraabdominal hemorrhage	0 (0.0%)	0 (0.0%)	$10^{\#@}$ (26.3%)	$2^{\$}$ (2.7%)	49.535^{*}	$^{MC}p < 0.001^{*}$
3.Subcutaneous hemorrhage	0 (0.0%)	$15^{\#}$ (20.8%)	$20^{\#@}$ (52.6%)	$5^{\#@\$}$ (6.7%)	62.008	$<0.001^{*}$

$\chi 2$: **Chi square test** MC: **Monte Carlo**
p: p value for comparing between the different studied groups
*: Statistically significant at $p \leq 0.05$

#: Significant with **Group 1**
@: Significant with **Group 2**
$: Significant with **Group 3**

Hemorrhage

Intra-abdominal hemorrhage: In control and ACR low dose groups, no intra-abdominal hemorrhage was found. In ACR high dose group, it was in 10 out of 38 (26.3%) (Figs. 3c, 2d). In ACR Vit. E group; it was in 2 out of 75 (2.7 %). There were significant statistical differences between control and ACR high dose group as well as between ACR high dose and ACR vit. E groups (Table 2).

Subcutaneous hemorrhage: no subcutaneous hemorrhage was reported in the control animals, but ACR groups (low and high dose) showed anomalies in the form of subcutaneous hemorrhage in the neck (Figs. 4a, 4b, 4c, 5a, 5b, 5c), in the forelimb (Fig. 4a), over side of abdomen (Fig .4d), hind limb and tail (Figs. 5d, 6d, 6e), left shoulder (Figs. 6a, 6b, 6c) and face region (Fig. 6e). In the control groups, it was shown in 0 out of 80 (0 %).

