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Morphological and Skeletal Malformations Induced by Gabapentin in Rat Fetuses and their Amelioration by Ginger

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ABSTRACT: The present study aimed to investigate the possible morphological and skeletal fetotoxicity of the new antiepileptic drug, gabapentin (GBP) during the organogenesis phase of the rat embryonic development and to examine the possible ameliorative role of ginger *(Zingiber officinale)*. Morphologically, there was evident growth retardation and a high frequency of malformations in the skin, head, trunk, limbs and tail of fetuses maternally injected with GBP. Skeletal investigation revealed that rat fetuses of the GBP group exhibited delayed ossification and various skeletal malformation. Limb deformities was the most prominent feature observed followed by coastal malformations, vertebral deformities, skull anomalies and sternal defects, respectively. The most evident skull malformation was maxillary and mandibular hypoplasia. Ginger extract caused an evident decrease in GBP-induced fetotoxicity at the investigated parameters. In conclusion, treatment with GBP should be approached with highly caution during pregnancy and ginger is recommended to be taken in parallel for its ameliorative role in this regard.

Keywords: Gabapentin; organogenesis; malformation; morphology; skeletal and ginger.

INTRODUCTION: Epilepsy is a commonly encountered serious chronic neurological problem which affects millions of people worldwide. Due to relapsing and severity of epilepsy, many women cannot stop using medications even during pregnancy [1]. It is a well-documented fact that teratogens affect the development of embryo, mostly during the process of organogenesis, which starts on GD 6 in rats and continues to GD 15 [2]. Currently, management of epilepsy mainly depends on antiepileptic drugs (AEDs), which are notoriously known for their adverse side effects and this explains the reason for their several generations. Infants of mothers treated with AEDs during pregnancy were found to have a greater incidence of congenital malformations than those of either normal control or non-treated epileptic women [3]. Therefore, it is strongly believed that AED therapy rather than the maternal disease or convulsions are the cause of malformations identified at birth [3]. It has been reported that AEDs should be chosen to be effective, safe and free from fetal toxicity as possible as it can [4].

Gabapentin (GBP) was originally introduced for the treatment of epilepsy but has achieved greater popularity as an adjunctive therapy for chronic pain [5]. There is a little information on the teratogenic effects of GBP [6]. It has been labelled category C on the basis of the adverse effects produced in rodent fetuses [7]. Despite expanding data on the usage of GBP, there is little information, so far, on its teratogenic effects [8]. It is well known that AEDs belong to the drugs which have traditionally been considered as bone damaging [9]. Administration of AEDs may lead to the development of osteomalacia or osteoporosis [10]. Studies on rodents have shown that oral consumption of GBP caused delayed ossification of several bones in the skull, vertebral column, upper, and lower limbs during the organogenesis period [8].

Traditionally, ginger has been applied for treating colic, indigestion, stomach ulcers, rheumatism and joint problems. Furthermore, ginger has anti-cancer, anti-inflammatory properties as well as anti-nausea/vomiting properties [11]. Moreover, no toxic effects on the morphology or endoskeleton of rat embryos maternally administered ginger were observed [12]. Weidner and Sigwart [13] examined the effect of oral administration of three different doses of ginger extract to pregnant female rats from GD 6 to 15 and

found no signs of embryotoxicity. The present study was designed to investigate the ameliorative role of ginger against morphological and skeletal malformations induced by GBP in rat fetuses.

MATERIALS AND METHODS:

Animals and grouping: Principles of animal care and use were carefully followed during the conducting of all experiments. Healthy mature virgin females and fertile males of Wistar albino rats (*Rattus norvegicus*), weighing 135 ± 15g and aged 17 ± 1 weeks, were obtained from Hellwan Animal Breeding Farm, Ministry of Health, Cairo, Egypt. Animals were kept in the laboratory for at least one week before initiation of the experiments for acclimatization. They were housed in specially designed plastic rodent cages at Faculty of Science, *Menoufia* University. They were maintained at 25 ± 2°C in 12h light: 12h dark cycle. Free access of water and standard diet composed of 50% ground barely, 20% ground yellow maize, 20% milk and 10% vegetables were supplied.

Mating was achieved by housing virgin females with fertile males at a ratio of one male with two females overnight. Females were checked daily in the morning for the presence of a copulatory plug and the presence of sperms in unstained native vaginal smears. Therefore, vaginal smears were carried out to give a precise determination of the onset of gestation. The day at which vaginal smear was positive has been considered as day zero of pregnancy. Day 20 was determined as the end point for experimentation. A total of 36 rats were used for the present study. The pregnant rats were divided into four groups, six rats each, as follows:

- Control group, administrated distilled water.
- Ginger group given oral injection of ginger (200 mg/kg).
- Experimental GBP group given intraperitoneal injection of GBP (162 mg/kg).
- Combined GBP and ginger injected group, received intraperitoneal injection of GBP first followed by oral injection of ginger one hour later.

GBP administration: GBP, with the trade name Gaptin, (Delta Pharma Company, Egypt) was employed for the study. The applied dose was 162 mg/kg per day during the organogenesis phase of gestation, *i.e.* starting on GD 6 and ending on GD 15 [7].

Water extraction of ginger: Fresh rhizomes of ginger (*Zingiber officinale)* were purchased from a local market at Shebeen El-Koom, Menoufia, Egypt and processed as described in our previous study [14].

Embryotoxicity estimation: On GD 20, the pregnant females were anaesthetized using ether and then sacrificed. A ventral incision in the abdomen was made, and then the whole uterus was removed, weighed and photographed. Fetuses were removed individually from each horn and living fetuses were anaesthetized by ether and examined grossly for investigating the morphological abnormalities under a dissecting microscope. A total of 187 fetuses were included in the present study. Embryotoxicity were initially assessed morphologically by counting the number of implants, live, resorbed and dead fetuses. Morphometric parameters were recorded. 59 of the fetuses were eviscerated and kept in 10 % formalin for detection of endoskeletal malformations.

Investigated parameters:

Morphological parameters:

- *Crown-rump length:* The crown rump length (cm) of the fetuses of different groups was recorded.
- *Body weight:* The weight (g) of the fetuses of different groups was recorded.

Skeletal investigation: For endo-skeletal preparations, double staining technique was applied as described in a recent study emerged from our laboratory [15].

Data evaluation and statistical analysis: All data sets were expressed as mean ± standard error of the mean (SEM). The data were analyzed statistically for normal distribution (student's T test) and homogeneity of variances (Levene test) using statistical package of social sciences (IBM SPSS) statistics software for Windows, Version 22 (IBM Corp., Armonk, NY, USA). Differences were considered insignificant whenever $P>0.05$. The significances of the obtained data were classified into three categories, i.e. $P<0.0001$, $P<0.001$ and $P<0.05$ according to P values.

