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RESEARCH ARTICLE

FOETUSES ABNORMALITIES CAUSED BYTEGRETOLDRUG ON THEIR PARENTS OF THE ALBINO RAT

El-Balshy R.M, Zowail M.E, Nafie E.H and El-Feky M.A.
Zoology Department, Faculty of Science, Benha University, Egypt.

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Abstract

The current study was performed to illustrate the harmful effects of different doses of Tegretol (CBZ) which has active ingredient (**Carbamazepine; CBZ**) on the prenatal foetuses of rat at 20th day of gestation. Tegretol (CBZ) noticeably reduced the body weight &length of foetuses in addition the percentage of dead foetuses increased; the early mortality of foetuscs which became resorbed besides late foetuses mortality (stillbirth) during the gestation phase. Tegretol (CBZ) administration caused delay of ossification of some bones and shortness of others .Some histo-pathological alterations were founded in livers, kidneys, and lungs of foetuses tissues which parentally administered with different doses of Tegretol (CBZ).

Introduction:-

Anticonvulsants were the props of seizure treatment till the 1990s, when the newer Anti-Epileptic Drugs (AEDs) with a good efficacy, lower toxic effects, more tolerability, and with no need for blood level monitoring were developed. A study of live-born infants in Danimark found that exposure to the newer-generation of AEDs in the first trimester of gestation was not associated to an increased risk in the major birth defects **[Molgaard et al. 2011].**

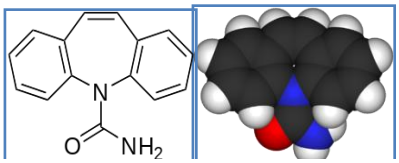
Carbamazepine ;(CBZ) became the drug of choice in primary generalized epilepsies and in the middle of 1990 was approved for treatment of partial seizures. (CBZ) also considered the drug of choice for the therapy of the trigeminal neuralgia [**Cheshire; 1997, Sindrup&Jensen; 2002**].

CBZ is significant protein bound to a high distribution, Most of this drug will stay bound to plasma protein due to the high protein binding property of it , it will enter the bloodstream from tissue stores. Approximately 72% of orally administered **(CBZ)** is absorbed, while 28% is unchanged and dis-charged through the faeces **[RxList; 2006].** After the absorption, **(CBZ)** is heavily metabolized by the liver: only about 1% of the dose leaves the body in an un altered form the metabolites of this drug make entero-hepatic cycling and finally are excreted with urine .The elimination half-life time of **(CBZ)** is dose-dependent but is usually in the range of 25–65 h after-administration **[Wishartet al.; 2006].** Its important urine metabolites are 10, 11-dihydro-10, 11-expoxy carbamazepine **(CBZ)**-epoxide and trans-10, 11-dihy-dro-10, 11-dihydroxy carbamazepine **(CBZ)**-diol **[Reith et al.; 2000]** Importantly, the former is pharmaceutically as active as its parent drug.CBZ do oxidation via CYP 3A4 and to a less extent CYP 2C8 to carbamazepine-lO, ll-epoxide, which is the active metabolite that is thought to make toxic effects.

CBZ toxicity can be divided into 3 levels:(1) disorientation and ataxia at levels of 11–15 mg/L;(2) aggression and hallucinations with levels of 15–25 mg/L; and(3) seizures and coma with levels above 25 mg/L [**Al khaliliY et**

Corresponding Author: -El-Balshy R.M
Address**:-**Zoology Department, Faculty of Science, Benha University, Egypt.

al.;2021]. Due to wide spectrum side effects of (CBZ) the differentiation of hyper sensitivity syndrome will be difficult, but it has been linked to the syndrome affecting the liver, lungs and kidneys in severe cases [**Schrieret al ;2004, Eguchiet al;2011, Elshamaet al** ;**2013**]. It has been found that using of antiepileptic (CBZ) during the gestational period may cause risk, associated with maternal toxicity hepatic and nephritic toxicity and chromosomal aberrations in pregnant rats, and intrauterine growth retardation which was appear as low body weight ,length reduction and malformations **[El-Gaafarawi&Abouel-Magd; 2015**].



Chemical structure of Carbamazepine (CBZ)

Chemically, **(CBZ)** is a neutral, lipid-soluble compound that can pass the blood–brain barrier and other membranes in the body easily **[Hansen et al.; 1996, Matalonet al.; 2002,].**

In many comparative clinical studies, it has been confirmed that; phenobarbital, phenytoin, primidone, or valproic acid **[Fritz et al.;1976]** antiepileptic administeration during pregnancy is associated with a 2 to 3 fold increase in the rate of congenital anomalies, mainly congenital heart defects; cleft lip;the cleft palate; anomalies of the urinary tract; and the syndromes of dys-morphism and developmental disability **[Matalonet al.;2002].** Here is a concerning of the teratogenic effects of **(CBZ)**, but most investigators believe that malformations associated with the maternal use of **(CBZ)** can be divided into major malformations as cranio-facialdefects, heart defects & neural tube defects and minor anomalies as growth retardation, developmental delay& hyperplasia of the nails or distal phalanges **[Matalonet al;2002, Artamaetal;2005].**

Severe **(CBZ)**-associated hepato-toxicity takes two shapes: a hyper sensitive reaction in the form of granulo-matous hepatitis that appear as fever and abnormal liver functions test ,and an acute hepatitis and hepato- cellular necrosis with fever, rashes , hepatitis and lymphoid enopathy simulating biliary tract infection **[Driefuset al;1987]**that may resultfrom direct drug toxicity . Hepatotoxic reactions of **(CBZ)** usually happen within 3-4 weeks after the administration of therapy and are independent of serum **(CBZ)** levels.

Carbamazepine (CBZ) use is accompanied with variable toxicities histopathological changes include,degeneration of renal tubules, inflammatory infiltration, congestion of blood vessels and the glomeruli have been fragmented and atrophied. Similarly[**El-Gaafarawi& Aboul-maged**;**2015**] reported that kidney of pregnant rat administered with daily dose 3.6 mg/100g of **(CBZ)** on day 20th of gestation manifested ,dilatation of proximal tubules , dilatation of Bowman's capsules dilatation of proximal tubules and shrinkage of some glomeruli and some were lobulated, while high daily dose10.8mg/100g of **carbamazepine (CBZ)** at day 20th of gestation showed, degeneration of distal-convoluted tubules and numerous hemorrhagic areas ,dilatation of Bowman's spaces. Also this finding supportive to other authors **[Eguchiet al; 2011]** who revealed thatgranulomatous interstitial nephritis (GIN) associated with **(CBZ)**-induced hypersensitivity syndrome**. [Elshamaetal.;2013]** reported that, Kidney of mice administered with **(CBZ)** showed complete distortion of glomeruli,atrophy of glomeruli, degeneration of renal tubules **. [Tatsunorietal;2010]** reported that **(CBZ)** concentration and its metabolites are higher in the brain, lung, liver, and kidney than of its concentration in the blood. so that the drug is retained in organ tissues and so this fact explains the toxic effect of carbamazepine in kidney which represented as histopathological changes.

(CBZ)-induced interstitial pneumonitis is a rare but well described complication **[Archibald et al; 2006**] in adults.The thoracic CT findings and the clinical improvement after **(CBZ)** administration, suggest that **carbamazepine (CBZ)**-induced interstitial pneumonitis. Despite the gradual improvement after its withdrawal patterns of lung disease take months to years after an initial **(CBZ)** exposing mainly bronchiolitis obliterans - organizing druginduced lupus and pneumonia**[Milesi-Lecat et al; 1997, Banka et al; 2002, Archibald et al; 2006**]. Clinical follow-up along with thoracic CT imaging will reveal any residual lung damage.

Method:-

For the present study Tegretol which has the active ingredient (Carbamazepine; CBZ) was obtained from El-ezaby pharmacy Egypt,Novartis Pharmaceuticals Corporation with a chemical formula $\underline{C}_{15}\underline{H}_{12}\underline{N}_{2}\underline{O}$and was dissolved in saline prior to oral administration The doses of the drug was calculated according to the value of LD50 for oral administration in rats described by Novartis Pharmaceuticals Corporation 2014&Safty Data Sheet.The oral LD_{50}in rats(3850-4025).The recentdosesare(98mg/kg; $1/8LD_{50}$)in G1 ,(16mg/kg; $1/50LD_{50}$)inG2,(8mg/kg;$1/100LD_{50}$)in G3 ,and (5mg/kg;$1/150LD_{50}$)in G4. The experimental animals were divided into 5 groups; Control group (C): was given saline orally. Group one (G1): administered with (1/8 LD50; 98 mg/kg) orally). Group two (G2): was given (1/50 of LD50; 16 mg/kg). Group three (G3): was administered with (1/100 of LD50; 8 mg/kg). Group four (G4): received (1/150 ofLD50; 5 mg/kg). Males and females were administered with tegretol (CBZ) for seven days before mating, after that they do copulation with each other.Pregnant females continued receiving Tegretol (CBZ) at 20^{th} day of gestation.