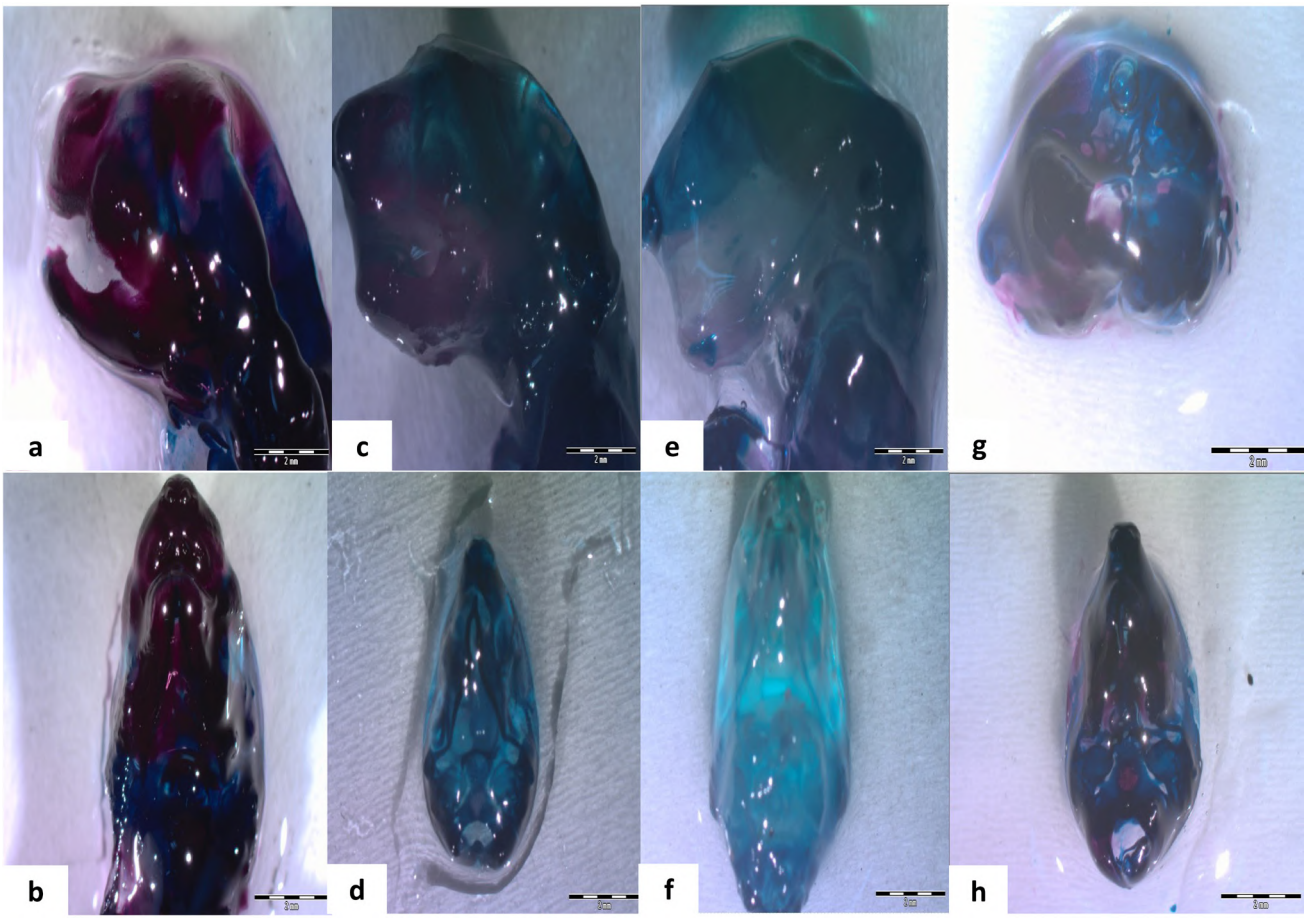

Fig. 7.- 20 GD rats' embryos skulls from different groups lateral and ventral views showing: **a, b**) normal ossification of skull bones in control group. **c, d**) decreased ossification of skull bones in ACR low dose group. **e, f**) very poor and almost non ossified skull bones in ACR high dose group. **g, h**) improved ossifications of skull bones in ACR vit. E group.

In ACR low dose it was in 15 out of 72 (20.8 %). In ACR high dose, it was in 20 out of 38 (52.6%). In ACR Vit. E group, it was present in 5 out of 75 (6.7%). There were significant statistical differences between the control and ACR groups and between ACR groups and ACR Vit. E group (Table 2).

Skeletal Abnormalities

Alizarin red stained skeletons of fetuses revealed many anomalies such as decreased ossification of the bones of the skull, poor ossifications of the vertebral column and the thoracic cage, reduction, non-ossification of the bones of the limbs and vertebrae (Figs. 7-12, Table 3).

Skull abnormalities

Reduced ossification of skull bones: in control group, normal ossification was found (Figs. 7a, 7b). In ACR low dose group reduced or decreased ossification was found in 12 out of 72 (16.7%) (Figs. 7c, 7d). In ACR high dose group, it was found in 16 out of 38 (42.1%) (Figs. 7e, 7f). In ACR Vit. E group, it was found in 3 out of 75 (4%) (Figs. 7g, 7h). There was a significant statistical difference between control and ACR groups and between ACR groups and ACR vit. E group (Table 3).

Non ossification of skull bones: normal ossification was found in control groups. In ACR high dose group, non-ossification was found in 8 out of 38 (21.1%) (Figs. 7e, 7f). In ACR vit. E group, it was in 0 out of 75 (0%). There were significant statistical differences between the control and ACR high dose group and between ACR and ACR vit. E group (Table 3).

Anomalies of the thoracic cage

In control group, normal thoracic cage was found with normal ossification (Figs. 8a, 8b). Bifid sternum was reported in 5 out of 38 (13.2%) in ACR high dose group (Fig. 8f). Incomplete ossification of ribs was reported in 7 out of 72 (9.7 %) in ACR low dose group and 15 out of 38 (39.5%) in ACR high dose group (Figs. 8c, 8d, 8e), while in ACR vit. E group, it was in 1 out of 75(1.3 %) (Figs. 8g, 8h). There were statistical significant differences between the control and ACR groups and between ACR groups and ACR vit. E group (Table 3).