RESULTS AND DISCUSSION:

Morphological investigation:

Body weight gain of mothers: The differences in mothers' weight gain of control and experimental groups are summarized in Figure (1). The dams that were administered ginger exhibited a gradually progressive increase in body weight gain similar or even more than that of the control group starting with 5.38 gm in GD 8 and ending with 64.70 gm in GD 20. Contrarily, there was reduction in body weight gain of GBP injected mothers just before the end of injection at the GD 15 then the weight gain increased slowly until GD 18 after which the body weight gradually increased in lower values until GD 20 (-3.52, -1.93, -

0.48, 5.23, 8.30, 13.83, 27.23 gm). The mothers of GBP plus ginger group exhibited a gradual increase in the body weights but in low values when compared with the control group (3.72, 8.25, 11.07, 17.27, 25.98, 36.28, 47.50 gm).

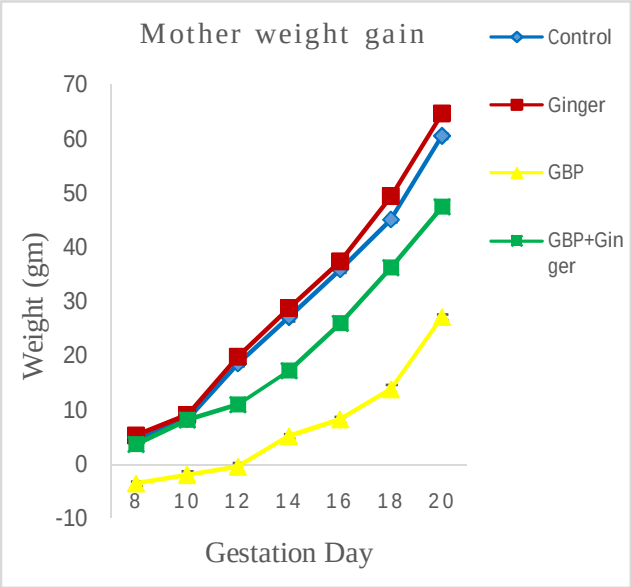

Figure 1: Graph showing changes in the body weight gain of mothers in different groups.

Average weight of uteri: No significant difference was recorded in fetal resorptions among different groups. Table (1) shows the difference of uterus weight in different groups. The average weight of the uteri of pregnant rats injected with ginger only showed insignificant difference (51.33±0.30) compared with control (52.03±0.28). On contrast, the average weight of uteri of pregnant rats injected with GBP exhibited a highly significant decrease (35.37±0.31) compared with that of the control group (52.03±0.28). The average weight of uteri of pregnant rats injected with both GBP and ginger exhibited a low significant reduction (46.52±0.32) compared with the control group and a highly significant increase when compared with the GBP group (35.37±0.31).

Table 1: Weight of uteri of pregnant rats at the end of experimentation, *i.e.* GD 20.

Groups	Average weight of uteri	C%
Control	52.03±0.28	0%
Ginger	51.33±0.30	-0.7 (-1.35%)
GBP	35.37±0.31***	-16.66 (-32.02%)
GBP + ginger	46.52±0.32*c	-5.51 (-10.60%)

C% = percentage of change compared with control; Asterisks (-***) refer to the P values compared with the control group; c= highly significant (P<0.0001) compared with GBP group; * P<0.05; *** P<0.0001*

It has been accepted that when embryos or fetuses become maternally subjected to a potentially teratogenic agent, developmental alteration occurs due to their interaction. The final result of this interaction can lead to a number of abnormalities including morphological, skeletal, anatomical, delay in intrauterine growth and development or even fetal death [15, 16]. Susceptibility of teratogenicity in an organism towards any teratogen depends on many factors such as critical developmental stage at which the organisms are exposed, the teratogen nature, the dose and route of teratogen and also on the types of initiating mechanism of teratogenesis [17]. It has been known that administration of higher dosages of old AEDs is associated with higher risks for anatomical teratogenesis in the embryo [18]. However, there is no sufficient knowledge concerning teratogenic effect of newer AEDs and few studies have assisted their teratogenic risks [18].

Padmanabhan et al. [19] observed much less weight gain in mice mothers treated with vigabatrin. A study of Etemad et al. [3] showed that pregabalin administration in mice decreased mother weight gain accompanied by reduced uterus weight. Morse et al. [20] also showed that oral injection of pregabalin resulted in reduced maternal body weight gain. The present study revealed that pregnant mothers injected with GBP during organogenesis exhibited a reduction in the uterine weights, though the number of implantation sites and resorption of most fetuses from the different groups were similar to that of the control group. Thus, changes in maternal weight gain in treated dams could be due to an effect on the uterine compartment rather than on the maternal weight.

Morphometric analysis: The reproductive toxicity data from the control, ginger, GBP and GBP plus ginger injected groups are presented in Table (2). The fetal growth parameters evaluated in this study were fetal crown-rump length and body weight. The results revealed that GBP led to proportionate intrauterine growth retardation relative to the control group as it affected the growth parameters of the fetuses with no mortality. On the other hand, a marked improvement in fetal growth parameters was recorded in fetuses maternally injected with GBP and followed by ginger compared with the GBP group.

Fetal crown-rump length: As Table (2) shows, the crown-rump length of fetuses exhibited insignificant increase in ginger group compared with that of the control group. The length of fetuses maternally injected with GBP displayed a significant shortening compared with control group. On the other hand, the fetuses maternally injected with GBP followed by ginger displayed a significant increase in the length

compared with GBP alone. This in return, led to a low significant difference between GBP plus ginger group when compared with the control group.

Fetal body weight: Table (2) illustrates the changes in body weight of fetuses in both control and experimental groups. The fetuses of the control and maternally ginger injected group had somewhat similar values (5.47 ± 0.17; 5.32 ± 0.07 for the two, respectively). There was a highly significant decrease in the body weight of fetuses of the maternally GBP injected group (3.53 ± 0.18). Administration of ginger after GBP injection led to a marked amelioration of body weight compared with fetuses of GBP group. This significant amelioration of body weight led to a low significant difference towards the control group.

Marchi et al. [21] reported reduction in body weight when lamotrigine was administered in rats at four times the median effective dose during the organogenesis period. Offspring of lamotrigine treated rat demonstrated relatively lower length and body weight [22]. Significant reduction in fetal body weight and an increase in the frequency of intrauterine growth retardation (IUGR) were evident in mice fetuses prenatally exposed to vigabatrin [23]. Fetal weight was significantly reduced when pregnant rats was administered topiramate orally [24, 25]. Of the old generation, AEDs, valproic acid was the most known drug to induce fetal growth retardation [26-28]. Decrease in fetal body weight may be due to its lower mitotic growth rate because of chemical administration to embryos [29].

Table 2: Crown-rump length and body weight of fetuses aged 20 days in different groups.

