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RESEARCH ARTICLE

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Morphological and osteological studies on the effect of Acrylamide (ACR) on parentally treated foetuses of albino rat

ABSTRACT:

Acrylamide (ACR) has become one of the major public health concerns since it was detected in widely consumed food items. The present study investigates the effects of ACR on the skeletal system of the prenatal foetuses of 20th day of gestation of rat. The used rats were arranged in five groups; Control group (C); males and females rats administered orally distilled water. G1; male rats orally administered 10 mg/kg of ACR for consecutive 10 days and then they allowed to mate with control females. G2 female rats orally administered 10 mg/kg of ACR for consecutive 10 days and then they allowed mating with control males. G3 male and female rats orally administered 10 mg/kg orally of ACR orally for 10 consecutive days and then they allowed mating with each other. G4 pregnant female orally administered 10 mg/kg of ACR orally at (1st - 20th) day of pregnancy. At the 20th day of gestation, the uteri were removed by caesarean sections. For each mother, the number of foetal swelling in each horn, living and dead foetuses and early of late resorptions were recorded. Early and late resorptions were discerned according to their size. For morphological studies, foetuses, either living or dead were counted, weight and length measured, then morphologically examined for any external malformation. Foetuses showed severe skeletal alterations included incomplete ossification for some bones of skull, vertebrae, fore and hind limbs and significant reduction in the length of most long bones of both limbs. The alterations indicated that ACR treatment induced growth retardation confirmed by great reduction in body weight and body length. The results suggest that ACR has teratogenic effects on parentally treated 20-day-old albino rat foetuses.

KEY WORDS:

Acrylamide, endoskeleton system, teratogenicity, 20-day-old Albino rat foetuses.

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INTRODUCTION:

Food supplied all the nutrients necessary for the organism to function properly biological function of body organs. Acrylamide is a chemical that can form in some food items during high-temperature cooking processes, such as frying, roasting, and baking. Acrylamide in food forms from sugars and an amino acid that are naturally present in food; it does not come from food packaging or the environment (Mottram *et al*., 2002).

Acrylamide (C3H5NO, according to IUPAC: 2-propenamid) is a reactive, small organic molecule with very high water solubility. These properties facilitate its rapid absorption and distribution through the body (Mannaa *et al*., 2006). ACR is a polar substance which easily dissolves in water or other polar solvents, e.g. in methanol or ethanol (Hogervorst *et al.,* 2009). High reactivity of ACR relates to the double bond and amide group. The compound may create hydrogen bonds and can react both with amide and vinyl groups (Girma *et al*., 2005;

Zyżelewicz *et al.,* 2010). Acrylamide is polymerized under the influence of temperature and UV radiation.

ACR has become one of the major public health concerns since it was detected in widely consumed food items such as fried bread (breakfast cereals), potato chips, and any carbohydrate-rich food items cooked at high temperatures (Guyton and Hall, 2006; Mahmood *et al*., 2015). ACR is present in everyday diet of most people. To make matters worse, some of the products containing acrylamide are attractive to children and young people (Semla *et al*., 2017). The mechanism of ACR formation in food has not been clearly described yet. Numerous researches have shown only hypothetical ways in which ACR is being formed in comestible products (Edegaard *et al.*, 2008; Mestdagh *et al.*, 2008). Most of the research point to asparagines presence as a significant factor contributing to ACR formation (Taeymans *et al.*, 2004; Zhang *et al.*, 2009). ACR formation is connected to the Maillard-reaction during food preparation above 120°C.This reaction occurs between reducing sugars and amino acids such as paragines (Elbashir *et al*., 2014; Muttucumaru *et al*., 2014). The content of ACR increases considerably during frying, grilling and roasting. Popular food stuffs such as coffee, high-in-starch potato products and cereal products contain large amounts of ACR (Claus *et al.*, 2008; Tajner-Czopek *et al.*, 2012).

Acrylamide can be prepared by the hydrolysis of acrylonitrile by nitrile hydratase. In industry, most of the acrylamide is used to synthesize polyacrylamides that are common in personal care and widely used in grooming products (e.g. lotions, cosmetics, deodorants), soil conditioning, waste water treatment, and paper and textile industries (Friedman, 2003; Exon, 2006; Parzefall, 2008). ACR is the main compound of Cosmetics, ACR gel electrophoresis, and textile production and sugar manufacturing and food packaging (Szczerbina, 2005; Exon, 2006). In addition to such industrial and laboratory uses, high levels of ACR were detected in tobacco smoke (Pruser and Flynn, 2011). Food and cigarette smoke are the major sources of acrylamide exposure for people in the general population (Urban *et al*., 2006; Çebi, 2016). High concentrations of Acrylamide are found in processed foods like: chips (50 - 3500 µg/kg), frites(170 - 2287 µg/kg), coffee (170 - 350 µg/kg), bread (70 - 430 µg/kg) or corn flakes (30 – 1400 µg/kg) (Friedman, 2003; Guyton and Hall, 2006). People are exposed to substantially more acrylamide from tobacco smoke than from food. People who smoke have three to five times' higher levels of acrylamide exposure markers in their blood than do non-smokers (Virk-Baker *et al*., 2004). Exposure from other sources is likely to be significantly less than that from food or smoking (Pelucchi *et al*., 2011).

Sub-acute toxic effects were experienced by individuals drinking well water contaminated with acrylamide (WHO, 2002). Occupational exposure mostly affects chemical plant workers, laboratory workers, construction industry workers, miners or workers of coal preparation plants (Pennisi *et al*., 2013). Irrespective of route, exposure to acrylamide rapidly occurs in blood plasma (Barber *et al.,* 2001). ACR delivered by the oral route is metabolized in the liver (Hammad *et al.,* 2013). Acrylamide is metabolized with the aid of the biotransformation takes place with the cytochrome P450. The resulting metabolite is an epoxide derivative, glycidamide, which is more reactive towards DNA and proteins than the parent compound ACR (El-Mottaleb and Rashed, 2008; Tareke *et al*., 2002 & 2008). Therefore, the most important pathogenic pathway is the oxidative biotransformation of ACR by cytochrome P450 2E1 (CYP2E1) (Hammad *et al*., 2013). The resulting metabolite is an epoxide derivative, glycidamide, which is more reactive towards DNA and proteins than the parent compound, ACR (Tareke *et al*., 2008). The majority of ACR is conjugated with glutathione while a lesser amount is activated via glycidamide (Emekli-Alturfan *et al.,* 2012).

Once absorbed, ACR may be conjugated by glutathione-S-transferase (GST) to N-acetyl S-(3-amino-3-oxopropyl) cysteine or it reacts with cytochrome P450 (CYP450) to produce glycidamide (Kopańska *et al*., 2015). Since ACR is formed during the cooking or frying of many commonly consumed foods at high temperatures, the general population is highly exposed to ACR in their diets (Mahmood *et al*., 2015). The present work aims to investigate the possible teratogenicity effects of ACR on rat foetuses. The work focused on the survival, morphological and osteological alterations of parentally treated 20- day old albino rat foetuses.

MATERIAL AND METHODS:

Administration of Acrylamide (ACR):

Highest purity Acrylamide obtained from the Egyptian company "LOBACHEME", Cairo, with a chemical formula C_3H_5NO and molar mass (71.079 g/mol). The applied single dose of ACR (10 mg/kg) was orally administered (Mahmood *et al*., 2015; Lai *et al.,* 2017).

Mating procedures:

The adult virgin females of approximate age 8 - 12 weak were selected. Each three of them were kept with one adult male in one cage overnight. In the next morning pregnancy was assured by the presence of vaginal plug. Each pregnant female was kept in a separate cage. In case of absence of the vaginal plug, a drop from the vaginal contents was prepared and examined for the presence of the spermatozoa; their presence was taken as a sign of copulation. This female was considered to be at the first day of pregnancy (Billet and Wild, 1975; McClain and Becker, 1975).

Animals groups:

Pure strain of Virgin male and females of Albino rat; *Rattus norvegicus* domestica, provided from Theodor Bilharz research institute; El Nile road, Warrak El Hadar, Embaba, Egypt were used for experimentation.