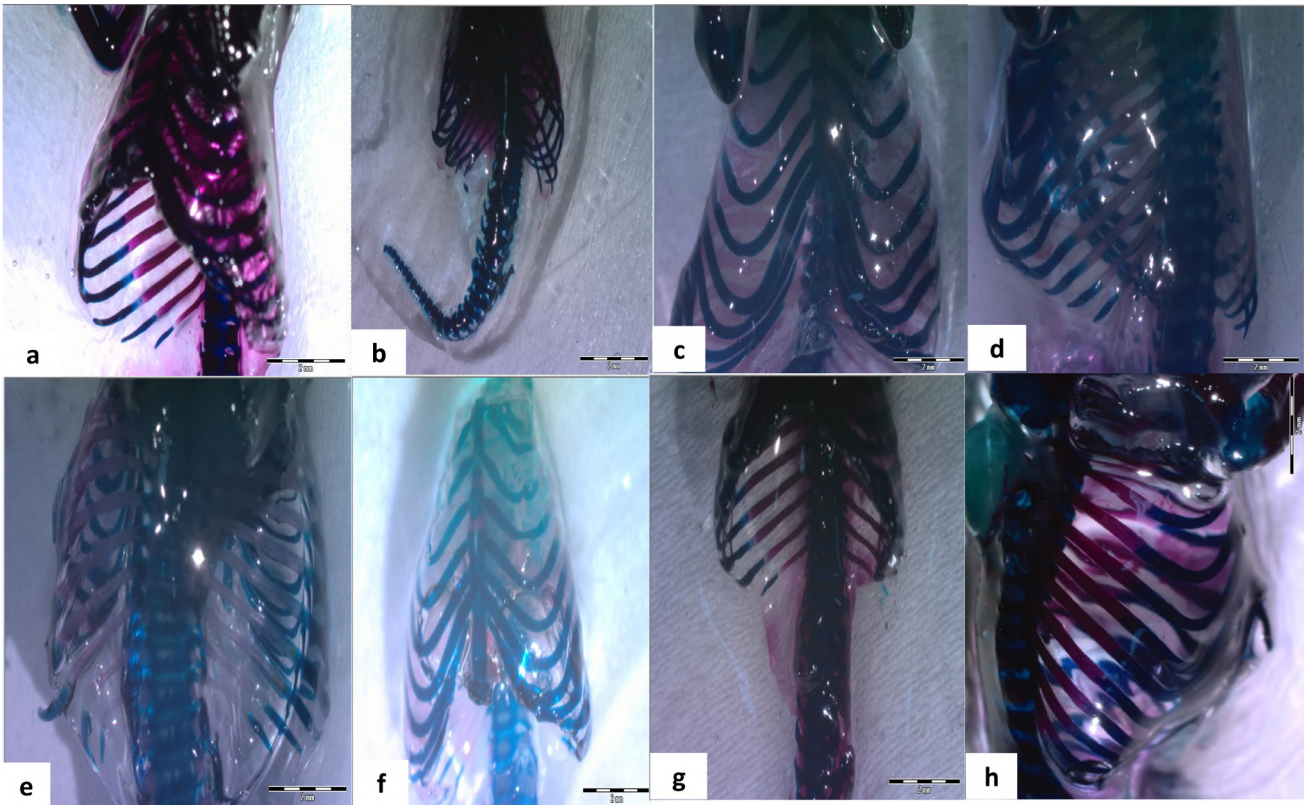

Fig. 8.- 20 GD rats' embryos from different groups showing: **a, b**) normal thoracic cage, normal vertebral centers of thoracic, lumbar and sacral vertebrae in control group. **c, d**) reduced ossifications of vertebral column and thoracic cage in ACR low dose group. **e, f**) poor ossification and bifid sternum in ACR high dose group. **g, h**) improved ossification of thoracic cage and vertebrae in ACR vit. E group.