Groups	Fetal Growth Parameters	
	Length	Weight
Control	5.02 ± 0.06	5.47 ± 0.17
Ginger	4.72 ± 0.05	5.32 ± 0.07
GBP	3.58 ± 0.05**	3.53 ± 0.18***
GBP + Ginger	4.12 ± 0.05* b	4.93 ± 0.10* c

Data are represented as mean ± SEM; Asterisks (- ** - ***) refer to the P value compared with the control group; c= highly significant (P<0.0001) compared with GBP group; b= significant (P<0.001) compared with GBP group; a= low significant (P<0.05) compared with GBP group; * P< 0.05, ** P<0.001, *** P< 0.0001*

Morphological abnormalities: Fetuses of control and ginger injected mothers taken on the day of scarifying, *i.e.* GD 20 displayed normal size, length and morphological appearance (Fig. 2A&B). However, there was a low incidence of subcutaneous hemorrhage and thin skin (2.6 %; 3% for the two groups, respectively) (Table 3). On the other hand, various malformations were detected in fetuses maternally injected with GBP. There was a high frequency of malformations in the skin, head, trunk, limbs and tail of fetuses of this group (Table 3). Co-administration of GBP and ginger resulted in an evident reduction in the incidence of the malformations observed in GBP injected group (Table 3).

Thin skin had the most prominent malformation in fetuses of the GBP group and GBP plus ginger group (36.1%; 14.8%, respectively) (Fig. 2C). Ear malformations came second in the incidence of teratogenic abnormalities which was in the form of small (microtia) or absent (anotia) ear pinna (Fig. 2C, D & F) (29.5%; 9.3% for the two groups, respectively). Subcutaneous hemorrhage was detected in the head and hind limb regions (Fig. 2E & F) and came in the third place (23%; 7.4% for fetuses maternally injected with GBP individually or in combination with ginger, respectively). The investigation showed that about 21.3% of fetuses maternally injected with GBP and 5.6% of fetuses of combined GBP and ginger group had malformed limbs. These deformities included malrotation and delayed development in fore and hind limbs which appeared as syndactyly, micromelia and angulated (Fig. 2D-H). In some fetuses, the tail was evidently malformed (19.7%; 7.4 for GBP and GBP plus ginger injected groups, respectively). These deformities can be described as angulated and short tails (Fig. 2I & J). Another common malformation observed was the evident backward deviation in normal curvatures or kyphosis (Fig. 2C) occurred with incidence of 19.7% in fetuses of maternally injected GBP group and 7.4% in fetuses of GBP and ginger co-administered mothers. Head malformations including exencephaly (Fig. 2H) and unequal jaws or brachygnathia (Fig. 2D), where the snout was narrow and pointed due to the small mandible, were clearly evident. About 18% and 4.9% of fetuses of mothers injected with GBP suffered from brachygnathia and exencephaly, respectively, whereas only 3.7 % of fetuses of combined GBP and ginger co-administered mothers had brachygnathia and none of them had exencephaly. Exomphalos was observed in fetuses of GBP and combined GBP and ginger injected mothers with incidence of 9.8% and 5.6%, respectively (Fig. 2K). Ginger extract caused an evident decrease in GBP-induced embryotoxicity when given after GBP injection. The fetuses of this group exhibited marked improvement in terms of shape, size and length (Fig. 2L).

These results were consistent with the work of Prakash et al. [7] in mice. Afshar et al. [8] found that maternal injection of GBP during different stages of pregnancy in mice caused brachygnathia, vertebral column deformity, limb anomalies, exencephaly and

severe trunk malformations. Vigabatrin, which is also a new AED similar to GBP, induced similar alterations in mice fetuses [30].

The lack of skin wrinkled appearance which led to its thinning was the most common morphological anomaly in the fetuses of the GBP group. This was also observed in the study of Fadel et al. [31] who found that maternal injection of diazepam during the organogenesis period induced loss of wrinkled appearance of the skin in the growth-retarded rat fetuses. Akhtar et al. [32] stated that valproic acid also is known to have teratogenic effects on the development of skin. Ear malformation was the second most observed malformation in the fetuses of the present study with 29.5% of the fetuses suffering from either anotia or microtia. In a case study, Montouris et al. [33] found that pregnant women who received GBP and lamotrigine during pregnancy had an incidence of having infants with congenital malformation in the left external ear canal.

Subcutaneous hemorrhage came third in the overall GBP induced gross malformations in the fetuses. Mohanty et al. [22] reported widespread of hemorrhages in offspring maternally treated with lamotrigine. Limb deformities were common in the fetuses maternally injected with GBP which included malrotation, syndactyly, micromelia and angulated fore and hind limbs. Prakash et al. [7] admitted that administration of GBP to pregnant female mice during the midgestation period resulted in malrotated and rudimentary limbs of the fetuses. Similar results were observed in mice fetuses maternally injected with GBP during the first 10 or 15 days of pregnancy [6, 8]. In the offspring of dams treated with topiramate or pregabalin during the period of organogenesis, the frequency of limb malformations was increased [3, 25].

Caudal abnormalities of the present study included short or angulated tails. Treatment with valproic during pregnancy induced similar malformation in the offspring who had reduced curled or kinked tails [34, 35]. The authors suggested that these tail abnormalities could be attributed to abnormal intrauterine position of the fetus. Deformities in vertebrae were another anomaly that appeared as abnormal curvatures along the vertebral column like scoliosis. GBP is known by its effect on the vertebral column curvature and it was proved to induce such alterations in many studies after its intraperitoneal injection in pregnant mice with different doses during the organogenesis period [6-8].

Brachygnathia was the most prevalent anomaly in mice fetuses maternally injected with GBP [8]. However, in the present study only 18% of the fetuses suffered from this alteration. Exomphalos or umbilical hernia was among the least GBP induced anomalies in the fetuses. This was consistent with the study of Abdulrazzaq et al. [30] who found a significant incidence of exomphalos in vigabatrin treated mice and attributed this to developmental defects in the abdominal wall. Afshar et al. [8] recorded the presence of exencephaly in mice fetuses maternally subjected to GBP during the first 15^{th} days of gestation. Lamotrigine, when orally administered to pregnant rats, was also found to induce exencephaly in the offspring [22].

Endo- skeletal investigation: In developmental studies, the fetal skeleton is an important indicator of embryonic development health and changes in skeletal development commonly reflect changes in the maternal-fetal environment [36]. Fetuses maternally injected with GBP had various skeletal malformations including mandibular hypoplasia, malformation in the ribs and delayed ossification of the vertebrae, skull and limbs. Table (4) summarizes the different skeletal malformations in the fetuses of different groups.

Table 3: Percentage of fetuses with morphological abnormalities (%) recorded at the end of experimentation, *i.e.* GD 20 in different groups.

Malformation	Groups			
	Control n=39	**Ginger n=33**	**GBP n=61**	**GBP + Ginger n=54**
Thin skin	(1) 2.6%	(1) 3%	(22) 36.1%	(8) 14.8 %
Ear malformations	0 %	0%	(18) 29.5 %	(5) 9.3 %
Subcutaneous hemorrhage	(1) 2.6 %	(1) 3 %	(14) 23 %	(4) 7.4 %
Limb deformities	0 %	0 %	(13) 21.3%	(3) 5.6 %
Tail abnormalities	0 %	0%	(12) 19.7%	(4) 7.4 %
Kyphosis	0%	0%	(12) 19.7%	(4) 7.4%
Brachygnathia	0 %	0 %	(11) 18 %	(2) 3.7 %
Exomphalos	0%	0%	(6) 9.8%	(3) 5.6%
Exencephaly	0 %	0 %	(3) 4.9 %	0 %

The percentage of every abnormality was calculated according to each group.