The used rats were arranged in five groups; Control group (C) was the group orally administered distilled water. G1 was the group of male orally administered with (10 mg/kg) of ACR for consecutive 10 days and then they allowed to mate with control females.G2 was the group of female orally administered with (10 mg/kg) of ACR for consecutive 10 days and then they allowed to mate with control males. G3 was the group of male and female orally administered with (10 mg/kg orally) of ACR for consecutive 10 days and then they allowed to mate with each other.G4 was the group of Pregnant females orally administered with (10 mg/kg) of ACR at (1^{st} - 20^{th}) day of pregnancy.

At the 20^{th} day of gestation, the uteri were removed by caesarean sections. For each mother, the number of foetal swelling in each horn, living and dead foetuses and early of late resorptions were recorded. Early and late resorptions were discerned according to their size. For morphological studies, foetuses either living or dead were counted, weight and length measured, then morphologically examined for any external malformation. For studying the skeletal malformations, Fresh foetuses were fixed in 95% ethyl alcohol and processed for double staining: alizarin red and alcian blue. After complete digestion of the soft tissues with 2% potassium hydroxide, the specimens were kept in ascending grades of glycerine, till bones visualized (Falkeholm *et al*., 2006).

RESULTS:

Foetuses mortality:

Acrylamide- administration increased the rate of dead foetuses and reduced the number of alive foetuses as mentioned in table1. The percent of change of the total foetuses of the treated group to those of the control group was found to be 87.5% at G1; 73.2% at G2; 57.1% at G3 and 51.8% at G4. In addition, the percentage of change of a live foetus in comparison with live foetuses of the control was 12.5%, 26.8%, 42.9%, and 48.2% in case of G1, G2, G3, and G4, respectively. Table 1 shows also two dead foetuses out of total 51 in G1, seven dead foetuses out of total 48 in G2, ten dead foetuses out of total 42 in G3 and eleven dead foetuses out of total 40 in G4. This result indicated that the more effective foetuses are in G4 that foetuses maternally orally administered with ACR (10 mg/kg) at (1^{st} - 20^{th}) day of pregnancy.

Table 1. Effect of Acrylamide on the percent of change of total foetuses and a live foetus in case of treated groups compared with the control.

		Total NO. of foetuses	Mean SD	% Change from control	% Change from alive
C	NO. of foetuses	56	4.4		
	Alive	56	4.4	0.00	0.00
	Dead	-	-		
G1	NO. of foetuses	51	4.2		
	Alive	49	3.9	87.5%	12.5%
	Dead	2	0.4		
G2	NO. of foetuses	48	2.6		
	Alive	41	2.8	73.2%	26.8%
	Dead	7	0.8		
G3	NO. of foetuses	42	3.9		
	Alive	32	2.3	57.1%	42.9%
	Dead	10	1.00		
G4	NO. of foetuses	40	4.6		
	Alive	29	3.8	51.8%	48.2%
	Dead	11	0.9		

External and endoskeleton Malformations:

External Morphology:

The external morphological examination of foetuses (20^{th} day of gestation) indicated that Acrylamide (ACR) administration caused growth retardation of foetuses assessed by the reduction of foetal body weight and length**.** Weight reaching to (5.3 ± 0.16) was 5.2 ± 0.1, 4.5 ± 0.13, 4.2 ± 0.1 and 4.2 ± 0.08 gm for G1; G2; G3; and G4, respectively (Fig. 1). G4 showed the highest reduction of body weight compared to the other studied groups (Fig. 1). The mean foetal length of parentally administered with ACR was significantly decreased. It was 4.6 ± 0.08, 4.45 ± 0.12, 4.2 ± 0.08, and 4.1 ± 0.09 cm for G1; G2; G3; and G4 respectively compared to the control (4.7 ± 0.08)

(Fig. 2). Superficial hematomas were clearly observed all over the body regions (Fig. 3 G1-G4).

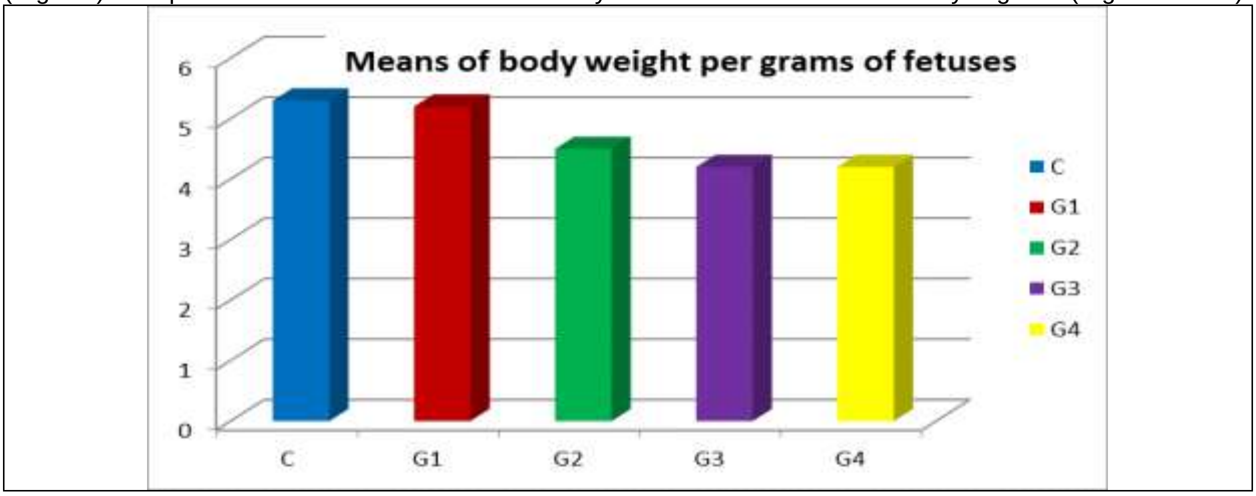

Fig. 1. Histogram showing the mean change in the body weight of the 20th day – old rat foetuses of the control and treated groups.

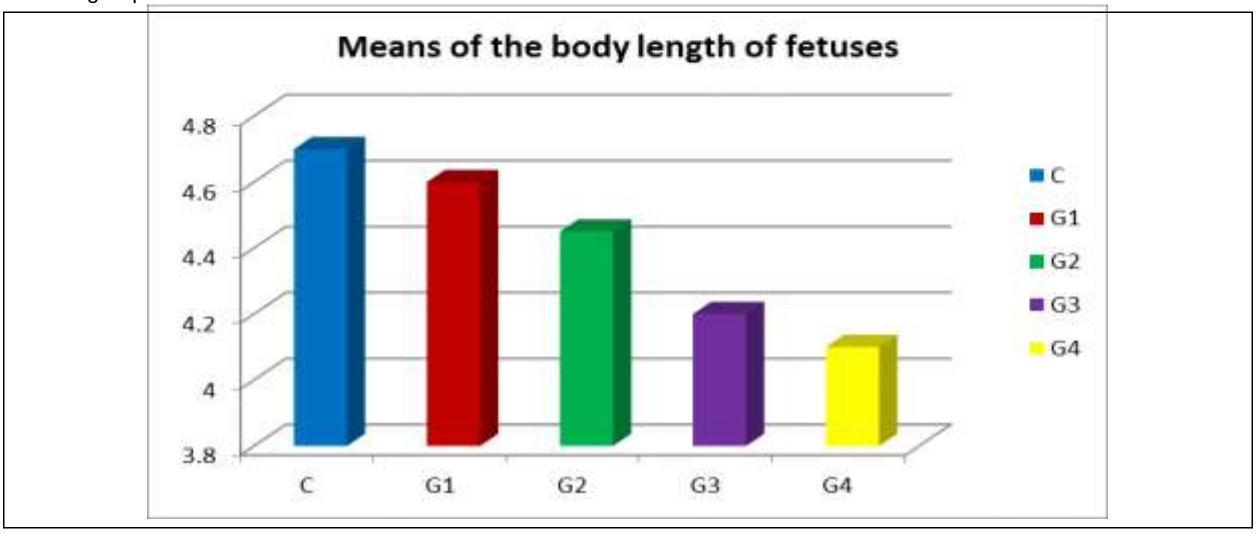

Fig. 2. Histogram showing the mean change in the body length of the 20th–day old rat foetuses of the control and treated groups.