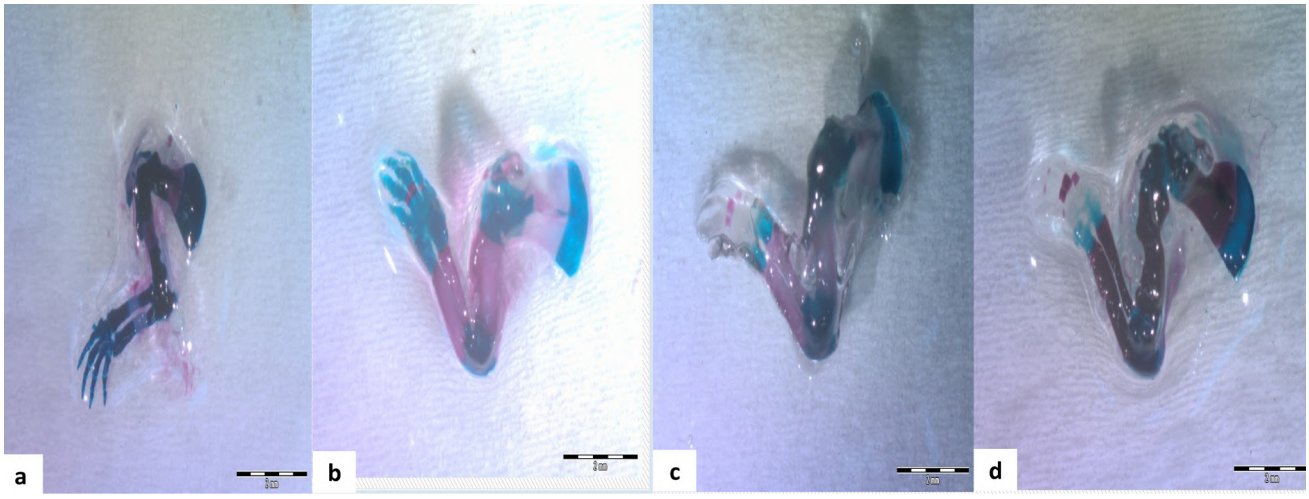

Fig. 9.- 20 GD rats' embryos lateral view of the pectoral girdle and forelimb from different groups showing: **a**) normal ossification in control group. **b**) reduced ossification in ACR low dose group. **c**) poor ossification with non- ossified areas in ACR high dose group. **d**) improved ossification in ACR vit. E group.

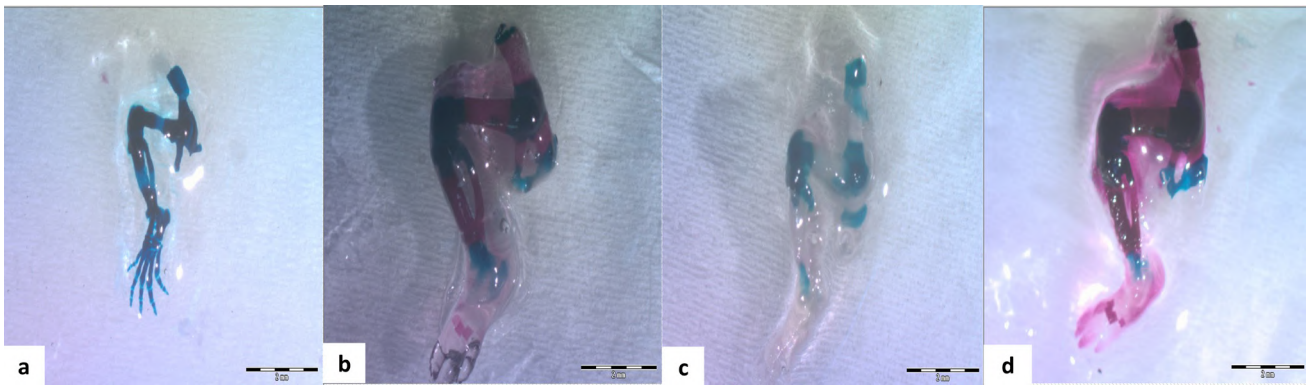

Fig. 10.- 20 GD rats' embryos lateral view of the pelvic girdle and hind limb from different groups showing: **a**) normal ossification in Control group. **b**) decreased ossification in ACR low dose group. **c**) very poor ossification with large non-ossified areas in ACR high dose group. **d**) improved ossification in ACR vit. E group.