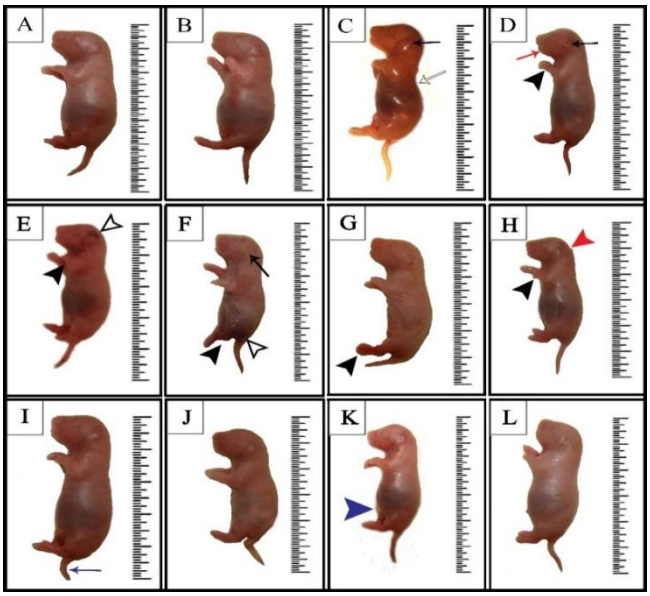

Figure 2: Photographs of representative fetuses sacrificed on GD 20 showing (A) control, (B) ginger, (C-K) GBP and (L) GBP + ginger groups.

Control group: Examining the double stained endoskeletal system of the 20-day-old control fetuses revealed that most of the calvaria bones are well ossified. In particular, at the level of the sternum, just six ossified parts (sternabrae) were visible. At the level of the ribs, only the portions articulating with the vertebrae were ossified, while those connected to the sternum were cartilaginous. The vertebral column was composed of number of vertebral segments, subdivided depending on their location and morphological characteristics into 7 cervical (Cv), 13 thoracic (Tv), 6 lumbar (Lv), 5 sacral (Sv) and caudal (Cav) vertebrae with variable number. Each vertebra was formed by a ventral corpus, two lateral arches and by the dorsal neural spine. Intervertebral discs and caudal vertebrae were mostly cartilaginous in nature and therefore stained blue. In some cases, however, the first two-three caudal vertebrae were partially ossified.

The anterior girdle was formed by the scapula and the clavicle. The scapula possessed a red-stained main body and a cartilaginous dorsal margin. Humerus, radius and ulna were partially ossified. Commencement of the ossification of metacarpal bones was noticed in a proximo-distal direction. The rest of the hand endoskeleton was still cartilage in nature.

The pelvic girdle was composed of a dorsal ilium, anterior pubis and posterior ischium. The ilium formed the typical iliac crest and articulated with the sacral vertebrae, fused together forming the sacral bone. The femur was articulated proximally with the pelvic girdle at the level of the acetabulum and distally with the tibia, fibula and the patella. Tibia and fibula articulated at their proximal ends but were separated along two-thirds of their length by a wide cleft and joined together distally. Femur, tibia, and fibula were only partially ossified (diaphysis), while the tarsal bones were in the shape of a cartilage draft and stained blue. Ossification was determined to begin in the diaphyses of the metatarsal bones. The proximal and medial phalanges were yet completely cartilaginous in nature. The endo-skeletal elements were well formed with no deformity and the ossification went on a proximo-distal direction (Fig.3 A).

Ginger group: Comparing the skeleton of the fetuses of the ginger group with that of the control group revealed no significant difference. The skull, vertebral column, sternum, ribs and limbs showed normal skeletal structure and had the same degree of ossification like that of the control group (Fig. 4 B).

GBP group: Fetuses maternally injected with GBP showed various skeletal abnormalities (Table 4; Fig. 3C-K). Malformation of calvaria bones constituted 40.9% of the endo-skeletal malformations. The most evident skull malformation was mandibular and maxillary hypoplasia (88.9% and 77.8%, respectively) (Fig. 3C, G & H) followed by delayed ossification of calvaria bones (Fig. 3C-F).

Vertebral column anomalies came in the third place among the induced skeletal malformations. About 63.6% of fetuses showed vertebral defects. These defects included kyphosis (50%, Fig. 3 G), abnormalities in the cervical arches, either non-fusion, absent parts or delayed ossification of the arches (42.9%, Fig. 3 D, E, F, H & I), delayed or absent sacral vertebral ossification (21.4%, Fig. 3 E, F & I). Malformations of the ribs were the second most common skeletal anomaly observed with incidence of 72.7% (Table 4). The most prominent costal malformation was costal separation (62.5%, Fig. 3 D, E, I & K). Other costal defects included wavy ribs (18.8%, Fig. 4 H) as well as costal curvature (18.8%, Fig. 3J). Delayed ossification of sternabrae was the only anomaly found in the sternum with incidence of 36.4% (Fig. 3 C & K).

Limb malformations were the most common skeletal anomaly observed with incidence of 77.3% in fetuses of GBP group (Table 4). The majority of limb malformation was higher in hind limb than the forelimb (100% and 23.5%, respectively). There were no evident malformations in the stylopodial or zeugopodial bones in the four groups, however, some fetuses had defects in humerus bone (Fig. 3K). Some fetuses showed decrease in the intensity of pelvic girdle as well as femur, tibia and fibula ossification pattern (Fig. 3 C&I). On the other hand, an evident delayed ossification was observed in autopod and appeared

predominantly in the metacarpal and metatarsal bones. Primary ossification centers in these bones and phalanges did not appear (Fig. 3 E & I).

GBP + Ginger Group: Administration of GBP followed by ginger caused an evident decrease in the skeletal malformations compared with the GBP group (Fig. 3L). Skull anomalies were reduced to 23.1% compared with the GBP group (Table 4).

Vertebral column deformities constituted 23.1% and varied between kyphosis (66.7%), cervical arches anomalies (33.3%) and thoracic and lumbar vertebral defects (33.3%). Malformations of the ribs were decreased to 23.1% and were in the form of separated ribs only (Fig. 3L). Only 15.4% of fetuses of this group had delayed ossification of sternabrae (Table 4). About 38.5% of fetuses of this group had limb malformations in the form of delayed ossification pattern, especially in the hind limb with higher incidence in the metatarsal and the phalangeal bones.