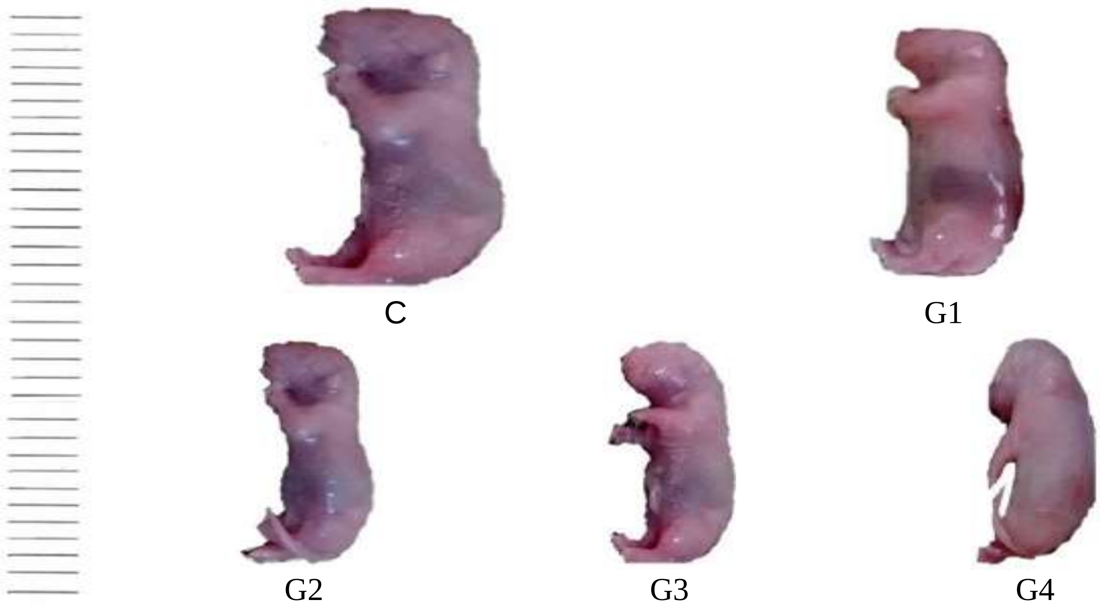

Fig. 3. Photographs of a lateral view of rat foetuses at 20thday of gestation showing: Control (C) and four Acrylamide (ACR) - treated groups (G1, G2, G3, & G4).

Endoskeleton Observations:

The skeletal system of the albino rat consists of two main structures: the axial skeleton and appendicular skeleton. The axial skeleton comprises bones of the skull, vertebral column, ribs and sternum while the appendicular skeleton consists of bones of pectoral girdle and fore limbs and pelvic girdle and hind limbs (Fig. 4 C). Acrylamide (ACR) affected the development of skeletal system of administered foetuses at the 20^{th} day of gestations compared with the control one at the same age. This effect included the reduction in lengths and decrement of bones and cartilages (Figs 4-12C&G1-G4).

Axial Skeleton:

The Skull:

Examining the double stained endoskeleton system at 20^{th} day of gestation of control foetuses of the albino rat, showed, complete ossification of the skull components (Figs 4, 5, & 6 C). Decrease of ossification of both cartilaginous and dermal bones of the skull for the treated groups. In relation to the change in length and the volume of skull of the parentally administered foetuses of which subjected to severe abnormalities, this lead to a clear shortage in length and volume of the skull as compared with the control group (Figs 5 & 6 G1-G4).

There is malformation in the skull of foetuses parentally administered with a single dose of ACR. i.e., incomplete ossification of the nasal, frontal, supraoccipital, parietal, interparietal, zygomatic process of squamosal, tympanic bulla, squamosal, periotic, supraoccipital, palatine, pterygoid and ethmoid bones (Figs 5 & 6 G1-G4). The most evident decrement was deposition of bone material that starts with first group, progresses with second group then third group and reached its final effects in the fourth group. In relation to the G3 & G4 treated group, the shape and volume of skull has a clear shortage and not ossified the most bones including dermal and cartilaginous one; basisphenoid and basioccipital as compared the control skull (Figs 5 & 6 G3 & G4). The bones of the lower jaw showed moderate ossification in foetuses of all treated groups; a gradual lack of ossification as can be shown from figure 6, since there is a slight ossification for dentary of the treated group, (Figs 4 & 6 G1-G4).

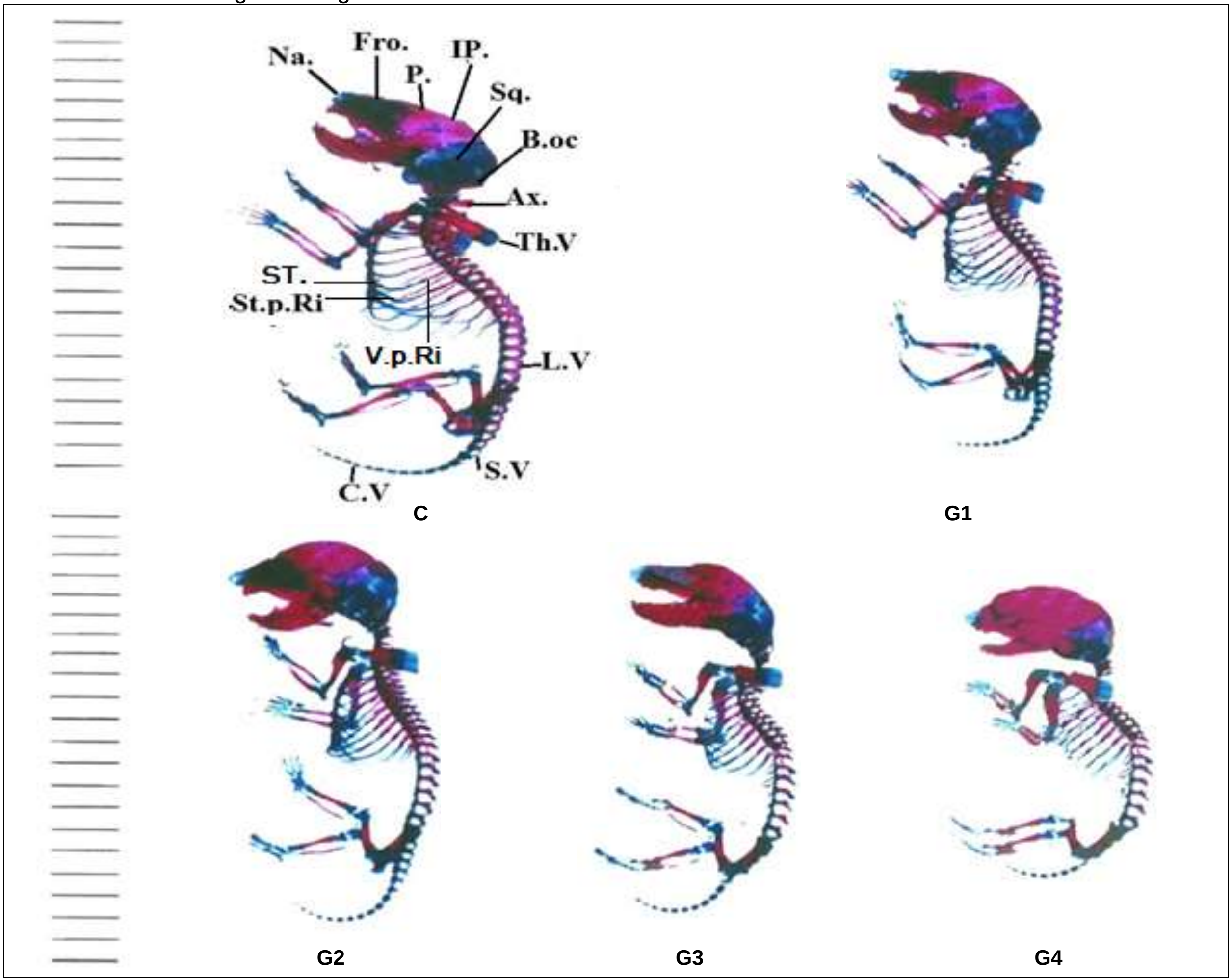

Fig. 4. Photographs of a lateral view of the skeletal system of 20^{th} of gestation of rat foetuses showing: Control (C) and four ACR- treated groups (G1, G2, G3, & G4).