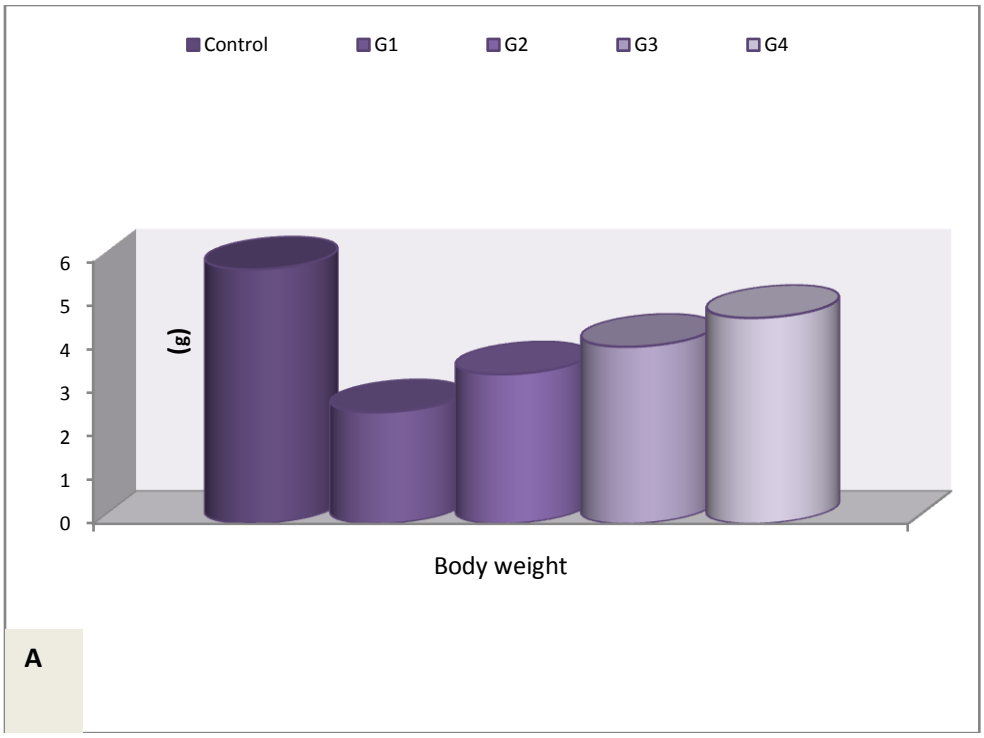
We brought Virgin males and females of age 8-12 weeks and About 200g weight were supplied **by TheodorBilharzResearchInstitute** (Giza,Egypt) .Rats were adapted for 7 days with :12-hour of light& dark cycle. All experiments introduced in this study were in compliance withthe international guidelines.Adult males were kept with adult females overnight. In the next morning pregnancy was exacted by the presence of vaginal plug **[McClain and Becker, 1975]**. Each pregnant female was kept in a separate cage.Males and females were administered withTegretol (CBZ) for a week (7 days) before mating, then pregnant females sustain administration with Tegretol (CBZ). At the 20^{th}day of her gestation, the uteri were obtained by caesarean sections. For each pregnant female, the number of foetuses bulge in each horn, living / dead foetuses. Early and late resorptions were detected due to their size. **For morphological studies**: Weights of control and Tegretol (CBZ) administered pregnant rat and the percentages of survival of their foetuses in all groups were recorded at the end of the experiment (20^{th} day of gestation). The percentage of body weight gain was calculated. Weights of gravid uterine and carcass were also recorded.The pregnant female of all groups were sacrificed on the morning of day 20^{th} of gestation. The lengths and the weights of the 20^{th} day foetuses were recorded.

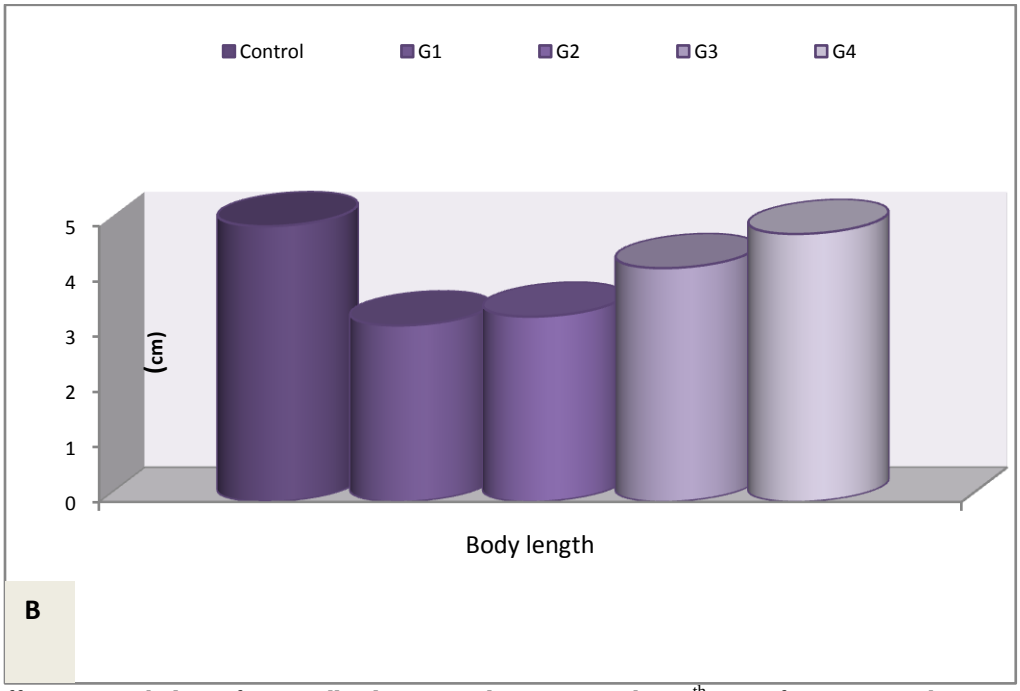
The mortality ratewas also recorded and then the morphological examination for any external malformations has been noted. **For the skeletal malformationsstudying:** Newly picked foetuses were fixed in 95% ethyl alcohol and handled to double staining; (alizarin red &alcian blue). Next to thecompleteelimination of the soft tissues by 2% (KOH), the specimens were put in increasing grades of glycerine, till bones disclosed [**Falkeholm*etal*. 2001**]. The bony tissues were take red color and the cartilaginous were stained with blue color. The transparent specimens were saved in 100% glycerin for examination and photography**.For histological examination:** Liver, kidney and lung tissues were obtained from the 20^{th}dayfoetusesof the control and parentally administered with Tegretol (CBZ) -groups. Samples were set in 10% formal- saline for a day, then treated due to [**Banchroft et al., 1996**], dehydrated then cleared and inserted in paraffin wax, after that 5µm sections were cut and stained with hematoxylin& eosin then examined**.For Statistical analysis:** The statistical analysis was carried out using one-way ANOVA using SPSS, ver. 25**(IBM Corp. Released 2013)**. Data was treated as a complete randomization design according to[**Steel *et al.*; (1997)]**. The significance level was set at < 0.05

Results:-

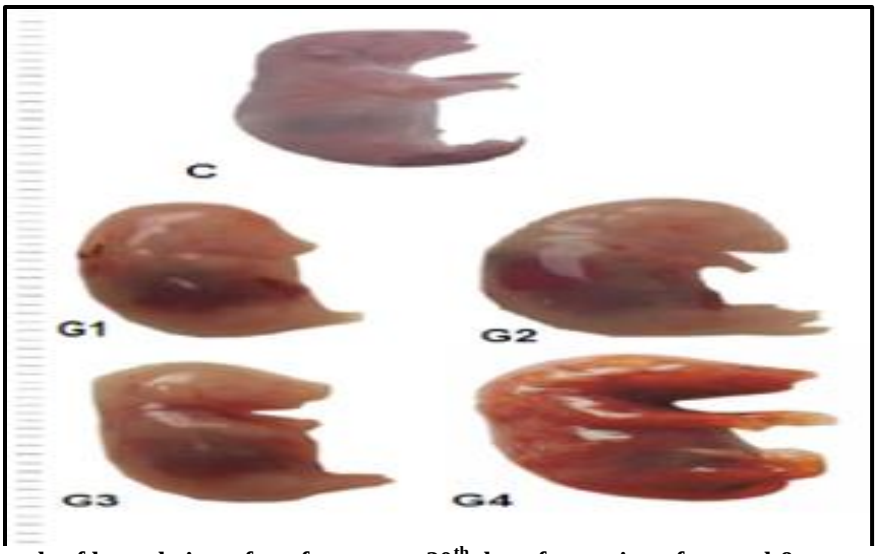
A) External morphological studies:

In the present study the external morphological investigation of the 20^{th} day foetuses showed that, Tegretol (CBZ) induces a growth retardation to the foetuses; decrease in thefoetal body weight & length (Fig.1). A considerable reduction in the body weight of foetuses:(*2.55±0.48*e), (*3.42±0.29*d) (*4.05±0.21c*)and(*4.71±0.50b*) werenoticed in G1, G2, G3 and G4 correspondingly as compared to the control (*5.82±0.66a*).plus a significant decrease in foetusesbody length in the administeredgroups;as it was *(3.20±0.16d) at G1;(3.36±0.15d) at G2 ;(4.24±0.44c) at G3 and (4.86±0.21b) cm at G4*respectively whencompared withcontrol group*(5.00±0.48a)*Examining the whole morphology of parentally administered foetuses with Tegretol (CBZ) indicated the occurrence of pattern of congenital malformations like; convexity body, bizarre tail and founding of superficial haematomas in different areas of the whole body (Fig.2).