The present results are in agreement with the study of Afshar and Golalipour [6] who found that intraperitoneal injection of GBP from GD 1 to GD 10 caused delayed ossification in some bones of the skull, vertebral column, upper and lower limbs. Similar results were reported by Afshar et al. [8] where maternal intraperitoneal injection of GBP, during the first 15th days of pregnancy in mice resulted in mandibular hypoplasia, malformations of calvaria and vertebral column as well as delayed ossification, especially in the metacarpal and metatarsal bones. In their study on the effects of GBP on the endo-skeletal system of rat fetuses, Singh et al. [37] reported that in utero exposure of GBP induced similar skeletal anomalies in limbs and vertebrae of developing rat fetuses.

Exposure to higher doses of pregabalin in animals caused a reduction in ossification rate, but not dose dependent [3, 20]. Mice and rats exposed to valproic acid exhibited abnormal limbs including long bone reductions and abnormal or missing digits [25, 27, 28, 38]. Similarly, rat fetuses maternally subjected to intraperitoneal injection of phenytoin suffered from deteriorated ossification of skull bones and costal separation anomaly [2, 39].

Probably, significant effects observed when administering GBP on GD 6 to GD 15 can be related to the fact that, during days 9 and 10 of the rat embryogenesis, the mesoderm changes drastically. The formation of the intraembryonic mesoderm and its differentiation in regions is fundamental for the formation of cartilaginous and membranous structures that will be the foundations for the skeletal system [40].

Table 4: Effect of GBP on the endoskeleton of the 20-day-old fetuses in different groups (Percentage %).

Endo-skeletal abnormality	Groups			
	Control n= 13	**Ginger n= 11**	**GBP n=22**	**GBP + Ginger n= 13**
Skull Mandibular hypoplasia Maxillary hypoplasia Delayed ossification	**0%**	**0%**	**(9) 40.9%** (8) 88.9% (7) 77.8% (5) 55.6%	**(3) 23.1%** (1) 33.3% (1) 33.3% (3) 100%
Vertebral column Kyphosis Cervical vertebrae Thoracic and lumbar vertebrae Sacral vertebrae	**0%**	**0%**	**(14) 63.6%** (7) 50% (6) 42.9% (3) 21.4% (3) 21.4%	**(3) 23.1%** (2) 66.7% (1) 33.3% (1) 33.3% 0%
Ribs Separated curved Wavy	**0%**	**0%**	**(16) 72.7%** (10) 62.5% (3) 18.8% (3) 18.8%	**(3) 23.1%** (3) 100% 0% 0%
Sternum	**0%**	**0%**	**(8) 36.4%**	**(2) 15.4%**
Limb malformations Fore limb Hind limb	**0%**	**0%**	**(17) 77.3%** (4) 23.5% (17) 100%	**(5) 38.5%** (1) 20% (5) 100%

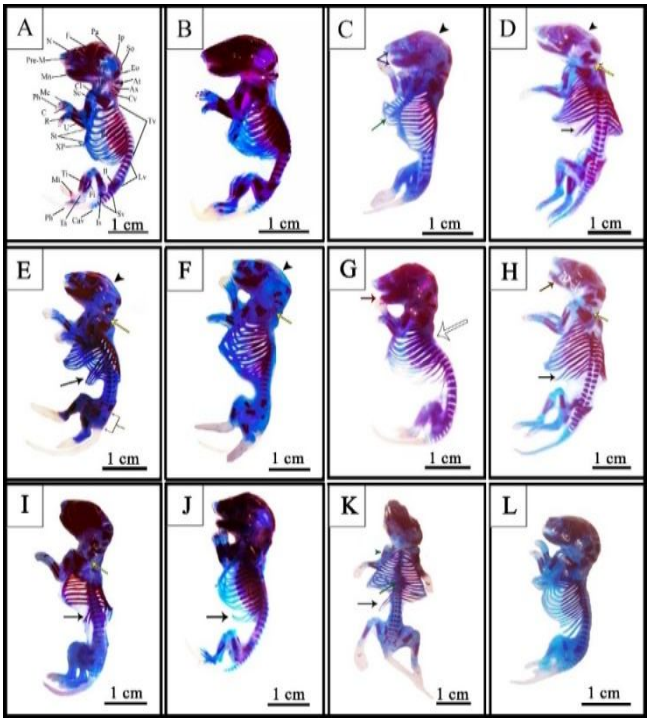

Figure 3: Photographs of the endo-skeletal system of double stained 20-day-old fetuses of control (A) ginger (B), GBP (C-K) and GBP + ginger (L) groups. All lateral view except K. Scale bar = 1 cm.

Along with different skeletal defects, the present study showed reduced length of the ossified parts of long bones of fore and hind limbs in the fetuses injected with GBP during the organogenesis period. The lengths of ossification parts of the long bones (humerus, radius, ulna, femur, tibia and fibula) in the four groups were demonstrated in Figure (4). The mean length of the ossified part of the fore limb bones was quite similar in both the control and ginger groups and showed the highest values with no significant difference in-between, humerus (0.37±0.011 cm and 0.36±0.007 cm), radius (0.27±0.013 cm and 0.27±0.010 cm), ulna (0.38±0.012 cm, 0.38±0.011 cm) for the control and ginger groups, respectively. Conversely, GBP group showed the lowest values for the lengths of ossification centers in the fore limb long bones with a highly significant difference compared with that of the control (0.29±0.005 cm, 0.18±0.005 cm and 0.21±0.005 cm, for humerus, radius and ulna, respectively). Co-administration of ginger after GBP injection resulted in no significant difference (in case of humerus) or low significant difference (in case of radius and ulna) between the control group, while it showed low (humerus) and high (radius and ulna) significant difference when compared with GBP group with overall moderate amelioration in the lengths of ossification centers in fore limb long bones (0.33±0.010 cm, 0.22±0.004 cm and 0.31±0.009 cm for the three bones).

Similar results were obtained when measuring the lengths of ossification centers in the long bones of hind limbs (femur, fibula and tibia). The control and ginger groups showed the highest lengths with insignificant difference between the two groups, femur (0.28±0.013 cm and 0.26±0.014 cm), fibula (0.32±0.015 cm and 0.31±0.010 cm) and tibia (0.37±0.011 cm and 0.35±0.016 cm) for the control and ginger groups, respectively. The lengths showed statistically high significant decrease in the GBP group when compared with the control group (0.19±0.004 cm, 0.15±0.009 cm and 0.20±0.004 cm for femur, fibula and tibia, respectively). The lengths of the ossification centers increased in the combined group and showed low significant difference compared with control and low (femur) and high (fibula and tibia) significant difference when compared with GBP group (0.23±0.013 cm, 0.28±0.006 cm and 0.29±0.005 cm for the three bones).

Administration of vigabatrin and valproic acid to 4-week old rat pups led to decrease in the mass, length and diameter of the isolated bones in comparison with the control rats [10]. Injection with phenobarbital also led to reduction in the length of the ulna, radius, tibia and phalanges as shown in the study of Yan et al. [41]. Numerous studies suggest that patients treated with AEDs may be at an increased risk for bone disease including changes in bone turnover, osteoporosis, alterations in bone quality, and fracture [42].