Abbreviations:

N., Nasal; Fro., Frontal; P., Parietal; IP., Interparietal; Sq.; Squamosal, B.oc :.,Basioccipital; Ax., Axis; Th.V., Thoracic vertebrae; St.p.Ri., Sternal portion of ribs; L.V., Lumbar vertebrae; S.V., Sacral vertebrae; C.V., Cervical vertebrae.

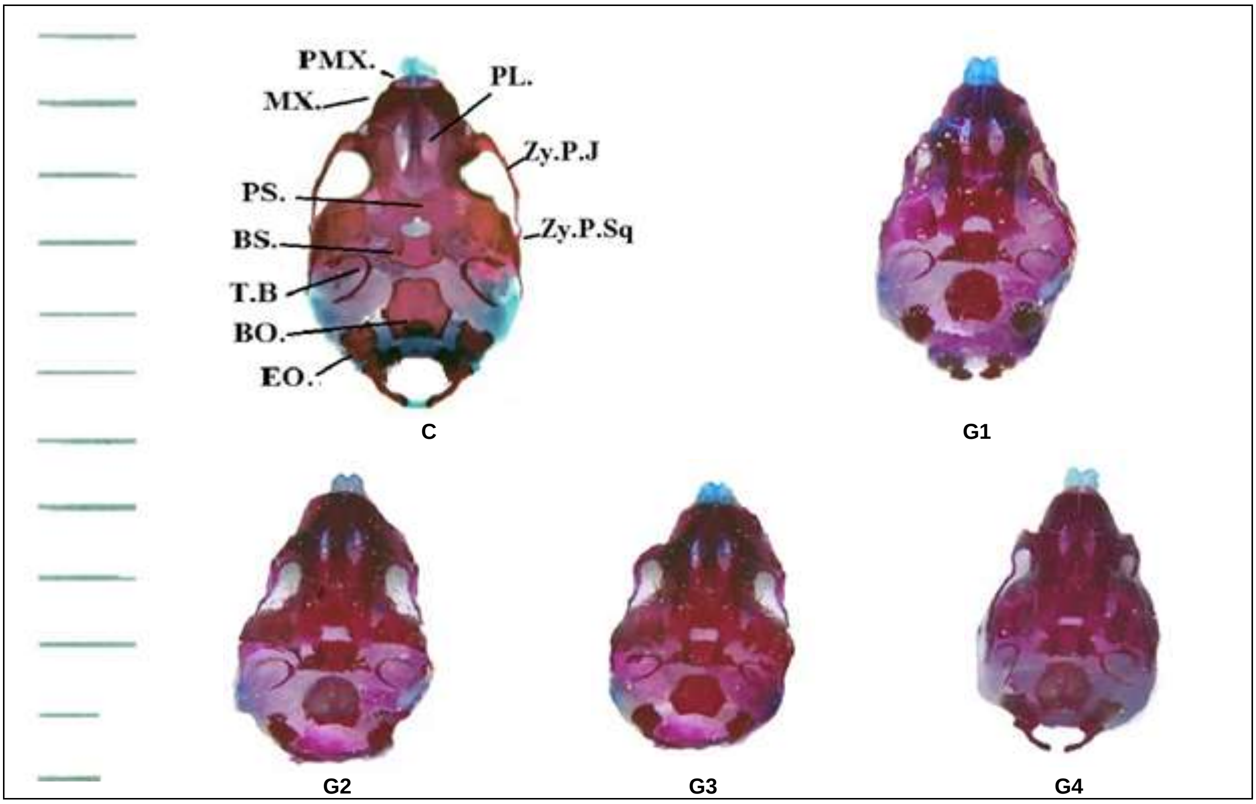

Fig. 5. Photographs of a ventral view of the skull of rat foetuses at 20th of gestation showing: Control (C) and four ACR- treated groups (G1, G2, G3, & G4).

Abbreviations:

MX., Maxilla; PMX., Premaxilla; PL., Palatine; BO., Basioccipital; PS., Presphenoid; BS. , Basisphenoid; T.B., Tympanic bulla; Zy.P.J., Zygomatic process of jugal ; Zy.P.Sq., Zygomatic process of squamosal; EO., Exoccipital.

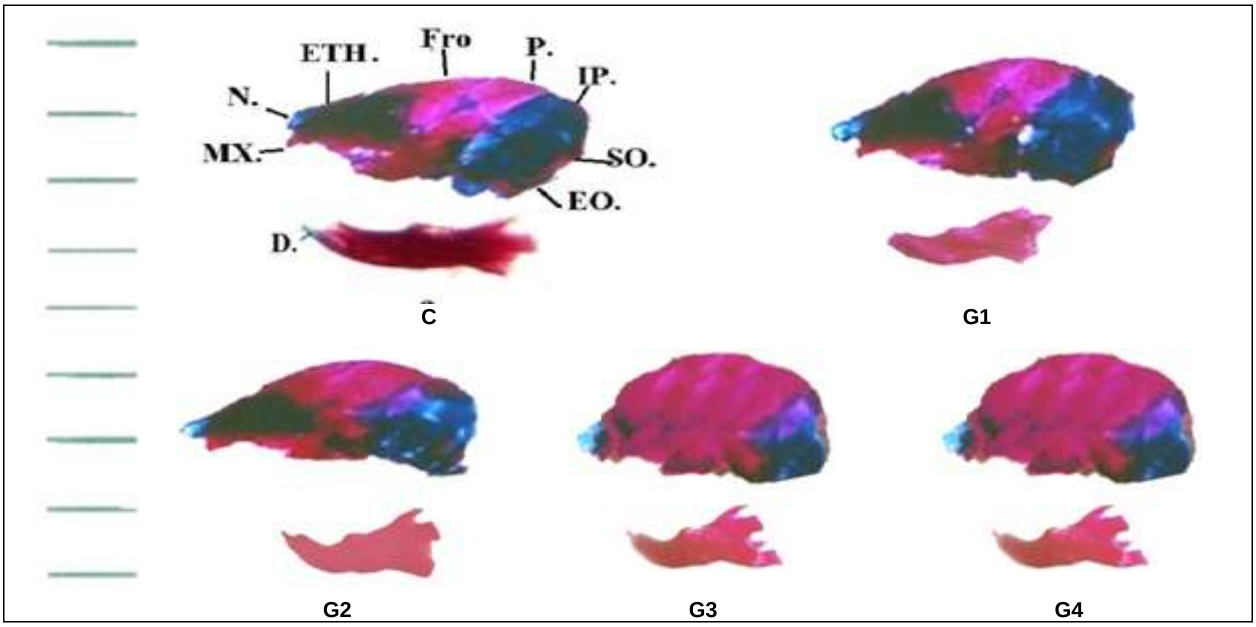

Fig. 6. Photographs of a lateral view of the skull of rat foetuses at 20th day of gestation showing: Control (C) and four ACR- treated groups (G1, G2, G3, & G4).

Abbreviations

D., Dentary; MX., Maxilla; N., Nasal; ETH., Ethmoid; Fro., Frontal; P., Parietal; IP., Interparietal; SO., Supraoccipital; EO., Exoccipital

The Vertebral Column:

The vertebral column of foetuses obtained from the control pregnant rat showed, more or less, ossified vertebrae which are represented by 7 cervical, 12 thoracic,7 lumber, 4 sacral and 10 caudal vertebrae (Figs 4 & 7 C).

Examination of the vertebral column of foetuses at 20th day of gestation parentally treated with single dose of ACR revealed that the atlas and axis vertebrae not well ossified. Vertebrae of "G1" and "G2" showed no alteration from the control except for the caudal vertebrae which showed more or less-ossification. Most of the examined foetuses of "G3" and "G4" showed severe lack of ossification in their cervical and lumbar. Moreover, sacral and caudal vertebrae were completely non-ossified (Fig. 7 G1-G4).