(Fig.1):- Effect onMorphologyof parentallyadministered Foetuses at the 20th Day of Gestationwith Various Doses of Tegretol (CBZ) when compared to Control Group (C). (A): Shows; the Mean variance in the Body Weight. (B): Shows; the Mean alteration in the Body Length.as (G1): 98mg/Kg; (G2): 16mg/Kg; (G3): 8mg/Kg & (G4): 5mg/Kg



(Fig.2):- Photographsof lateral view of rat foetuses at 20th day of gestation of control & parentally Tegretol (CBZ)-administered groups showing; control (C) and Tegretol (CBZ)-administered groups. (G1): 98 mg/kg 1/8 LD50, (G2): 16 mg/kg 1/50 LD50, (G3): 8 mg/kg 1/100 LD50 and (G4): 5 mg/kg 1/150 LD50.

B) Foetusesmortality:

Tegretol (CBZ)administeration enhanced the ratio of dead foetuses and decreased the quantity of alivefoetuses as showed in table (1). A considerable reduction in the whole amount of foetuses in allTegretol (CBZ) administered groups; was (37.50 %) at G1 ;(27.08 %) at G2; (12.50%) at G3 and (10.40 %) at G4. On other hand; the percentage of change of a live foetuses in comparison with the live foetuses of the control was (75.00 %; 68.70%; 31.30 % and 25%) in case of (G1, G2, G3 &G4) respectively.

The External morphology of uteri was taken from control and Tegretol (CBZ) administered group's revealednoticeable variations (Figs.3). TheControl group showed a natural large uterus enclosing large embryos. Also thedied at the end of gestation stage andcontinued as stillbirth, the early died of foetusesthatbecame resorbed at the remnant of gestation period. Group (1) showeda verysmall uterus with some resorbed embryos. Group (2) produced less embryosenclosed in a small uterus and some resorbed. (G3& G4) which took lower doses of Tegretol (CBZ) exposed larger uteri contain in more embryos than (G1) & (G2) (Fig.3).

Fig.3:-

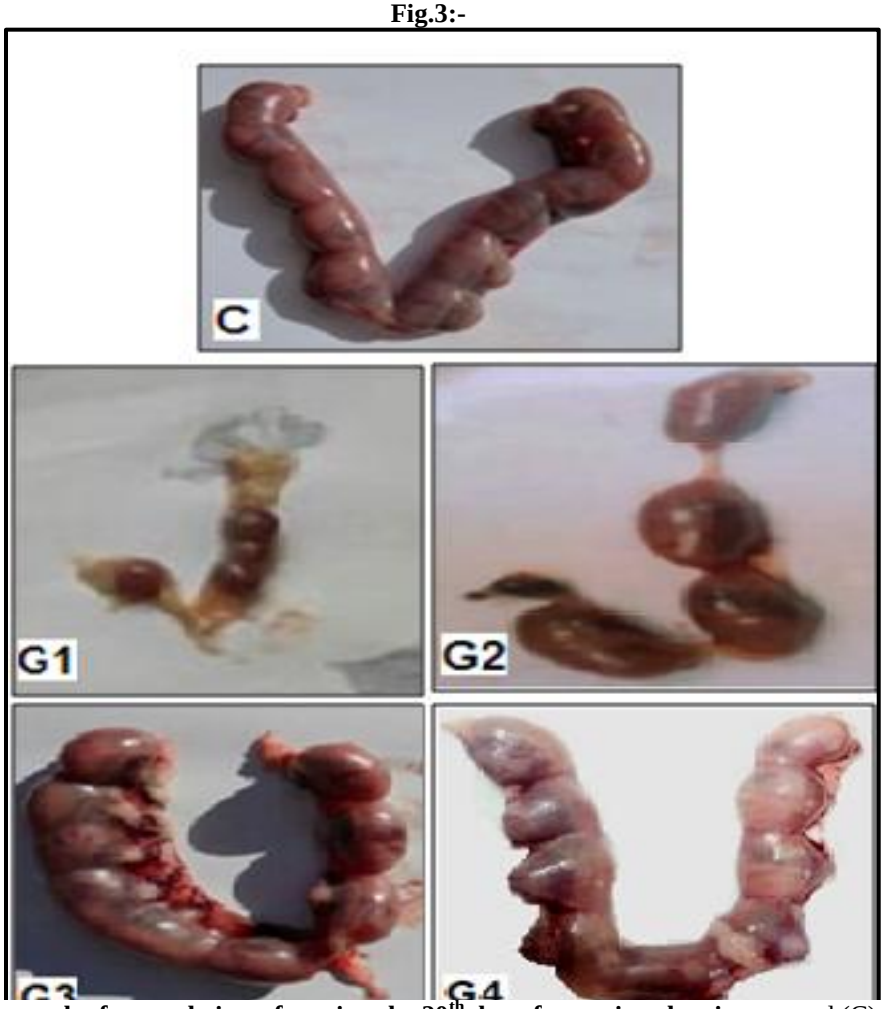


Fig. (3):- Photograph of ventral view of uteri at the 20th day of gestation showing;control (C) and Tegretol (CBZ)-administered groups. (G1): 98 mg/kg 1/8 LD50, (G2): 16 mg/kg 1/50 LD50, (G3): 8 mg/kg 1/100 LD50 and (G4): 5 mg/kg 1/150 LD50.

Table 1:-

Group		Total no. of Fetuses	Mean SD	Changes from control (%)	Changes from alive (%)
C	No of foetuses Alive Dead	48 48 -	9.6	0.00	0.00
G1	No of foetuses Alive Dead	30 12 18	6 2.4 3.6	37.50	75.00
G2	No of foetuses Alive Dead	35 15 20	7 3 4	27.08	68.70
G3	No of foetuses Alive Dead	42 33 9	8.4 6.6 1.8	12.50	31.30
G4	No of foetuses Alive Dead	43 36 7	8.6 7.2 1.4	10.40	25.00

Table (1):-The percent of change of total foetuses and a live foetuses at the 20th day of gestation in case of Tegretol (CBZ) administered groups compared with the control:
Values presented in this table are the mean of five pregnant rats.
*.The mean difference is significant at the 0.05 level Vs Control, p< 0.05.

C) Endoskeletonobservations:

1) Axial Skeleton:

In general, the skeletal system of albino rats consist of 2 main parts; axial and appendicular skeleton.The axial skeleton includes bones of the skull,the vertebral column,the ribs &the sternum.Although,the appendicular skeleton consists of pectoralgirdle & fore limbs and pelvic girdle & hind limbs (Figs 4-10 C). Osteological malformation of 20th day foetusesrevealed that administration with different doses of Tegretol (CBZ)to the parents caused several objectionable effects ranging between moderate and severe malformations (Figs 4-10G1-G4).

Ossification of the skull:

Examining the skull at 20thday of gestation of the control foetuses of albino rat showed; complete ossification of its components (Figs 4, 5, & 6 C). There is malformation in the skull of foetuses parentally administered with different doses of Tegretol (CBZ). As, incomplete ossification of the nasal, frontal, supra-occipital, parietal, inter-parietal, zygomatic process of squamosal, tympanic bulla, squamosal, periotic, supra-occipital, palatine, pterygoid&ethmoid bones (Figs 5 & 6 G1-G4). In relation to the change of length and volume of the skull of the parentally administered foetuses of which focusedon severe abnormalities, this lead to a clear shortage in the length and volume of the skull in comparing with the control (Figs 5& 6 G1-G4) The bones of the lower jaw displayed moderate ossification in foetuses of all Tegretol (CBZ) administered groups; a gradual lack in ossification as shown from figure 6, while there is a slight ossification ofdentary of the administered group, (Figs 4 & 6 G1-G4).