Yan et al. [41] assumed two possibilities to give rise to the shortened length of long bones. First is the small cartilage template induced by treatment as it has been proven that the formation of a proper cartilage model is a prerequisite for normal endochondral ossification [43]. The other possible explanation for the shorter long bones is that the process of mineralization was defective due to AED treatment which causes the blockage of calcium channels, and eventually leads to a loss in bone mineral density [44].

It has been emphasized that one of the main mechanisms of teratogenic action which affects ossification is oxidative stress [27]. Prenatal bone development is known to be sensitive to many environmental conditions, which may negatively impact fetal skeletal development [45]. Adverse events during the rapid development of the appendicular skeletal during mid-gestation can result in reduced neonatal size, weight and growth rates [46]. Changes in prenatal bone health and fetal osteogenesis have been positively correlated with excessively elevated reactive oxygen species (ROS) that may lead to improper skeletal formation [47, 48]. The embryonic and fetal development periods are believed to be extremely sensitive to high levels of ROS in part because effective free radical

scavenging systems are not yet fully developed [27]. Tung and Winn [49] experimentally found valproic acid exposure resulted in increased ROS levels and attributed developmental defects in head and neck region to these increased ROS levels.

Combined treatment of ginger and GBP in the present study resulted in increased mother weight gain and greatly diminished the deleterious effects of GBP. This finding agrees with many studies which prove the ability of ginger in regaining body weight loss. Gastric intubation of different doses of ginger from GD 6 to GD 15 didn't affect the maternal body weight gain in rats [13]. Several reports indicated the effectiveness of ginger in the protection against characteristic diabetic weight loss in rats as there was highly significant increase in their body weight when compared with that of the diabetic non-treated groups [50] and they attributed this effect to the hypoglycemic potential of ginger.

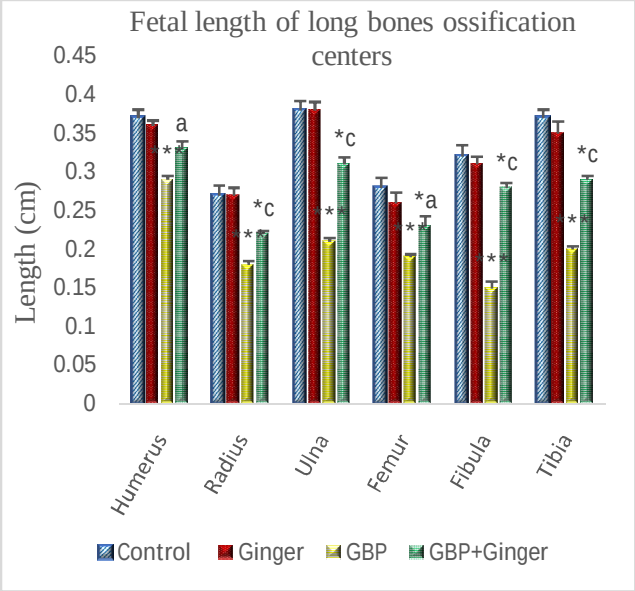

Figure 4: Graph showing the effects of maternal GBP administration on the lengths of ossification centers of the long bones in 20-day-old fetuses.

Administration of ginger also ameliorated the fetal toxic effect of GBP during pregnancy evidenced by reduced the morphological abnormalities and growth retardation of the fetuses. These data are in accordance with many other investigations on the effect of ginger during pregnancy. Weidner and Sigwart [13] reported that no significant differences were seen in fetal body weight, the external, the skeletal or visceral examination of the fetuses maternally treated with ginger in doses up to 1000 mg/kg body weight during mid gestation.

Related studies have indicated that antioxidant treatments can prevent or reduce growth retardation and/or the occurrence of malformations as a consequence of xenobiotics exposure during development [51]. In line with the use of antioxidant and folic acid therapy for reduction of the frequency and severity of AED-induced teratogenic effects, it was found that concomitant vitamin E administration significantly attenuated valproic acid and phenytoin induced decrease in crown-rump length, fetal weight and malformations [26, 28, 39]. The study of Abd El-Aziz et al. [36] showed that the co-administration of vitamin E with MeHg was also associated with an improvement in the fetal crown-rump length, body weight, head length, and biparietal diameter of the rat fetuses. The study of Abou-El-Naga [52] on pregnant mice injected with polycyclic hydrocarbons and curcumin from the GD 6 till the parturition showed improved neonatal growth retardation and reduced malformation rate.

All of the above mentioned ameliorative and protective effects of ginger could be attributed to its antioxidant properties. Several studies reported that ginger was demonstrated to be a strong antioxidant [53]. Its antioxidant activity has been attributed to its major active phenolic ingredients, especially, 6-gingerol, 8-gingerol, 10-gingerol, and 6-shogaol [54]. In addition, the administration of ginger has been shown to improve oxidative stress by decreasing lipid peroxidation and protein oxidation as free radical generating sources and elevating the levels of enzymes implicated in the antioxidant defense [55]. The increase in body weight gain following ginger treatment may be explained by the fact that ginger contains vitamin A which contributes to regulation of body growth and fat reserves. It also contains vitamin B_6 which can result in body weight gain by intensification of the protein synthesis [56].

In the current study, administration of ginger after GBP during the organogenesis phase of the rat embryonic development was associated with decrease and improvement of skeletal malformation and increase in ossification at various level in the whole endoskeleton together with improvements in the lengths of long bones of both fore- and hind-limbs in the fetuses towards the control figures. In support of the present results, some *in vivo* and *in vitro* studies showed the ability of ginger to increase bone ossification and reduce bone turnover. Ginger administration was found to cause improvements in bone microarchitectures and structure and decrease the osteoporotic changes in femur diaphysis and metaphysis caused by cadmium chloride and bilateral ovariectomy in the study of Mustafa et al. [57]. The authors explained this ameliorating effect of ginger and improvement in bone structure to be related to the antioxidant characters of ginger, as well as its bone protection against

oxidative damage induced by cadmium. The ameliorative effect of ginger observed in the present study pointed to its high antioxidant ability.

CONCLUSION: In the light of morphological and endo-skeletal outcome it can be concluded that administration of gabapentin during rat organogenesis was found to have adverse side effects on the gross morphology, morphometric parameters and endoskeleton. On the other hand, co-administration of ginger improved these adverse effects. Therefore, treatment with gabapentin should be restricted to the necessity and in such cases, ginger should be taken in parallel. The evident effect of ginger demonstrated in this study is possibly related to its antioxidant and free radical-scavenging properties. The outcome of the present study called for more investigation regarding the mechanism of action of ginger.