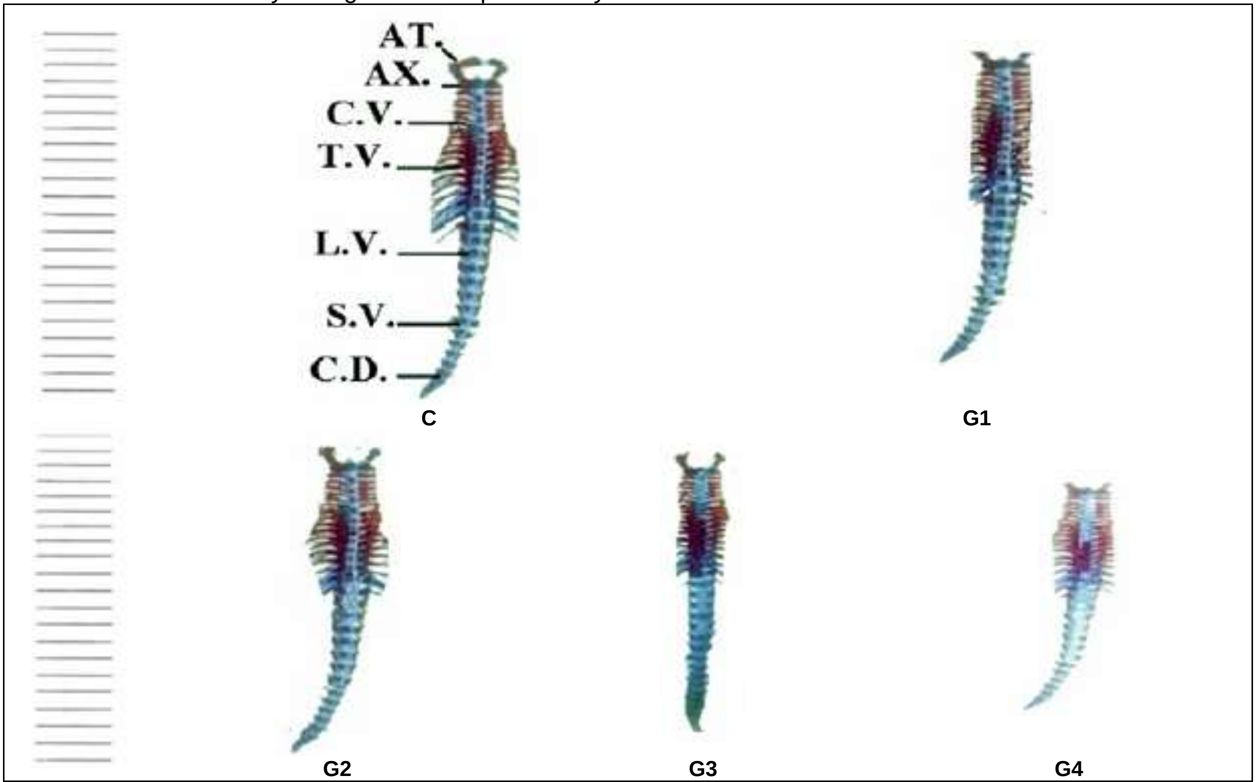

Fig. 7. Photographs of a ventral view of the vertebral column of rat foetuses at 20thof day of gestation showing: Control (C) and four ACR- treated groups (G1, G2, G3, & G4).

Abbreviations:

AT., Atlas; AX., Axis; C.V., Cervical vertebrae; T.V., Thoracic vertebrae; L.V., Lumbar vertebrae; S.V., Sacral vertebrae; C.D., Caudal vertebrate.

Sternebrea and Ribs:

The sternum of the control foetuses consists of six rod-like pieces of well-ossified sternebrae arranged in a straight line and the last one of them is the xiphisterum (Fig. 8 C). The sternebrae of foetuses parentally administered orally with ACR were shorter than the control group (Fig 8G1-G4). The most affected sternebrae were observed in the third and the fourth group (Fig. 8G3-G4).

The control foetuses (20th day of gestation) possess 13pairs of ribs (Figs 4 & 7). Each rib consists of a bony vertebral portion and a cartilaginous sternal one (Figs 4&8C). The sternal parts of the ribs, except the last three pairs, articulate with the sternum. The ribs which reach the ventral side are independently attached to the breastbone are named as a true rib. The unattached ribs are called false or floating ribs (Fig. 4C). The ribs of foetuses in all treated groups were shorter than the control one. The length of ribs of foetuses of the fourth group was shorter than the length of ribs of foetuses of the third one. The cartilaginous portion of the ribs exhibited less blue coloration than the normal referred to reduction in its chondrification (Figs 4 & 7 G1-G4).

The ribs of foetuses of G1 appeared well ossified as compared with the control one. Also, the sternal portion of last three ribs appeared as wavy shaped (Fig. 4 G1). But those of G2 exhibited obviously regression of ossification process for the most part of vertebral portion of ribs. The phenomenon of wavy sternal portion of ribs is found approximately in last six ribs (Figs 4 & 8G2).

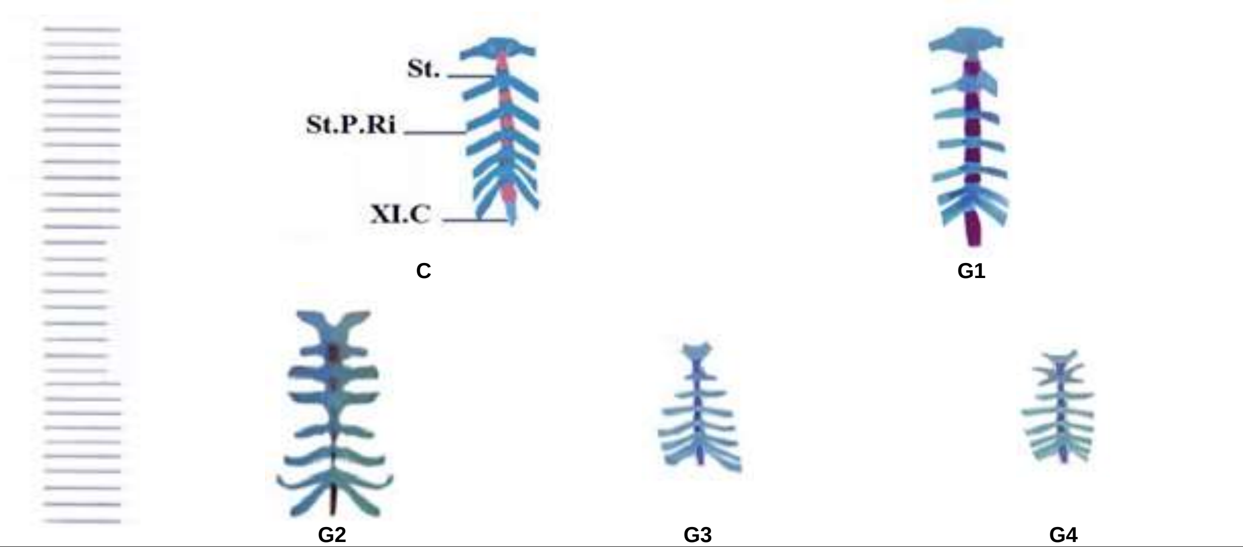

Fig. 8. Photographs of a ventral view of the sternum of rat foetuses at 20th day of gestation showing: Control (C) and four ACR- treated groups (G1, G2, G3, & G4).

Abbreviations:

ST., Sternum; ST.P.Ri., Sternal portion of ribs; XI.C., Xiphoid cartilage.

The Appendicular Skeleton:

The Pectoral Girdle and Fore Limb:

The pectoral girdle of the foetuses of 20th day of gestation obtained from untreated groups consists of a well ossified scapula and clavicle, while the supra-scapula still cartilaginous in nature (Fig. 9 C). The fore limb of the control foetuses of 20th day of gestation consists of ossified humerus, radius and ulna, phalanges with five digits and cartilaginous carpalia and metacarpalia (Fig. 9 C).

The components of the pectoral girdle and fore limb of foetuses obtained from parentally treated with ACR are manifested by reduction in size and the degree of ossification in reference to the control (Figs 9 & 10 G1-G4). Pectoral girdle and fore limbs of all examined foetuses of (parentally);“G3” and (maternally); “G4” showed severe lack of ossification and highest decreases in their lengths (Figs 9 & 10 G3&G4).