The vertebralColumn, Sternebrae and Ribs:

The vertebral column of control foetuses displayed well ossified vertebrae which are symbolized by 7 cervical; 12 thoracic; 7 lumber; 4 sacral & 10 caudal vertebrae (Fig.7C). Examination of the vertebral column of parentally Tegretol (CBZ)administeredfoetuses with different doses considerably decreased ossification of atlas and axis in foetusesoptainedfromthe four groups (Figs 4&7G1:G4). Most of the foetusesof theadministered groups showed more / less ossification in their cervical, thoracic, and lumbar, sacral and caudal (Figs 4&7G1:G4). Foetuses of control group have 13 pair of ribs, each pair consists of bony vertebral region and cartilaginous sternal one. The sterna area of the ribs, except the last 3 pairs, communicate with the sternum (Figs. 4 & 8C). No changes in the number and in ossification ofribs in the wholeTegretol (CBZ) groups in compared to control (Figs. 4 & 8G1: G4).

Sternum of thecontrolfoetuses consists of 6 rod-like parts of ossified sternebraearranged in a straight line, the last one is theXiphi-sternum (Fig. 8C). The sternebrae of the parentally administered foetuses with different doses of Tegretol (CBZ) showedmore / less ossificationsthan of the control group (Fig.8G1:G4)

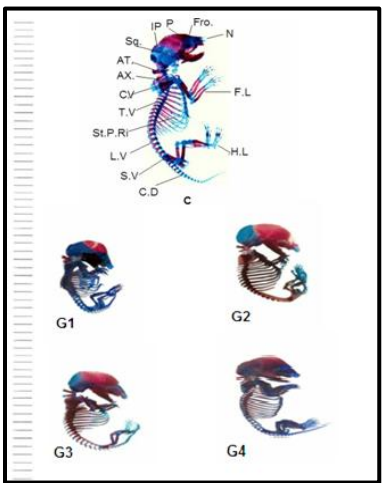


Fig.4

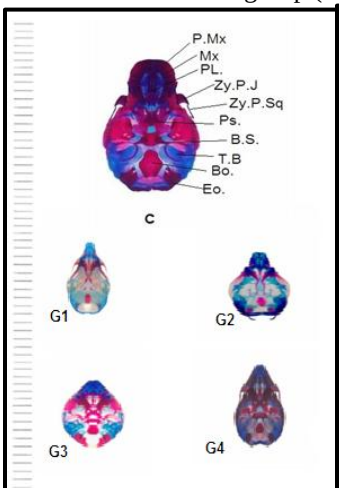


Fig.5

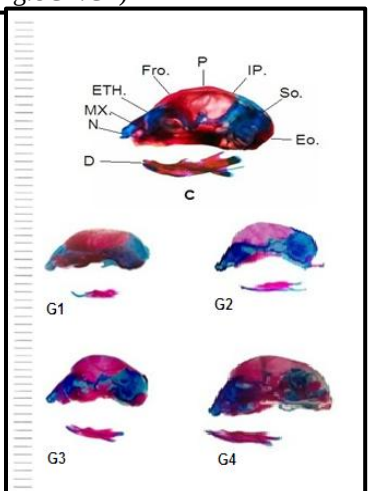


Fig.6

Fig. 4:- Photograph of Lateral View of the Skeletal System of rat Foetuses at the 20th Day of Gestation showing; Control (C) and Parentally Tegretol-administeredgroups. G1: (1/8 LD50; 98mg/Kg), G2: (1/50 LD50; 16mg/Kg), G3: (1/100 LD50; 8mg/kg) &G4 (1/150 LD50; 5mg/kg).

Abbreviations: Axis(Ax); Caudal vertebrae(C. D); Cervical vertebrae (C. V); Fore limb(F. L);Frontal (Fro); Hind limb (H. L);Interparietal(IP); Lumbar vertebrae (L. V); Nasal (N); Parietal (P); Ribs (R); Sacral vertebrae (S. V) ; Squamosal (Sq); Sternal portion of ribs (St. p. Ri); Thoracic vertebral(T.V).

Fig. (5): Photographs of a ventral view of the skull of foetuses at 20th of gestation showing; Control (C) and four Tegretol- administered groups. G1: (1/8 LD50; 98mg/Kg), G2: (1/50 LD50; 16mg/Kg), G3: (1/100 LD50; 8mg/kg) &G4 (1/150 LD50; 5mg/kg).

Abbreviations: Basioccipital (BO), Basisphenoid (B.S), Exooccipital (EO), Maxilla (MX), Palatine (PL), Premaxilla (P.MX), Presphenoid (P.S), Tympanic bulla (T.B), Zygomatic process of jugal (Zy.P.J), Zygomatic process of squamosal (Zy.P.Sq).

Fig (6): Photographs of a lateral view of the skull of mice foetuses at 20th day of gestation showing; Control (C) and four Tegretol-administered groups. G1: (1/8 LD50; 98mg/Kg), G2: (1/50 LD50; 16mg/Kg), G3: (1/100 LD50; 8mg/kg) &G4 (1/150 LD50; 5mg/kg)

Abbreviations: Dentery (D), Ethmoid (ETH), Exooccipital (EO), Frontal (Fr), Interparietal (IP), Maxilla (MX), Nasal (N), Parietal (P), Supraoccipita (SO).

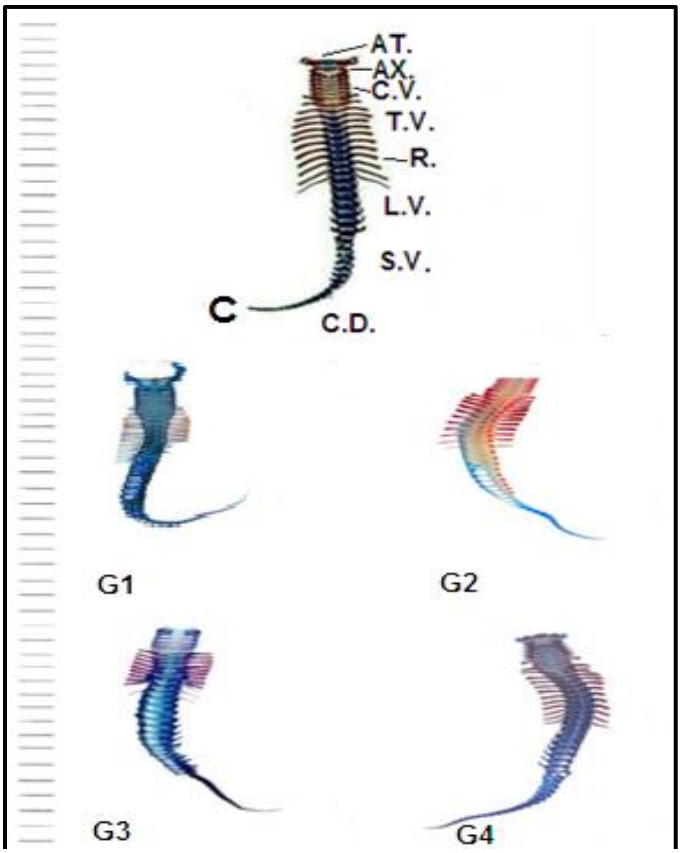


Fig (7):- Photograph of a ventral view of the vertebral column of rat foetuses at the 20th day of gestation showing;control (C) and parentally Tegretol-administered groups. G1: (1/8 LD50; 98mg/Kg), G2: (1/50 LD50; 16mg/Kg), G3: (1/100 LD50; 8mg/kg) &G4 (1/150 LD50; 5mg/kg)

Abbreviations: Atlas (AT), Axis (AX), Caudal vertebrate (C.D), Cervical vertebrae (C.V), Lumbar vertebrae (L.V), Ribs (R), Sacral vertebrae (S.V), Thoracic vertebrae (T.V.)

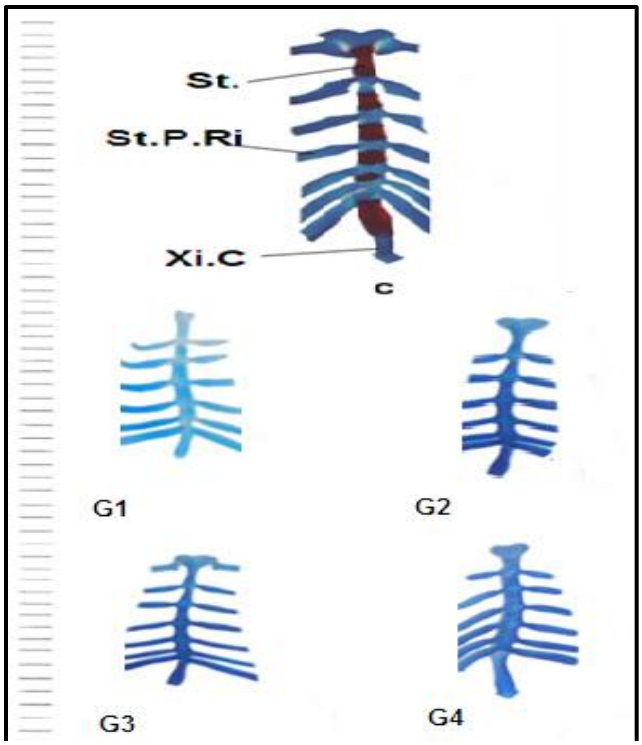


Fig (8):- Photographs Photograph of a ventral view of the sternum of rat foetuses at the 20th day of gestation showing; control (C) and parentallyTegretol-administeredgroups G1: (1/8 LD50; 98mg/Kg), G2: (1/50 LD50; 16mg/Kg), G3: (1/100 LD50; 8mg/kg) &G4 (1/150 LD50; 5mg/kg).