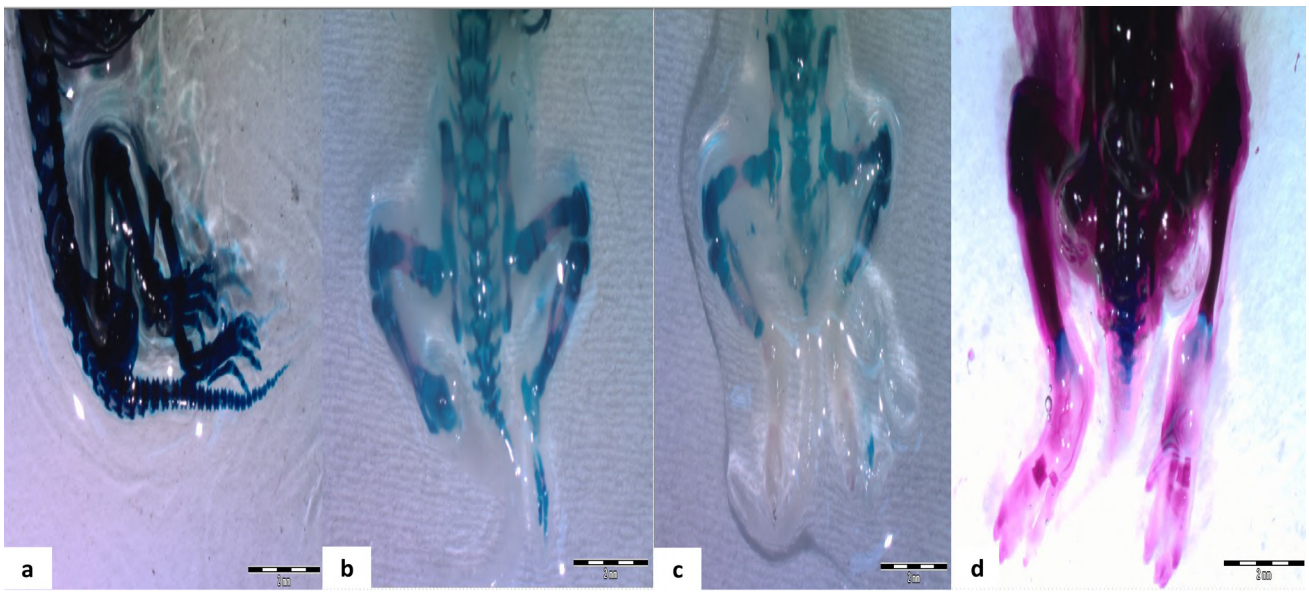

Fig. 11.- 20 GD rats' embryos lower part of the trunk, tail, pelvic girdle and hind limb from different groups showing: **a**) normal ossification in Control group. **b**) reduced ossification in ACR low dose group. **c**) very poor ossification in pelvic girdle lumber vertebrae and non-ossification of tail leg and feet in ACR high dose group. **d**) improved ossification in ACR vit. E group.