Abbreviations: AED, anti-epileptic drugs; At, atlas; Ax, axis; C, carpus; Cav, caudal vertebrae; Cl, clavicle; Cv, cervical vertebrae; Cor, cornified cells; Eo, exoccipital; Ep, epithelial cells; F, frontal; Fe, femur; Fi, fibula; GBP, gabapentin; GD, gestation day; Hu, humerus; Hy, hyoid; Il, Ilium; Ip, interparietal; Is, ischium; Le, leukocytes; Lv, lumbar vertebrae; Mc, metacarpus; Mn, mandible; Mt, metatarsus; N, nasal; Pa, parietal; Ph, phalanges; Pre-M, premaxilla; R, radius; Ri, ribs; S, squamosal; Sc, scapula; SEM, standard error of the mean; So, supraoccipital; St, sternum; Sv, sacral vertebrae; T, Tympanic; Ta, tarsus; Ti, tibia; Tv, thoracic vertebrae; U, ulna; XP, xiphoid process; Zy, zygomatic.

REFERENCES:

1. Barrett, C., & Richens, A. (2003) Epilepsy and pregnancy: report of an epilepsy research foundation workshop, *Epilepsy Res, 52*, 147-187.
2. Soysal, H., Unur, E., Düzler, A., Karaca, O. & Ekinci, N. (2011) Effects of intraperitoneal administration of the phenytoin on the skeletal system of rat fetus, *Seizure, 20*, 187-193.
3. Etemad, L., Afshar, M., Mohammadpour, A., Vahdati, N. & Moallem, S. (2013) Teratogenic effects of pregabalin in mice, *Iran J Basic Med Sci*, *16*, 1065-1070.
4. Nasar, M., Gupta, R. & Subhani, T. (2014) Teratogenicity of gabapentin in mice, *JMSCR, 2*, 3378-3385.
5. Krasowski, M. (2010) Therapeutic Drug Monitoring of the Newer Anti-Epilepsy Medications, *Pharmaceuticals, 3*, 1909-1935.
6. Afshar, M. & Golalipour, M. (2008) Teratogenic effects of gabapentin on neural tube and limb development in mice, *Neurosciences, 13*, 321-323.
7. Prakash, Prabhu, L., Rai, R., Pai, M., Yadav, S., Madhyastha, S., Goel, R., Singh, G. & Nasar, M. (2008) Teratogenic effects of the anticonvulsant gabapentin in mice, *Singapore Med J., 49*, 47-53.
8. Afshar, M., Hassanzadeh-Taheri, M., Moallem, S. A., Tamizi, A., & Golalipour, M. (2009) Teratogenic effects of gabapentin on the skeletal system of Balb/C mice fetuses, *Neurosciences, 14*, 239-244.
9. Mazziotti, G., Canalis, E. & Giustina, A. (2010) Drug-induced osteoporosis: mechanisms and clinical implications, *Am J Med, 123*, 877-884.
10. Nowińska, B., Folwarczna, J., Dusiło, A., Pytlik, M., Śliwiński, L., Cegieła, U., Kaczmarczyk-Sedlak, I., Pietryka, W., Hanke, T. & Trzeciak, H. (2012) Effects of vigabatrin on the skeletal system of young rats, *Acta Poloniae Pharmaceutica-Drug Research, 69*, 327-334.
11. Young, H., Luo, Y., Cheng, H. & Hsieh, W. (2005) Analgesic and anti-inflammatory activities of [6]- gingerol, *J Ethnopharmacol, 96*, 207-210.
12. Wilkinson, J. (2000) Effect of ginger tea on the fetal development of Sprague-Dawley rats, *Reprod Toxicol, 14*, 507-512.
13. Weidner, M. & Sigwart, K. (2001) Investigation of the teratogenic potential of a *Zingiber officinale* extract in the rat, *Reproductive Toxicology, 15*, 75-80.
14. Badawy, G., Atallah, M. & Sakr, S. (2019) The ameliorative role of ginger administration against gabapentin-induced hepatotoxicity in rat fetuses, *ejpmr, 6* (1), 622-631.
15. Badawy, G., Sakr, S., & El-Borm, H. (2018) The ameliorative role of curcumin on the morphological and skeletal malformations induced by betamethasone in rat fetuses, *Egypt J Exp Biol (Zoo), 14* (1): 53 – 68.
16. García-Peláez, I., Aguirre-Luna, O., Saavedra-Ontiveros, D. & Arteaga-Martínez, M. (2010) Teratogenic effect of ethylene glycol-methyl cellosolve mixture in rats. II. Craniofacial and limb abnormalities, *Int J Morphol, 28*, 1173-1180.
17. Bhaskar, N., Shahani, L. & Bhatnagar, P. (2014) Morphological and skeletal abnormalities induced by commercially available insecticides colonel-s-« and decis-« in the developing embryo of *Gallus domesticus*, *Int J Pharm Sci Rev Res, 26*, 140-148.
18. Meador, K., Penovich, P., Baker, G., Pennel, P., Bromfield, E., Pack, A., Liporace, J., Sam, M., Kalayjian, L., Thurman, D., Moore, E. & Loring D. (2009) Antiepileptic drug use in women of childbearing age, *Epilepsy Behav., 15*, 339-343.
19. Padmanabhan, R., Abdulrazzaq, Y., Bastaki, S. & Nurulain, M. (2010) Vigabatrin (VGB) adminis-

tered during late gestation lowers maternal folate concentration and causes pregnancy loss, fetal growth restriction and skeletal hypoplasia in the mouse, *Reprod Toxicol, 29*, 366-377.

20. Morse, D., Henck, J. & Bailey, S. (2016) Developmental toxicity studies with pregabalin in rats: significance of alterations in skull bone morphology, *Birth Def Res. (Part B), 107*, 94-107.

21. Marchi, N., Azoubel, R. & Tognola, W. (2001) Teratogenic effects of lamotrigine on rat fetal brain: a morphometric study, *Arq Neuropsiquiatr, 59*, 362-364.

22. Mohanty, C., Shah, N., Dhungel, S. & Das, B. (2011) Effect of lamotrigine on fetal rat brain, *People's J Sci Res, 4*, 5-7.

23. Padmanabhan, R., Abdulrazzaq, Y., Bastaki, S. & Shafiullah, M. (2008) Intrauterine growth restriction and skeletal variations in mouse fetuses induced by vigabatrin administered at preimplantation stages of development, *Congenit Anom, 48*, 29-39.

24. Fadel, R., Sequeira, R., Abu-Hijleh, M., Obeidat, M. & Salem, A. (2012) Effect of prenatal administration of therapeutic doses of topiramate on ossification of ribs and vertebrae in rat fetuses, *Rom J Morphol Embryol, 53*, 321–327.

25. Wlodarczyk, B., Palacios, A., George, T. & Finnell, R. (2012) Antiepileptic drugs and pregnancy outcomes, *Am J Med Genet A, 158*, 2071-2090.

26. Baran, O., Nergiz, Y. & Tuncer, M. (2006) The effects of valproic acid, vitamin E and folic acid on ribs of rat fetuses in the prenatal period, *Ann Anat., 188*, 117-125.

27. Ornoy, A. (2009) Valproic acid in pregnancy: how much are we endangering the embryo and fetus, *Reprod Toxicol, 28*, 1-10.