Abbreviations: Sternal portion of ribs (ST. P. R), Sternebrae (St), Xiphoid cartilage (XI. C).

2) Appendicular Skeleton:

The Pectoral Girdle and Fore Limb:

The pectoral girdle of controlfoetuses at the 20th day of gestation comprises a well ossified scapula and clavicle stained with alizarin red and cartilaginous supra-scapula stained with alcian blue. The fore limb of control foetuses at the 20thday of gestation consists of ossified humerus, radius, ulna and phalanges with five digits, as well as cartilaginous carpalia and meta-carpalia (Fig. 9C)

The components of the pectoral girdle and fore limb of foetuses obtained from parentally administered with different doses of Tegretol are manifested by decrease in size, length and the level of ossification in reference to the control group (Fig.9G1-G4) .At thehigh doses of Tegretolthe pectoral girdle and fore limbs of all foetuses of "G1" and "G2" showed acute lack of ossification (Fig.9G1&G2).. Moreover, the phalanges and the meta-carpalia of fore limbs G1&G2 appeared arched in shape.

The Pelvic Girdle and Hind Limb:

The pelvic girdle of control foetuses is consisted of 3 well ossified bones which are (ilium, ischium and pubis). The pubic symphysis remains cartilaginous in nature. The hind limb of controlfoetuses consists of femur, tibia, fibula, and tarsals, metatarsals and phalanges Fig. (10 C).

Examining of the pelvic girdle and hind limbs of Tegretol- parentally administered foetuses showed significant shortening & thinning and also displayed incomplete & lack of ossifications of their components in all administered groups when comparedwith control. In addition, deformation was noticed in the cartilage drafts of the metacarpal bones and phalanges infoetuses from administered group's comparedto the control Fig.10 G1-G4).

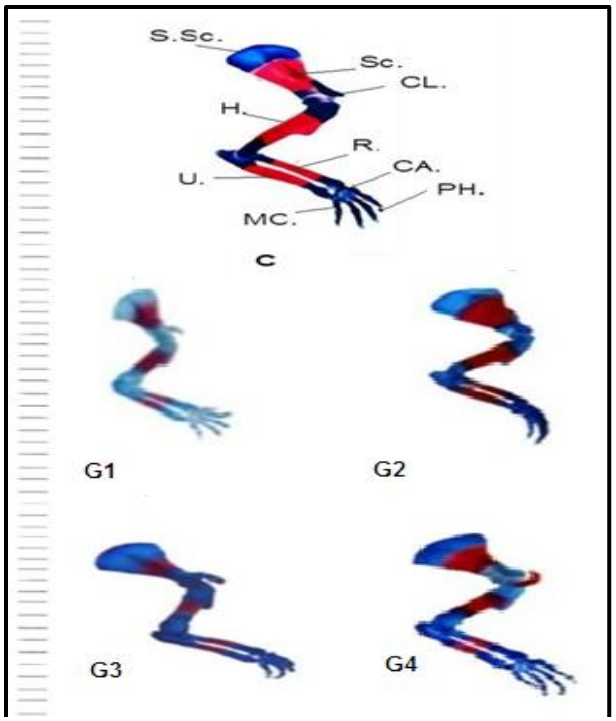


Fig. (9) Photograph of a lateral view of the pectoral girdle and fore limb of rat foetuses at the 20th day of gestation Showing;control (C) and parentally Tegretol (CBZ)- administered groups .(G1): 98 mg/kg 1/8 LD50, (G2): 16 mg/kg 1/50 LD50, (G3): 8 mg/kg 1/100 LD50 and (G4): 5 mg/kg 1/150 LD50

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

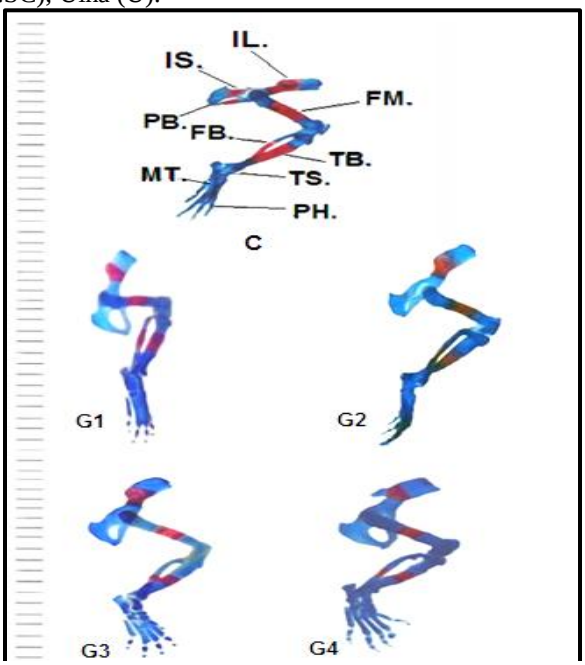


Fig. (10):- Photographs of a lateral view of the pelvic girdle and hind limb of ratfoetuses at 20th day of gestation showing;Control (C) and theTegretol-administered groups G1: (1/8 LD50;98mg/Kg), G2: (1/50 LD50;16mg/Kg), G3: (1/100 LD50;8mg/kg)&G4 (1/150 LD50;5mg/kg).

Abbreviations : Femur (FM), Fibula (FB), Ilium (IL), Ischium (IS), Metatarsalia (MT), Phalanges (PH), Pubis (PB), Tarsalia (TS), Tibia (TB).

D) Histological examination:

1) The liver: the liver from control group at 20th day of gestation cleared that the hepatic lobule is the unitofanatomical structure of the liver, the connective tissue is dividing the liver into many lobules which structure is incomplete. The hepatic lobulewas consisted of an epithelial parenchyma and a community of blood sinusoid. The parenchyma was made up of: hepatic cells organized in irregular and branching inter-connected plates & the hepatic cordswhich arranged in a radial direction around the central vein which has a circular outline in general, being limited by a thin cover of endothelial cells (Fig. 11; C). The hepatic strands are intervenedby many of blood sinusoids. Two types of cells could be seen on the wall of blood sinusoid, defined by endothelial flat cells, with flattened nuclei and scanty cytoplasm, and Kupffer cells having dark nuclei and branching pseudopodial process and a clear vacuolar appearance (Fig. 11; C). Hepatocytes are large in size, and are polygonal in shape with granular cytoplasm. The nuclei are large and located in the center. Groups of cells which darkly stained are of frequent occurrence in the hepatic tissue they reveals the different types of blood forming cells named, erythroblasts, lymphocytes and mega-karyocytes (Fig. 11; C).

The liver sections obtained fromTegretol- parentally administered foetusesof rats at different doses as; G1 was given (1/8 LD50; 98 mg/kg); G2 (1/50 LD50; 16 mg/kg); G3 (1/100 LD50; 8 mg/kg) and G4 (1/150 LD50; 5 mg/kg) showed clear degenerative alterations (Fig.11 G1-G4). In general, the hepatic cells were pale stained and between the noticeable signs of damage encountered is the general destruction of the normal structural organization of the hepatic lobules. This also inferred the similarity the characteristic plate /cord-like arrangement of the normal liver cells. The hepatocyte injury was clearly manifested by noticeable cytoplasmic vacuolization. Such vacuolization may be attributed to either lipolytic (fatty) or hydropic degeneration (Fig.11 G1 & G3). The central&portal veins were much swollen. They were inflamed with dull blood with clear marks of hemorrhage (Fig.11G&G3). Some cells showed histological features of necrosis; the nucleus of each necrotic cell displayedpyknotic, being smaller, condensed, and deeply stained with haemato-xylin (Fig.11G1-G4). Additionally, most of these nuclei are showingclear signs of nuclear pyknosis, karyorrhexis, pluskaryolysis (Fig.11G3). In some samples, severe necrosis of liver was noticedaround the central vein accompanied with acomplete loss of the cellular outlines & of nuclear staining (Fig.11G1&G3). The endothelial lining of the central vein was completely eroded causing perceptible hemorrhage in the whole tissue (Fig.11 G1& G3). As well congestion blood vessels appears in the central& portal veins (Fig. 11 G2 &G4). The blood sinusoids are dilated, with eroded lining and hypertrophied Kupffer cells were pushed into the internal lumina (Fig.11G2&G4).