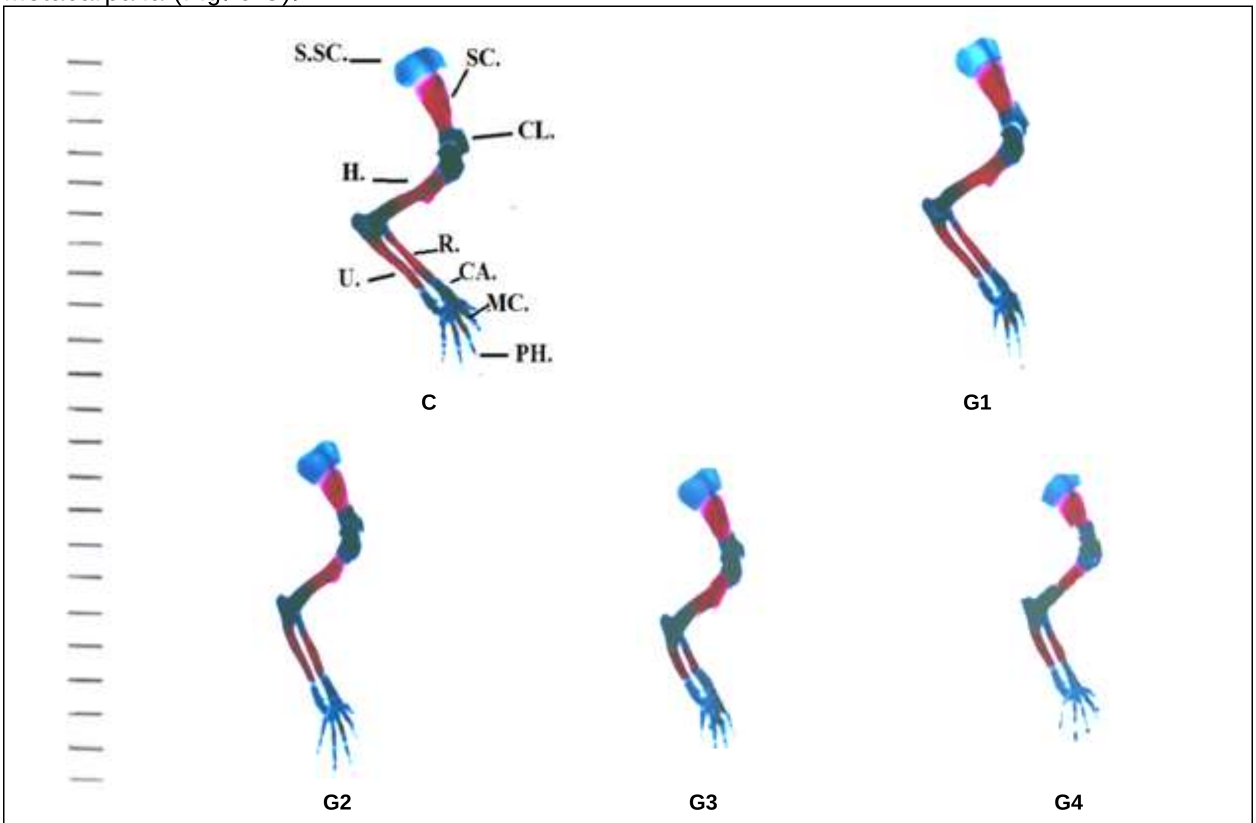

Fig. 9. Photographs of a lateral view of the pectoral girdle and fore limb of rat foetuses at 20th day of gestation of Control (C) and four ACR- treated groups (G1, G2, G3, & G4).

Abbreviations:

S.SC., Supra-scapula; SC., Scapula; CL., Clavicle; H., Humerus; R., Radius; U., Ulna; CA., Carpales; MC., Metacarpalia; PH., Phalanges.

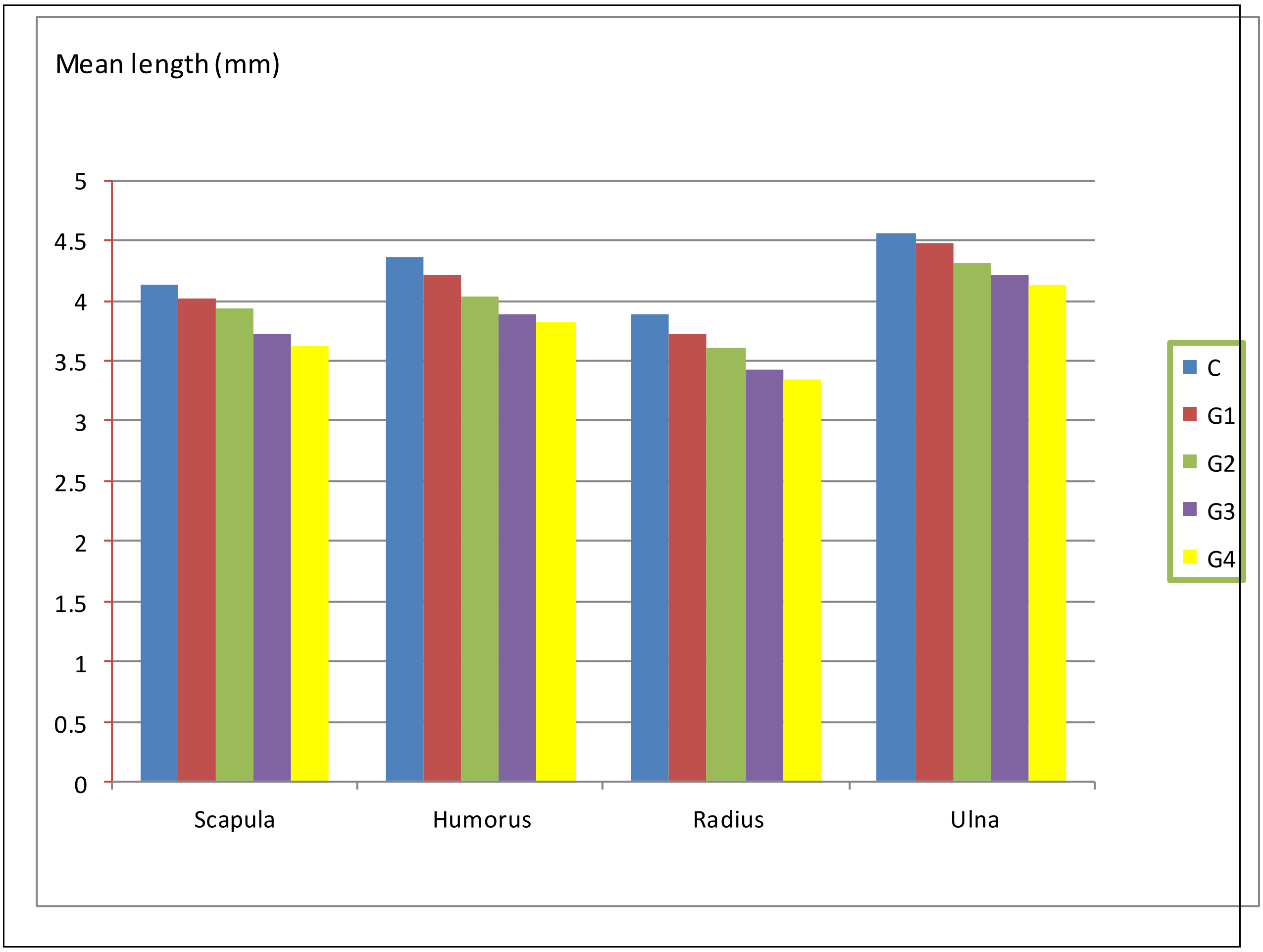

Fig. 10. mean ossified length of scapula, humerus, radius and ulna in control and treated groups of rat foetuses at 20th day of gestation.

The Pelvic Girdle and Hind Limb:

The pelvic girdle of the control foetuses of 20th day of gestation consists of three well ossified bones; ilium, ischium, and pubis. The pubic symphysis remains cartilaginous in nature. The hind limb of the control foetuses consists of well ossified bones; femur, tibia and fibula, a series of phalanges in the four digits and cartilaginous tarsals and metatarsals (Fig. 11 C).

The pelvic girdle and hind limb of the foetuses at 20th day of gestation parentally administered with ACR showed that the degree of ossification of ilium, ischium, pubis, femur, tibia and fibula was affected. Also, a series of phalanges was affected in all the treated groups (Fig.11 G1-G4). The degree of the chondrification of the pubic symphysis, tarsals and metatarsals was also affected especially at the third and fourth groups. The length of the components of the pelvic girdle and fore limb was shorter than the control. The highest decreases in lengths of such bones were observed in G3 and G4 (Figs 11 & 12 G3&G4).