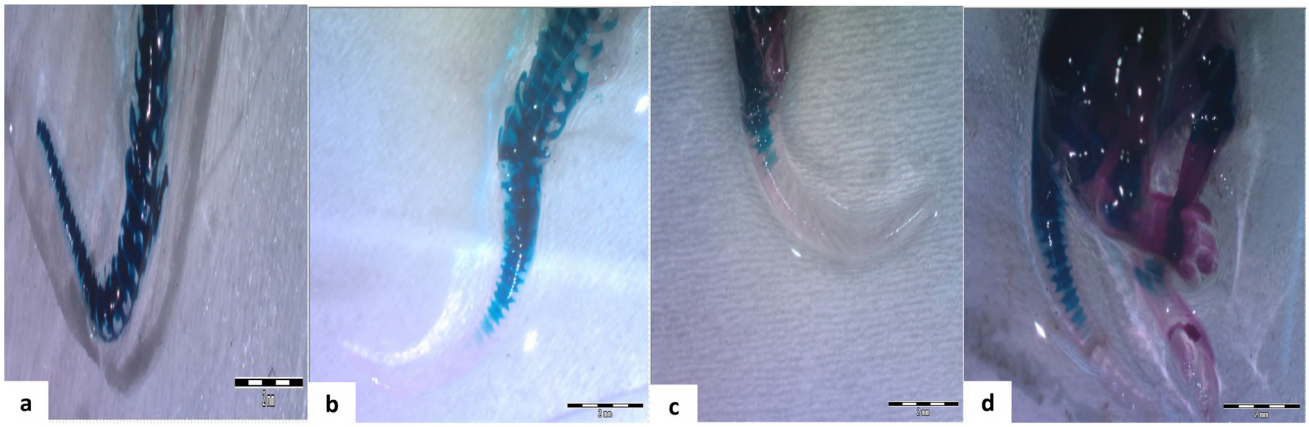

Fig. 12.- 20 GD rats' embryos lower part of vertebral column and hind limb from different groups showing: **a**) normal ossification in Control group. **b, c**) reduced ossification of lower lumber and sacral vertebrae and non- ossification of tail vertebra in ACR treated groups low and high doses respectively. **d**) improved ossification in ACR vit. E group.

Table 3. Comparison between the different studied groups according to incidence of skeletal anomalies.

Number of examined fetuses	Group 1 (n = 80)	Group 2 (n = 72)	Group 3 (n = 38)	Group 4 (n = 75)	χ^2	p
1. Incomplete ossification of skull	0 (0.0%)	12# (16.7%)	16#@ (42.1%)	3$ (4.0%)	50.635*	<0.001*
2. Unossified skull bone	0 (0.0%)	0 (0.0%)	8#@ (21.1%)	0$ (0.0%)	26.488*	MCp<0.001*
3. Bifid sternum	0 (0.0%)	0 (0.0%)	5#@ (13.2%)	0$ (0.0%)	15.314*	MCp<0.001*
4. Incomplete ossification of ribs	0 (0.0%)	7# (9.7%)	15#@ (39.5%)	1$ (1.3%)	58.273*	<0.001*
5. Incomplete ossification of forelimbs	0 (0.0%)	9# (12.5%)	18#@ (47.4%)	1$ (1.3%)	70.967*	<0.001*
6. Incomplete ossification of hind limbs	0 (0.0%)	8# (11.1%)	30#@ (78.9%)	3$ (4.0%)	140.310*	<0.001*
7. Incomplete ossification of sacral vertebrae	0 (0.0%)	7# (9.7%)	27#@ (71.1%)	2$ (2.7%)	128.009*	<0.001*
8. Incomplete ossification of tail	0 (0.0%)	14# (19.4%)	29#@ (76.3%)	2@$ (2.7%)	122.473*	<0.001*

χ^2: **Chi square test** MC: **Monte Carlo**
p: p value for comparing between the different studied groups
*: Statistically significant at $p \leq 0.05$

#: Significant with **Group 1**
@: Significant with **Group 2**
$: Significant with **Group 3**

Forelimb anomalies

Incomplete ossification of metacarpus and phalanges: in the control group, the metacarpus and phalanges were normal (Fig. 9a). In ACR low dose group, incomplete ossification of forelimb bones was reported in 9 out of 72 (12.5%) (Fig. 9b). In ACR high dose group, it was in 18 out of 38 (47.4%) (Fig. 9c), while in ACR vit. E group, it was 1 out of 75 (1.3%) (Fig. 9d). There were significant statistical differences between the control and ACR groups and between ACR groups and ACR vit. E group (Table 3).

Hind limb anomalies

Incomplete ossification of metatarsals and phalanges: in the control group, the metatarsals and phalanges were normally ossified (Fig. 10a). In ACR low dose group, incomplete ossification of hind limb bones was reported in 8 out of 72 (11.1%) (Fig. 10b). In ACR high dose group, it was found in 30 out of 38 (78.9%) (Fig. 10c), while in ACR vit. E group, it was 3 out of 75 (4%) (Fig. 10d). There were significant statistical differences between the control and ACR groups and between ACR groups and ACR vit. E group (Table 3).

Vertebral and tail anomalies

Incomplete ossification of sacral vertebrae: control group exhibited normal ossification (Fig. 11a). In ACR low dose group, incomplete ossification, non-ossification of caudal vertebrae, and reduced pelvic elements were reported in 7 out

of 72 (9.7%) (Fig. 11b). In ACR high dose group, it was reported in 27 out of 38 (71.1%) (Fig. 11c), while in ACR Vit. E group, it was reported in 2 out of 75 (2.7%) (Fig. 11d). There were statistical significant differences between the control and ACR groups. as well as between ACR groups and ACR vit. E group (Table 3).