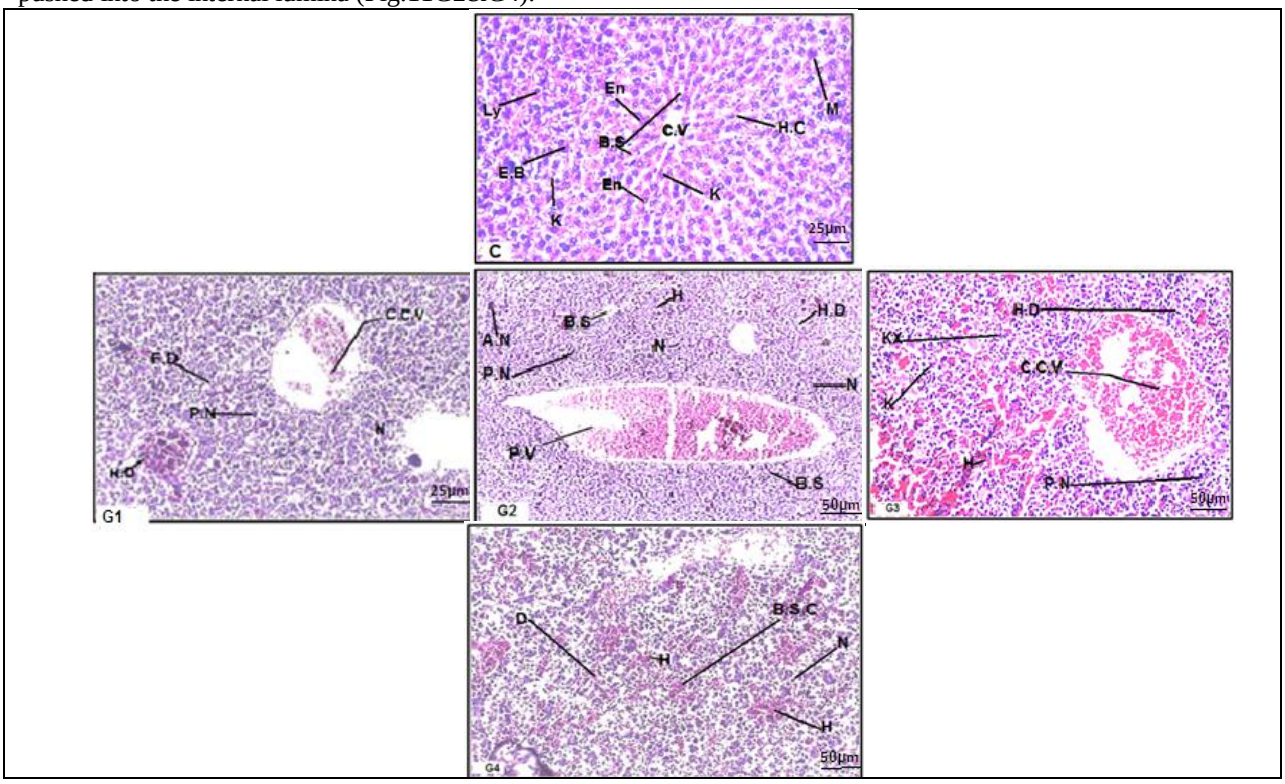
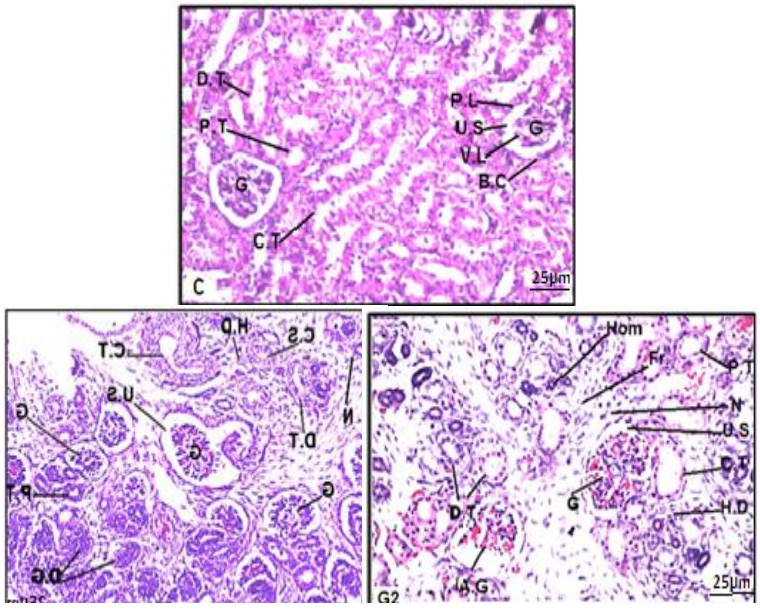


Fig (11):- Photomicrograph of a Section in the Liver of rat Foetus at the 20th Day of Gestation Stained with H&E. [C] Control Group Showing ;Blood Sinusoids (B.S), Central Vein (C.V), Erythroblast (E.B), Endothelial Cells (En), Hepatocytes (H.C), Kupffer Cells (K), Lymphocytes (Ly) and Megakaryocyte (M).**Group One [G1]: Parentally administeredFoetus with 98 Mg/Kg of TegretolShowing;**central vein congestion (C.C.V), fatty degeneration (F.D),hydropic degeneration (H.D), necrosis (N) and pyknotic nucleus (P.N).**Group Two [G2]: Parentally administeredFoetus with 16Mg/Kg of Tegretol Showing;**apoptotic nuclei (A.N), distended blood sinusoids (B.S), hemorrhage (H), hydropic degeneration (H.D), necrosis (N) , pyknotic nucleus (P.N)and congested portal vein (P.V). **Group Three [G3]: Parentally administeredfoetus with 8 Mg/KgTegretol Showing;** Congestion in Central Vein (C.C.V), Hemorrhage (H), Hydropic Degeneration (H.D), Karyolysis (K), Kayorrhexis (Kx) and Pyknotized Nuclei (P.N). **Group Four [G4]: Parentally administered foetus with 5 Mg/Kg of Tegretol Showing;** Blood sinusoid congestion(B.S.C), Cell Degeneration(D), Hemorrhage (H) and necrosis (N).

The kidneys:

The kidneys of control foutuses consists of closely crowded uriniferous tubules, each one consists of initial un-branched nephron & a branched collecting tubule. The nephron initiates as a renal corpuscle which consists of a double walled Bowman's capsule partially surrounding the glomerulus .Bowman's capsule consists of 2 simple epithelial layers; an inner / visceral layer covering the glomerulus, & an outer / parietal layer. The two layers are attached with each other at the rim of the opening to the renal corpuscle (Fig. (12C).The tubular part of the nephron is composed of 2 portions; a proximal convoluted tubule, & a distal convoluted tubule. The proximal tubule is lined by a single layer of low columnar or pyramidal cells with round nuclei and acidophilic granular cytoplasm. The distal tubule is lined by cuboidal cells which are lower and narrower than those of the proximal tubule (Fig. 12C). The collecting tubules are lined with simple cuboidal epithelium and have arelatively, wide lumena.

The kidneys of the parentally administered Tegretolfoetuses have caused clear changes in comparewith the control. The degenerative symptoms include glomerulo-nephritis of the Malpighian corpuscles and cloudy swelling, hydropic degeneration and necrosis of the epithelial cells that lining the convoluted [proximal & distal] and the collecting tubules of the cortex (Fig.12G1- G4). In many cases, some glomeruli lost its normal circular shape and became irregular and decreases in size (Fig.12G2, G3&G4). In the other cases, the parietal and visceral layers of Bowman's capsule may fuse together (Figs.12G1&G3); these two cases are known as glomerulonephritis. A large number of the epithelial cells that lining the convoluted [proximal & distal] and collecting tubules became swollen and project inwards forming a conical shaped appearance and the lamina of the tubules became small.This case is identified as cloudy swelling (Fig.12G1&G2). Cells of the renal tubules showed necrosis; these cells detach in the lumen of the tubules, their nuclei are pyknotized or completely disappeared (Fig.12G1-G4). Other cells are fused together in homologous mass (Fig. 12G1- G4). Few cells undergo fragmentation, a case which is known as tubulo-rrhexis (Fig.12G1-G4).