28. Abdel Salam, G. & Allam, O. (2015) The effects of valproic acid on the skeletal development and the ameliorative effects of combined use of folic acid and vitamin E in pregnant albino rats, *IJCDA, 1*, 70-78.

29. Farag, A., Elhalwagy, M. & Farid, H. (2010) Effect of ginger supplementation on developmental toxicity induced by fenitrothion insecticide and/or lead in albino rats, *Pest Biochem Physiol, 97*, 267-274.

30. Abdulrazzaq, Y., Bastaki, S. & Padmanabhan, R. (1997) Teratogenic effects of vigabatrin in mouse fetuses, *Teratology, 55*, 165-176.

31. Fadel, R., Dessouki, S., Mostafa, N. & Dawoud, A. (2003) Growth retardation and malformations induced in albino rat fetuses after treatment with diazepam, *Suez Canal Univ Med I, 6*, 5-19.

32. Akhtar, L., Khan, M. & Minhas, L. (2015) The effect of prenatal administration of valproic acid on the survivability and day of hatching of chick embryo, *J Pak Med Assoc, 65*, 175-178.

33. Montouris, G. (2003) Gabapentin exposure in human pregnancy: results from the Gabapentin Pregnancy Registry, *Epilepsy Behav, 4*, 310-317.

34. Favre, M., Barkat, T., LaMendola, D., Khazen, G.,Markram, H. & Markram, K. (2013) General developmental health in the VPA-rat model of autism, *Front Behav Neurosci, 7*, 88.

35. Saft, P., Toledo-Cardenas, R., Coria-Avila, G., Perez-Pouchoulen, M., Brug, B., Hernandez, M. & Manzo, J. (2014) Characterization of four types of tail abnormalities in rats treated prenatally with valproic acid, *Revista Neurobiología, 5*.

36. Abd El-Aziz, G., El-Fark, M. & Saleh, H. (2012) The prenatal toxic effect of methylmercury on the development of the appendicular skeleton of rat fetuses and the protective role of vitamin E, *Anat Rec., 295*, 939-949.

37. Singh, K., Gupta, K. & Singh, M. (2014) Effect of in utero exposure of gabapentin and valproic acid on skeletal anomalies in rat fetuses, *Natl Acad Sci Lett, 37*, 117-121.

38. Okada, A., Kurihara, H., Aoki, Y., Bialer, M. & Fujiwara, M. (2004) Amidic modification of valproic acid reduces skeletal teratogenicity in mice, *Birth Defects Res (B) Dev Birth Defects Res B, 71*, 47-53.

39. Essa, T., Gabr, A., Mohamed, A. & Meki, A. (2015) Protective effect of maternal vitamin E supplementation on phenytoin-induced teratogenicity in rat pups, *Anatomy, 9*, 1-12.

40. Pownall, M., Gustafsson, M. & Emerson, C. (2002) Myogenic regulatory factors and the specification of muscle progenitors in vertebrate embryos, *Ann Rev Cell Dev Biol, 18*, 747-783.

41. 41- Yan, Y., Cheng, X., Yang, R., Li, H., Chen, J., Ma, Z., Wang, G., Chuai, M. & Yang, X. (2016) Exposure to excess phenobarbital negatively influences the osteogenesis of chick embryos, *Front Pharmacol, 7*, 349.

42. Carbone, L., Johnson, K., Robbins, J., Larson, J., Curb, J., Watson, K., Gass, M. & Lacroix, A. (2010) Antiepileptic drug use, falls, fractures, and BMD in postmenopausal women: findings from the women's health initiative (WHI), *J Bone Miner Res, 25*, 873-881.

43. Knudson, C. & Knudson, W. (2001) Cartilage proteoglycans, *Semin Cell Dev Biol, 12*, 69-78.

44. Hernández-Díaz, S., & Levin, M. (2014) Alteration of bioelectrically controlled processes in the embryo: a teratogenic mechanism for anticonvulsants, *Reprod Toxicol, 47*, 111-114.

45. Liang, C., Oest, M., Jones, J. & Prater, M. (2009) Gestational high saturated fat diet alters C57BL/6

mouse perinatal skeletal formation, *Birth Defects Res B Dev, Reprod Toxicol*, *86*: 362-369.

46. Cooper, C., Westlake, S., Harvey, N., Javaid, K., Dennison, E. & Hanson, M. (2006) Review: developmental origins of osteoporotic fracture, *Osteoporos Int*, *17*, 337-347.

47. Fiore, G. & Capasso, A. (2008) Effects of vitamin E and C on placental oxidative stress: an in vitro evidence for the potential therapeutic or prophylactic treatment of preeclampsia, *Med Chem*, *4*, 526-530.

48. Scifres, C. & Nelson, D. (2009) Intrauterine growth restriction, human placental development and trophoblast cell death, *J Physiol.*, *587*, 3453-3458.

49. Tung, E. & Winn, L. (2011) Valproic acid increases formation of reactive oxygen species and induces apoptosis in postimplantation embryos: a role for oxidative stress in valproic acid-induced neural tube defects, *Mol Pharmacol*, *80*, 979-987.

50. Faried, M., Mansour, F., Zolfakar, A. & El-Kholy, W. (2013) Experimentally induced diabetic keratopathy in albino rats and the possible protective role of ginger, *J Am Sci*, *9*, 206-220.

51. Joya, X., Garcia-Algar, O., Salat-Battle, J., Pujades, C. & Vall, O. (2015) Birth defects, *Res A Clin Mol Teratol*, *103*, 163-177.

52. Abou-El-Naga, A. (2006) The role of curcumin to ameliorate the toxic effect of benzo (a) pyrene on some organs of adult and newborn mice, *Egypt J Exp Biol (Zoo)*, *2*, 61-72.

53. Saeid, J., Mohamed, A. & Al-Baddy, M. (2010) Effect of aqueous extract of ginger (*Zingiber officinale*) on blood biochemistry parameters of broiler, *Int J Poultry Sci*, *9*, 944-947.

54. Si, W., Chen, Y., Zhang, J., Chen, Z. & Chung, H. (2018) Antioxidant activities of ginger extract and its constituents toward lipids, *Food Chem*, *239*, 1117-1125.

55. Abd-Allah, O. & Sharaf El-Din, A. (2013) The possible protective effect of ginger against intestinal damage induced by methotrexate in rats, *Med J Cairo Univ*, *81*, 1073-1084.

56. Ali, B., Blunden, G., Tanira, M. & Nemmar, A. (2008) Some phytochemical, pharmacological and toxicological properties of ginger (*Zingiber officinale* Roscoe): a review of recent research, *Food Chem Toxicol*, *46*, 409-420.

57. Mustafa, M., Omayma, M., Mahmoud, O. & Hussein, H. (2013) Histological and morphometric effects of CdCl2 and ginger on osteoporosis induced by bilateral ovariectomy in adult albino rats, *Eur J Anat*, *17*, 102-114.