Incomplete ossification of the tail: control group showed normal ossification (Fig. 12a). In ACR low dose group, incomplete ossification of the tail was reported in 14 out of 72 (19.4%) (Fig. 12b). In ACR high dose group, it was reported in 29 out of 38 (76.3%) (Fig. 12c), while in ACR vit. E group, it was reported in 2 out of 75 (2.7%) (Fig. 12d). There were statistical significant differences between the control and ACR groups and between ACR groups and ACR vit. E group (Table 3).

DISCUSSION

A reactive, tiny chemical molecule with a high water solubility is called acrylamide (ACR). These characteristics make it easier for the body to quickly absorb and distribute (Mannaa et al., 2006).

ACR has become a primary public health issues: it was identified in frequently eaten products, like breakfast cereals, potato chips, and other carbohydrate-rich items cooked at temperatures higher than 200°C (Guyton and Hall, 2006; Mahmood et al., 2015).

Wistar albino rats were used as the experimental animals in this investigation, because of their bigger size, which facilitates handling, sampling, and procedure execution. In addition, they are immune to infections because of their resemblance to humans in anatomical, physiological, and genetic terms (Bryda, 2013; Paxinos and Watson, 2006).

Natural dietary antioxidants may offer protection from toxicity brought on by dietary toxins (Hamdy et al., 2017). Our research evaluated the possible protective effects of vitamin E while examining the toxicological impact of acrylamide exposure on the development of embryos.

Our work revealed that oral administration of Acrylamide to pregnant rats caused significant decrease in embryos weight and length.

Zebrafish exposed to acrylamide may develop embryonic dysplasia (Huang et al., 2018). The birth weight was shown to be inversely correlated with the presence of acrylamide and glycidamide hemoglobin in the cord blood samples, according to epidemiological investigations (Barrett, 2012).

Schettgen et al. (2004) demonstrated that, in the perfused human placenta, acrylamide and glycidamide may cross the placental barrier and generate N-2-carbamoylethylvaline.

At a dosage of 25 mg/kg per day, Friedman et al. (1999) found higher rates of abortion and still births and diminished body mass in embryos of Wistar rat females.

According to Sorgel et al. (2002), up to 18.8 g/L of dietary acrylamide could be found in breast milk, and 10-50% of it is transmitted to the fetus through the placenta through blood in pregnant women.

A strong connection between ACR exposure prenatally and decreased birth weight and head circumference has been shown in humans by a sizable population-based investigation conducted in Europe (Pedersen et al., 2012).

Researchers that looked into the effects of ACR found that it lowered levels of reduced glutathione, caused DNA damage, and increased oxidative stress. These harmful effects of ACR were attributed to loss of balance between formations of reactive oxygen species (ROS) and antioxidant capacity (Erdemli et al., 2019).

The findings of the current investigation demonstrated that rats given ACR and vitamin E showed improved bone growth. Vitamin E is a potent antioxidant that prevents cell membrane oxidation by interacting with free radicals generated and preventing the propagation response, according to Erdemli et al. (2019).

Packer et al. (2001) reported that the histology and biochemical markers improved with vitamin E administration together with ACR. Vitamin E is regarded as a key chain-breaking antioxidant that reduces the reactivity of oxygen molecules and aids in cell signaling during chemical information transfer between cells or between other cell structures.

Subcutaneous and intra-abdominal hemorrhages were documented in the current study. With comparison control group, they were much more noticeable in ACR treated groups (high and low), and their incidence was significantly lower in ACR vit. E treated group.

Regarding skeletal modifications, several skeletal abnormalities, including inadequate ossification of skull bones and failure to do so, were found in the current study. These anomalies were much more noticeable in ACR treated groups and less frequent in ACR vit. E group.

Vertebral and rib anomalies such as decreased and un-ossification of vertebrae, or unossified atlas, were significantly evident in ACR treated groups and dropped among the ACR vit. E group.

Incomplete ossification of the metacarpus, metatarsus, and phalanges was found in the current research.

This findings on acrylamide administration supported earlier researches done by Tyl et al. (2000a, b).