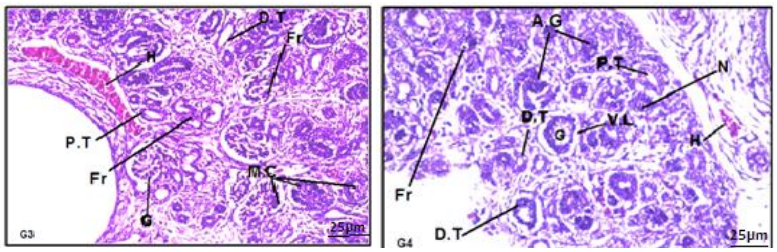
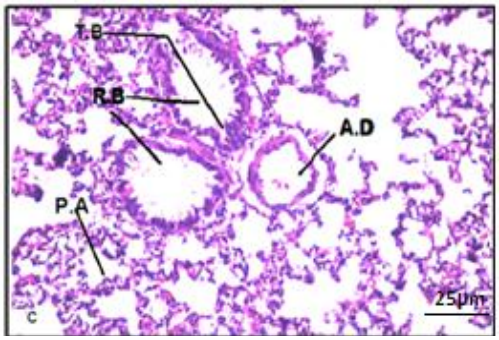


Fig. (12):- Photomicrograph of a Transverse Section in the Kidney of rat Foetus at the 20th Day of Gestation Stained with H&E. C; Control Group Showing ; normal structure of kidney: Bowman's capsule (B.C), collecting tubule (C.T), distal tubules (D.T), glomerulus (G), parietal layer (P.L), proximal tubules (P.T), urinary space (U.S) and visceral layer (V.L).**Group One [G1]: Parentally administered Foetus with 98 Mg/Kg of Tegretol Showing**; ;cloudy swelling (C.S), collecting tubule (C.T), degeneration of distal tubule cells (D.T), deteriorated glomerulus (G), hydropic degeneration (H.D), necrosis (N), degeneration of proximal tubule cells (P.T), dilated urinary space (U.S) and vacuolated cytoplasm (V). **Group Two [G2]: Parentally administered Foetus with 16Mg/Kg of Tegretol Showing;** atrophic glomerulus (A.G), collecting tubule (C.T), degeneration of distal tubules (D.T), fragmentation of cells (Fr.), deteriorated glomerulus (G), homologous layer (Hom.), hydropic degeneration (H.D), necrosis (N), degeneration of proximal tubules (P.T). and dilated urinary space (U.S).**Group Three [G3]: Parentally administered with 8 Mg/Kg Showing**; degeneration of distal tubules (D.T), fragmentation of cells (Fr.), deteriorated glomerulus (G),hemorrhage (H),degeneration of the Malpighian corpuscles (M.C) and degeneration of proximal tubules (P.T). **Group Four [G4]: Parentally administered with 5 Mg/Kg of Tegretol Showing** ; atrophic glomerulus (A.G), degeneration of renal tubule cells (D.T), fragmentation of cells (Fr.), deteriorated glomerulus (G),hemorrhage (H), necrosis (N) ,degeneration of proximal tubules (P.T) and Veciral layer(V.L).

Lungs:

Pulmonaryalveolusis a cup- shaped structure cell. The mouth of each alveolus opens inside the lumen of the respiratory bronchiole, an alveolar duct or sac. An Inter-alveolar septum is found among neighbouringalveoli. This septum is consisted of thelining cells of closest alveoli and the structures introduced between the alveoli. Capillaries inhabit a major part of the septum, & theyare shared by the lining cells of the next alveoli. Terminal bronchioles are outlined by asimple columnar ciliated & non-ciliated epithelialcells. Among the alveoli, the wall of the respiratory bronchiole is crinkled by cuboidal epithelium. The walls of the alveolar ducts are composed of pulmonary sacs and alveoli and only a few squamous to low cuboidal epithelial cells prevailing. The alveolar ducts conductsto a blindalveolar sacs that are thin-walled structures which are popper with pulmonary alveoli (Fig. 13C).

The tissue of the lungs of the administered foetuses is confused; parental administration of Tegretol (G1) and (G2) with (1/8 LD50; 98 mg/kg) and (1/50 LD50; 16 mg/kg), respectively revealed hemorrhage, pyknosis of nuclei, dilation and severe congestion of blood vessels (Fig.13G1&G2). In addition to, pulmonary oedema appears faintly stained eosinophilic fluid in the terminal and respiratory bronchioles and alveolar ducts in the above mentioned two groups (Fig13.G1&G2).Lung sections from (G3) and (G4) which were administered with the lower doses of Tegretol (1/100 LD50; 8 mg/kg) and (1/150 LD50; 5 mg/kg) respectively, displayed less histopathological alteration in foetallung than (G1) and (G2). Group (3) showed necrosis in the respiratory structures of the lung with pyknosis of nuclei in the lining epithelium, dilation and congestion of blood vessel (Fig.13G3&G4).



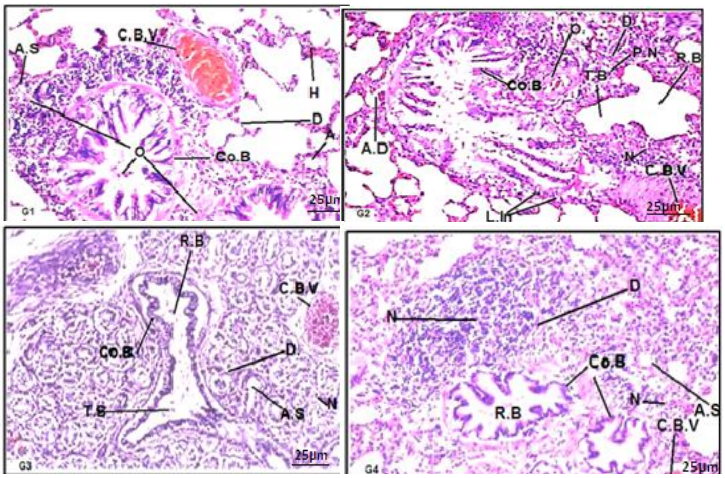


Fig. 13):- Photomicrograph of a Transverse Section in the lung of rat Foetus at the 20th Day of Gestation Stained with H&E. C; Control Group Showing ; normal alveolar duct (A.D), pulmonary alveoli (P.A), respiratory bronchiole (R.B) and terminal bronchioles (T.B).**Group One [G1]: Parentally administered Foetus with 98 Mg/Kg of Tegretol Showing;** degeneration of alveolar duct (A.D), destructed alveolar sacs (A.S), congestion of blood vessels (C.B.V) , Corrugation of the bronchiolar wall (Co. B.), degeneration of the respiratory bronchioles (D), hemorrhage (H) and oedema (O.) **.Group Two [G2]: Parentally administered Foetus with 16Mg/Kg of TegretolShowing;**alveolar duct (A.D), severe congestion of blood vessel (C.B.V), Corrugation of the bronchiolar wall(Co. B.), degeneration of respiratory bronchioles (D),Lymphatic Infiltration(L.In), necrosis (N) of air spaces, oedema (O.) and pyknosis of nuclei (P.N) in the lining epithelium of both respiratory bronchiole (R.B) and terminal bronchioles (T.B).**Group Three [G3]: Parentally administeredFoetus with 8 Mg/Kg of TegretolShowing;**alveolar sacs (A.S), congestion of blood vessel (C.B.V), Corrugation of the bronchiolar wall(Co. B.), degeneration (D) , necrosis (N) , respiratory bronchioles (R.B) and terminal broncholies (T.B).**Group Four [G4]: Parentally administered Foetus with 5 Mg/Kg of Tegretol Showing** ; alveolar sacs (A.S), congestion of blood vessel (C.B.V), corrugation of the bronchiolar wall (Co.B), degeneration (D) of the respiratory bronchioles (R.B) and necrosis (N).

Discussion:-

Anti-epileptic drugs (AEDs) have some clear teratogenic effects, and there are theoretical &evidence based risks for obstetrical complications ,weak off springs , congenital malformations and cognitive effect on child in their late life**[Gedzelman& Meador ; 2012]**.AEDs as Carbamazepine (CBZ) is used in the treatment of epilepsy, neuropathic pain and bipolar disorder**. Ornoy&Cohen ;1996** said that ;although carbamazepine has been available for many years, it is only during the last few years that its pharmacokinetics have been investigated. Carbamazepine (CBZ); which is the active ingredient of Tegretol has wide variety of side effects have been attributed to its use ,including haematological , hepatic , cardiac,pulmonary and renal disorders [**Salzmanet.al**.;**1997**]. **Artamaetal; 2005** registered that exposure to Carbamazepine (CBZ) during pregnancy may bring postnatal severe growth retardation and may induce "a Carbamazepine Syndrome" characterized by facial dysmorphic features and soft developmental disability.