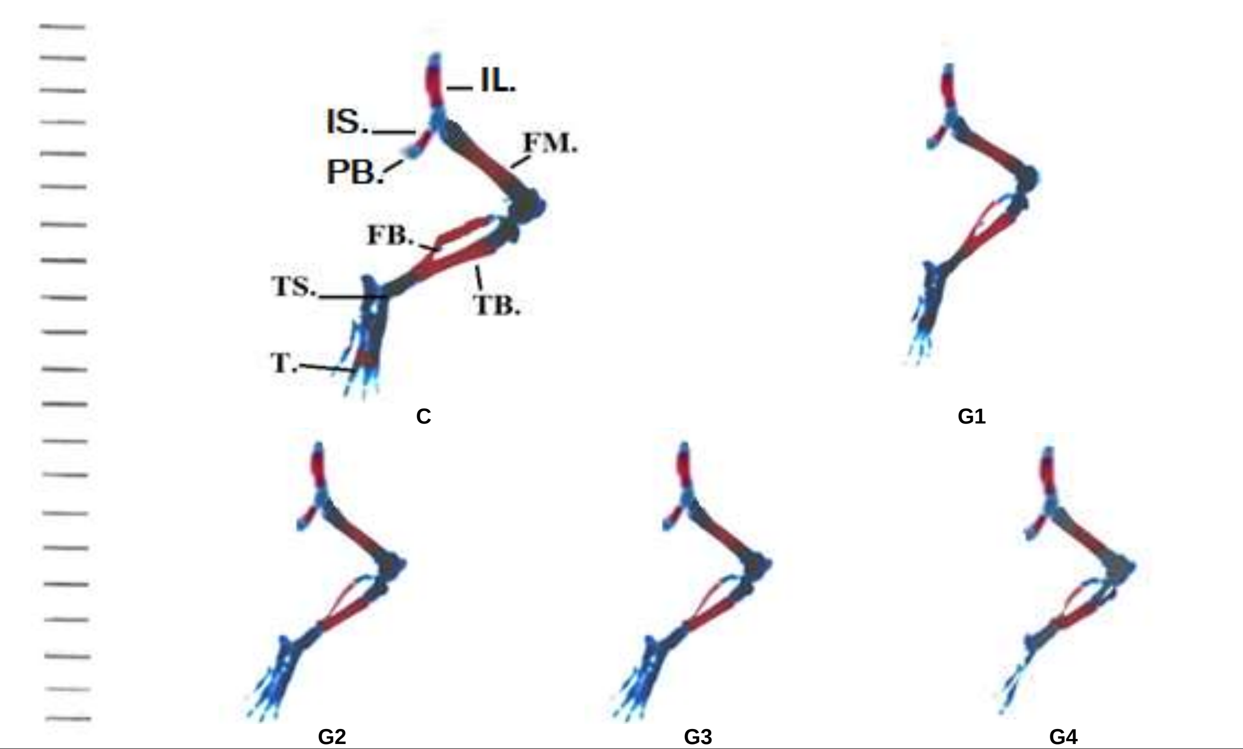

Fig. 11. Photographs of a lateral view of the pelvic girdle and hind limb of rat foetuses at 20th day of gestation showing: Control (C) and four ACR-treated groups (G1, G2, G3, & G4). ilium, ischium and pubis.

Abbreviations:

IL., Ilium; IS., Ischium; PB., Pubis; FM., Femur; FB., Fibula; TB., Tibia; TS., Tarsalia; T., Toes.

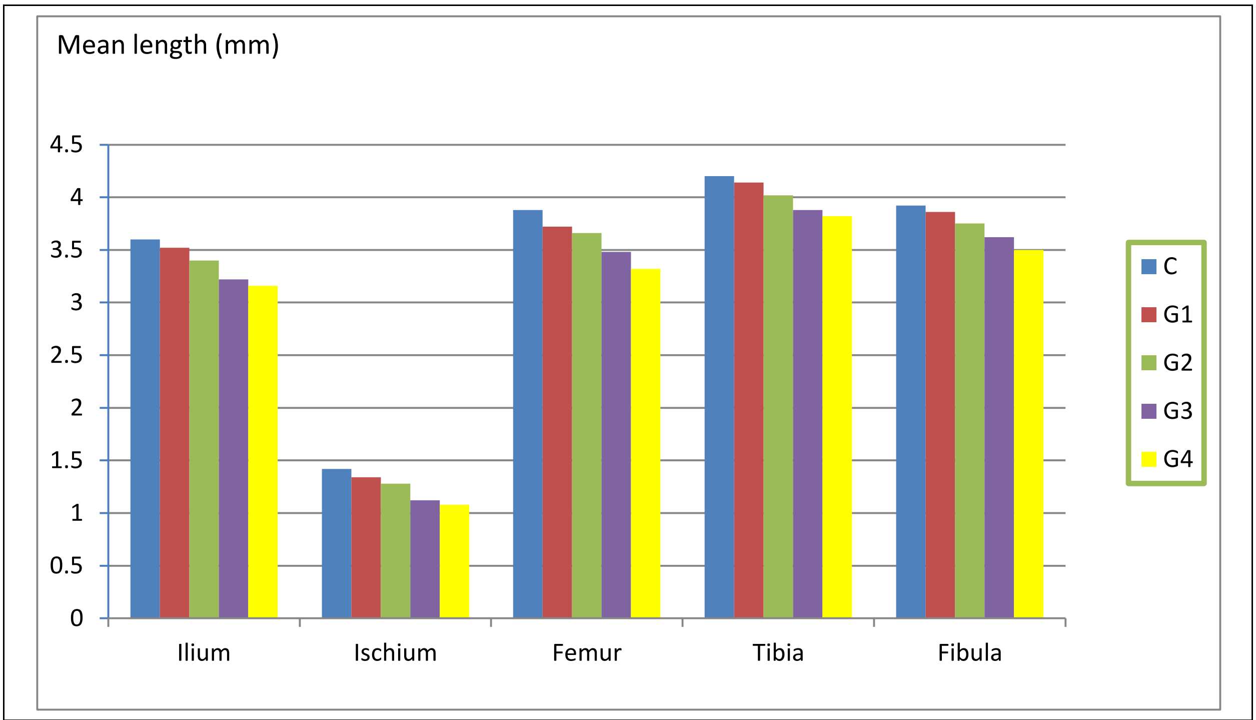

Fig. 12. Mean ossified length of ilium, ischium, femur, tibia and fibula in control and treated groups of rat foetuses at 20th day of gestation.

DISCUSSION:

Acrylamide (ACR) is a reactive, small organic molecule with very high-water solubility. These properties facilitate its rapid absorption and distribution through the body (Mannaa *et al*., 2006). ACR has become one of the major public health concerns since it was detected in widely consumed food items; for example, fried bread (breakfast cereals), potato chips, and any carbohydrate-rich food items cooked at high temperatures (higher than 200°C) (Guyton and Hall, 2006; Mahmood *et al*., 2015). The present study showed that oral administration of ACR with the single dose (10 mg/kg) caused toxic effects on the parentally treated foetuses (20th day of gestation). These effects were observed in the body weight, length of foetuses and number of alive and dead foetuses. The foetal body weight and length of ACR administered mother was less than that observed in the control animals. This result indicated that the most decrease in the weight and length of foetuses maternally administered orally daily with (10 mg/kg) of ACR was in the G4 at (1st - 20th) day of gestation. As a low molecular weight compound easily dissolving in water, acrylamide passes through the placenta in animals and human organism. It was also found in breast milk of women. Thus, it may have the influence on the normal prenatal and early postnatal development of infants (Sörgel *et al*., 2002). Embryo and foetal toxicity of ACR was not observed in rats (15 mg/kg/day). In mice (45 mg/kg/day) foetal weight was reduced (Field *et al*., 1990). Interesting studies over acrylamide influence on embryonic and early postnatal development of rats were performed by El-Sayyad *et al*. (2011). In this study, pregnant females were orally administered to high ACR doses of 30 mg/kg b.w. from day 6 of gestation until parturition and throughout lactation. The young pups derived from acrylamide exposed females had lower body size and weight in comparison to control animals. In human, a large population-based study in Europe has provided the epidemiological evidence showing a significant association between prenatal exposure to ACR and reduction in birth weight as well as head circumference (Pedersen *et al*., 2012)

The present study showed that the number of live foetuses obtained from parentally administered ACR was decreased in compared with the control. The percent of reduction was increased in G3 and G4 and dead foetuses were pronounced at G4 that administered orally ACR (10 mg/kg) at (1st - 20th) day of gestation. Exposure of pregnant females of rodents to ACR doses ≥5 mg/kg b.w./day, administered orally, resulted in increased post-implantation loss of embryos and decreased number of live pups. Exposure of pregnant females to higher doses of ACR (≤ 15 mg/kg b.w./day) resulted in reduced pup weight and survival (NTP, 2011). It was found that administered of male rats to acrylamide doses of 19 mg/kg for eight days and next mated to unexposed females led to reduced fertility rates and increased frequency of resorption of embryos (Sakamoto and Hashimoto, 1986).