El-Sayyad et al. (2007) assessed prenatal fetuses and newborns of mothers who consumed fried potato chips. They found increased occurrence of congenital anomalies such as vertebral deformities, limb and tail anomalies and subcutaneous hemorrhage. Additionally, El-Sayyad et al. (2007) provided evidence that pregnant mice that eat acrylamide and potato chips from GD 6 to GD 14, 16 or 17 showed congenital deformity and slowed ossification of the bones of fetuses. Therefore, it is proposed that acrylamide toxicity emerges primarily in relation to fetal growth and development. El-Sayyad et al. (2007) found that premaxilla, frontal, parietal, exoccipital, and mandibular ossification were all significantly delayed in 14-day-old babies whose mothers received oral doses of ACR daily at a rate of 25 g/kg/day from the sixth to the fourteenth day of gestation.

Development of skeleton is carefully regulated, and secretory substances released from bone cells are important for controlling angiogenesis and homeostasis in addition to being essential for the coordination of motor function. The correct production of growth plates depends on the differentiation of MSCs into chondrocytes, which is followed by the construction of cartilage models, as opposed to the differentiation of MSCs straight into osteoblasts that give birth to osteocytes (Zelzer, 2003).

The removal of ageing or damaged bones is carried out by osteoclasts derived from hematopoietic stem cells (Han et al., 2018). Thus, the assembly and creation of skeleton is facilitated by the secretion of various growth factors by these bone cells, which regulates a dynamic equilibrium between osteoblasts, osteocytes, and osteoclasts (Hartmann, 2006).

The current study illustrated the toxicological effects of acrylamide on skeletal development. Previous research demonstrated that acrylamide inhibited expression of genes involved in development of skeleton as sox9, bmp2, runx2, and col2a1, during various pregnancy periods and resulted in aberrant cartilage formation and body length reduction. Col2a1's expression is controlled by Sox9, a protein-coding transcription factor involved in cartilage development (Fanghuan et al., 2021; Sekiya et al., 2000). Sox9 is an essential signaling protein that controls cartilage differentiation in rat embryos throughout the formation of the skeleton (Sekiya et al., 2000).

Sox9 regulates the early embryonic MSCs' differentiation into chondrocytes and promote the expression of col2a1, comp, and mia in the extracellular matrix, resulting in the formation of a cartilage matrix. The most fundamental collagen in cartilage, type II collagen, which is controlled by col2a1, indicates the activity of chondrocytes and can result in problems of the bones and joints in the case of mutation (Jimenez and Dharmavaram, 1994).

A thick cartilage matrix may be created by the interaction of cartilage oligomeric matrix protein (COMP) with fibrin (Di Cesare et al., 2002). Chondrocytes release melanoma inhibitory activity (MIA), which is present throughout cartilage formation, to aid in chondrocyte differentiation and maturation (Bosserhoff et al., 1997).

The primary defensive mechanisms to oxidative stress brought on by free radicals are antioxidants. The main antioxidant and membrane

protector against ROS is vitamin E (Agarwal et al., 2005; Yousef et al., 2003).

In the current work, ACR-exposed rats and treated by Vit E showed significant improvement in the development of skeleton. The claims made by Lee et al (2005) and Talebi et al (2012) are consistent with our findings.

CONCLUSION AND RECOMMENDATION

Acrylamide causes evident skeletal anomalies in rat embryos. Providing Vit E to ACR treated rats significantly reduced such effects. It is recommended to have Vit E rich foods and or Vit E supplements to decrease the harmful effects of ACR rich food on fetal development.

Ethical approval

The research protocol approved by Ethics Committee of Faculty of Medicine, Alexandria University [IRB No: 00012098 (expires 6-10-2025) - FWA No: 00018699 (expires 21-1-2026)]. Serial number 0305642 and followed the guidelines for use of animals with adherence to ARRIVE guidelines.

Authors'contribution: Melad N.Kelada, Marwa Mahmoud Mady and Sally Mahmoud Mohamed Hussein Omar contribute to the study design. Experiments and data collection were carried out by Melad N. Kelada and Sally Mahmoud Mohamed Hussein Omar. All authors approved the final manuscript.

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