In the present work ACR administration "G1, G2, G3, and G4" was found to cause skeletal retardation and circulatory disorders in the form of hematoma in different parts of the foetal body. However, ACR doesn't cause any change in body shape of foetuses. El-Sayyad *et al*. (2007) examined the gross morphology of prenatal foetuses and newly born of mothers fed on fried potato chips from the 6th day of gestation till partition revealed the incidence of some congenital malformations including kyphotic body, uni- and bilateral malformation of both fore and hind limbs, reduced neck region, kinky tail and presence of superficial spotty regions of skin haemorrhage in head, neck and trunk regions (superficial hematomas). However, no increase in the incidence of malformations was observed in rat (15 mg/kg) and mice (45 mg/kg) treated with ACR; however, the incidence of variations (predominately extra rib) increased (Field *et al*., 1990).

The present study dealt with the effect of ACR on the skeletal malformations produced in rat foetuses that examined on day 20th of gestation. It has been found that ACR in the single used dose (10 mg/kg) caused variable deformities in some elements of the examined skeletal system and absent ossification of some parts of the components of the skeleton.

The present results showed that ACR delayed the development of the skull bones according to the intensity of the red coloration denoting decrease in the process of osteogenesis. The bones of the skull that revealed retardation in ossification were parietal, interparietal, zygomatic process of squamosal, tympanic bulla, squamosal, periotic, supraoccipital, palatine, pterygoid and ethmoid bones. The most evident decrement was deposition of bone material that starts with first group, progresses with second group then third group and reached its final effects in the fourth group. The bones of the lower jaw showed moderate ossification in foetuses of all treated groups. El-Sayyad *et al*. (2007) observed that in 14-day-old foetuses, maternally treated with ACR daily with oral doses of 25 μ g/kg from 6th till 14th day of gestation, showed a marked retardation of ossification in premaxilla, frontal, parietal, exoccipital and mandibular regions. Dietary administration to acrylamide was associated with reduced birth weight and head circumference in human. Consumption of specific foods during pregnancy was associated with higher acrylamide exposure in utero (Pedersen *et al*., 2012).

In relation to the vertebral column, the present study revealed that. Vertebrae of "G1" and "G2" showed no alteration from the control with the exception of the caudal vertebrae which showed more or less-ossification. Most of the examined foetuses of "G3" and "G4" showed severe lack of ossification in their cervical and lumbar vertebrae. Acute skeletal malformations were observed in cervical, lumbar, sacral and caudal vertebrae in the foetuses of the third and the fourth groups. El-Sayyad *et al*. (2007)

showed that the length of ossification centres of axial and appendicular skeletons of 14-days old foetuses maternally administered with acrylamide with oral doses of 25 µg/kg from 6th till 14th day of gestation was significantly decreased as compared with the control foetuses. Histological investigation of tympanic region, cervical vertebrae of 14-days old foetuses of maternally treated with ACR daily with oral doses of 25 µ g/kg from 6th till 14th day of gestation revealed much more retarded growth which still in the form of chondrified pattern (El-Sayyad *et al*., 2007).

The present study reported that the ribs and sternebrae were shorter than the control group. The cartilaginous portion of the ribs exhibited less blue coloration than the control referred to reduction in its chondrification. The components of the pectoral girdle and fore limb of foetuses obtained from parentally treated with ACR are manifested by reduction in size and the degree of ossification in reference to the control. The degree of ossification of scapula, clavicle, humerus, radius and ulna were affected especially at "G3" and "G4". The highest decreases in lengths of such bones were observed in G3 and G4. The size of scapula of pectoral girdle of 14-days old foetuses maternally treated with ACR with oral doses of 25 µg/kg from 6th till 14th day of gestation was reduced (El-Sayyad *et al*., 2007).

The pelvic girdle and hind limb of foetuses of 20th day of gestation parentally administered orally with ACR showed that the degree of ossification of ilium, ischium, pubis, femur, tibia and fibula was affected. Also, a series of phalanges was affected especially at the second and fourth group. The degree of the chondrification of the pubic symphysis, tarsals and metatarsals was also affected especially at the second and fourth group. The length of the components of the pelvic girdle and fore limb was shorter than control. The highest decreases in lengths of such bones were observed in G3 and G4. El-Sayyad *et al*. (2007) said that the size of ilium of pelvic girdle of 14-days old foetuses maternally administered acrylamide 25 µ g/kg oral doses from 6th till 14th day of gestation was comparatively reduced. The present findings on acrylamide administration confirmed the previous work carried out by Tyl *et al*. (2000) and Tyl and Friedman (2003) after treating pregnant rats & mice with acrylamide. On the other hand, the present results contradicted with the work of Field *et al*. (1990) who reported that pregnant mice administered with higher dose of acrylamide (45 mg/ Kg /day) resulted in both maternal and foetal toxicity was not associated with increased malformation incidence. The contradiction based on a fact that the authors used very highly embryotoxic dose which cause cell death and not interfere with cell growth pattern during organogenesis.

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التشوهات الناتجة من تأثير الأكريلاميد على الشكل الظاهري والجهاز الهيكلي لأجنة الجرذان البيضاء

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يختص هذا البحث بدراسة تأثير مادة الأكريلاميد على أجنة الفئران البيضاء قبل الولادة (اليوم العشرين من الحمل) وذلك بحقن أباءهم وأمهاتهم بجرعة فردية من مادة الأكريلاميد عن طريق الفم، الأكريلاميد مركب كيميائي يتشكل نتيجة تسخينها لدرجات حرارة عالية في بعض المواد الغذائية التي تحتوي على الكربوهيدرات أو المواد النشوية، مثل البطاطس المحمرة والمقلية و الشيبسى والخبز المحمص وحبوب القهوة المحمصة وخصوصاً الداكنة، ومنتجات الحبوب مثل البسكويت. وقد تبين من الدراسة وجود زيادة في نسبة الوفيات في المجاميع المعاملة. أيضا لوحظ نقصان في وزن وطول الأجنة مع وجود تشوهات في الشكل الخارجي للأجنة المعاملة أباءها وأمهاتها بمادة الأكريلاميد. أظهرت الدراسة تشوهات متوسطة الي شديدة في الجهاز الهيكلي للأجنة في اليوم العشرين من الحمل مثل عظام الجمجمة، كما أوضحت عن تشوهات في العمود الفقري لهذه الاجنة ومنها عدم تعظم اجسام بعض الفقرات العنقية والصدرية والقطنية والعجزية والذيلية. كما كشفت الدراسة ايضا قصر عظام الضلوع والغضروف السيفي بالمقارنة بالحالة العادية للأجنة في اليوم العشرين من الحمل، وبالنسبة لعظام الحزام الصدري والحوض فقد لوحظ قصر هذه العظام مع نقص احجامها بالمقارنة بالأجنة العادية وهذه العظام هي اللوحي، الترقوي، الفخذي والعظم القصبي الشطبوي. كما أن هذه العظام تظهر نقصا في شدة الصبغة الحمراء مما يدل على انها أقل كثافة مع انعدام ترسيب الخلايا العظمية بشكل طبيعي بالمقارنة بالحالة العادية.