It is necessary to point up that the main intracellular antioxidant element, glutathione, only reaches its maximum production at the end of gestation **[Wells et al. ;2005, Gracyet al. ;2009&Calabrese et al. ;2010].**As (CBZ) transferred by the placenta accumulated in foetal tissue, making intra-uterine growth restriction , **[Luef ;2009, Bath &Scharfman ;2013, Nieet al. ;2016].**Both of epilepsy and AEDs, like (CBZ), have been associated to poor pregnancy, & neonatal outcomes **[Li etal. ;2019].**As our main objective was to obtain the developmental malformations of Tegretol; with the active ingredient CBZ in the rat'sfoetuses more than studying the safe side of the drug doses on animals. We had to choose high doses for our study which could be okay for the rat dams and also cause malformations in their foetuses. Our results show that oral dose of Tegretol (CBZ) to rat had a notable effect on the weight of the foetuses compared with that of control. The weight of the foetuses that were antenatally exposed to Tegretol (CBZ) was reduced when compared to control foetuses. As well, most of these foetuses were not alive when they were delivered.

This study helped us to regulate the way of administration and dose of Tegretol (CBZ) which can be tolerated by rats and also sured that Tegretol (CBZ) causes low weight in foetuses which parentally exposed to the drug. This result can be useful to clinicians to explain the decreased birth weight of infants of mothers were on Tegretol (CBZ) administration during pregnancy.The present study focused on the effect of Tegretol (CBZ) on the morphology, mortality , skeletal system and the histology of the liver ,kidneys & lung of thefoetuses at 20^{th} day of gestation.Tegretol (CBZ administrated orally with The doses (98mg/kg; 1/8 LD50),(16mg/kg; 1/50 LD50), (8mg/kg ;1/100 LD50),(5mg/kg;1/150 LD50) to the male and female for 7 days before mating then the pregnant female continuous to administrated with tegretol at 20^{th} day of gestation.Our study also showed that administration of Tegretol (CBZ) significantly affected morphology of foetuses with regard to length and weight. This result is in harmony with **[Luef; 2009, Bath &Scharfman; 2013, Nieet al.; 2016]**as they said that CBZ is transferred by the placenta accumulated in tissue, making intrauterine growth restriction. There is a distinctive correlation between CBZ usage during pregnancy& developmental defects and " (CBZ)-syndrome " due to epidemiological and experimental examinations **[Matalonetal. ; 2002, Afsharetal.;2010&Wlodarczyketal. ;2012**]. As Clinical signs showed an increase in free (CBZ) blood levels, also facilitated its passage through the placenta **[Johnson et al. ; 2014].**

In the present study, low body weight and the crown-rump length, high resorption and malformations were founded in all Tegretol (CBZ) administered groups compared to the control . Tegretol (CBZ)-high dose groups caused malformation that should be listed as severe growth retardation. Malformation that has been described as severe growth retardation are with the doses (98mg/kg; 1/8 LD50) ,(16mg/kg; 1/50 LD50) . This result was ensured by **[Suchestonetal.;1986, DiavCitrinet al. ;2001, ElGaafarawi&Abouel-Magd ;2015]**.who appreciated that toxic effects of the high dose of (CBZ) induced intrauterine growth retardation that was marked by the low body weight and length , they also admitted that reduction in length may be according to the delayed ossification of the long bones and there was a association between the reduced long bone ossification centers and the foetal body weight reduction.Exposing to Tegretol (CBZ) during the gestational period induced a decrease in foetal crown-rump length & body weight, indicating the alteration in intrauterine foetal growth is the cause of foetal& prenatal mortality [**Brodsky&Christou; 2004, Frøenetal.; 2004 , Flenadyetal. ;2011**]. The intrauterine growth in mammals is the phase of proliferation and differentiation of active cells. This period is very sensitive to chemical abuse. An amount of effects, ranging from growth delay to high organ anomalies and functional deficiency, have been reported as an effect of chemical exposure to embryos **[Walker etal.;2000].** The intrauterine growth retardation can be related to oxidative stress-make adverse intrauterine surroundings to the embryo-foetal growth **[Krishna&Bhalera;2011, Sharma;2016].**This is dependable to the present results for intrauterine and growth retardation in all the 4 doses of Tegretol (CBZ). In addition, **[Artamaet.al. ;2005]** gave an account that prenatal exposure to (CBZ) may make postnatal beginning of serious growth retardation, **[Jones etal.; 1989]** showed a pattern of malformations same to those founded in hydentoinsyndrome.CBZ is metabolized into epoxides. Appearance of CBZ and CBZ- 10,11epoxide reveals theleast change during the pregnancy and there is confirmation of increased free drug. Free CBZ is more readily crossing the placental barrier & is extra clinically significant for foetal exposure Infants with a decreased hydroxylase enzyme activity would be at a high risk to this pattern of malformations if the mother used (CBZ) during pregnancy **[Buehler etal.;1990 , Nulmanet.al.;1997, Blackburn ;2013 & Johnson etal.; 2014].**

Suchestonet al.;1986 revealed that toxic effects of high CBZ dose caused intrauterine growth retardation which was cleared by decrease in bodyweight and length reduction of foetuses and they also noted that decreased foetus length may be due to retarded long bone ossification and there was a relevance between decreased long bone ossification centers and foetus low weight.In studies of developmental toxicity, a decrease in foetal body weight is in usual considered as a result of prenatal growth retardation **[Chahoud&Paumgartten; 2005].**

In the current work, Tegretolalso increased the number/percentage of deadand resorbed fetuses in the administered groups as compared to control group. These results are in agreement with **[Waters etal. 1994]** found 3 times more abnormal outcomes between offspring of epileptic women who treated with anticonvulsants, when compared to control. These included the neonatal death and congenital anomalies. The higher rate of poor outcome was found in pregnant mothers treated with phenol-barbitone .Neonatal of women with epilepsy who were CBZ administered during pregnancy have high rates of congenital malformations particularly, cardiovascular and urinary tract anomalies, a cleft palate & a cognitive decline. In different studies performed previously, they confirmed a growth-restricting effect of CBZ **[Hiilesmaaetal. 1981, Jones etal. 1989].** There are many studies to conclude that CBZ is a

teratogen **[Jones etal.; 1989, Shepard et al.; 2002].** However**, [Mastroiacovoet al.; 1988, Gaily et al. 1989]** found no effect of (CBZ) on the foetal growth.

The effect of Tegretol (CBZ); in our study on foetalgrowth, organogenesis, and intrauterine growth retardation and malformations inskeleton in compare with foetuses of control group. These alterations were more severe in groups administered with high doses of Tegretol than in groups administered with the low doses. As regard, the axial skeleton, our results revealed that Tegretolconsiderably decreasedlength and size of the skull, reduced the ossification of skull components and bones of the lower jaw. Also, Tegretolclearly decreased ossification of atlas, axis, and cervical, sacral and caudal vertebrae. Additionally , Tegretol diminished the length and size of ribs, sternebrae and xiphoid that also showed loss of ossification. The undesirable effects of Tegretol on the appendicular skeleton includes reduction in length and thickness of humerus, radius and ulna which also showed low ossification. Same , Tegretol significantly declined the length and thickness of the hind limb elements, delayed ossifications of the components of pelvic girdle and pubic bone, deterioratedthe cartilage drafts of the metacarpal bones and phalanges.This result agreed with**[Suchestonetal. ;1986, El-Gaafarawi&Abouel-Magd ; 2015]** .

On the other hand Kimmel et al. ;1987 said; even though it was noticed a decrease in foetal weight and length according to prenatal exposure of (CBZ), there was no significant changes were founded during the inspection of skeletal anomalies. A decrease in foetal body weight may be related to changes in the ossification development, especially the later ossification centers as sternebrae of the prenatal sternum, anterior and posterior phalangeal section of the paws, and growth delay .Even though skeletal examination is extra confused by the changes between mouse and rat, the time of laparotomy corresponds to the phase of the highest osteogenesis. The ossification of the rodent foetal bones happens rapidly throughout the last 48 hours of gestation. According to these aspects, the skeletal system in rodent foetuses is not mature when being evaluated. So, the decreased level of ossification of some bones in foetal skeleton does not essentially mean that a growth retardation has happened. Even if bone ossification progresses with gestational age, destruction of calcification of a particular bone is not essentially secondary to a time-consuming development of the skeleton in total. The delay in pre-natal ossification can be transient and does not last in the post-natal stage [**Carney &Kimmel; 2007**]. It could also be believed that the delay in ossification related to the age at which the foetuses were collected **[Daston&Seed; 2007].** Additionally , delaying in ossification usually occurs in involvement to other effects on the foetal growth, mainly reduced foetal body weight, as resulted in this study. Generally, delayed ossification and decreased foetal body weight are symptomatics of wide spread effect on the foetalmaturity**[Daston&Seed; 2007].**

In our study, cesarean sections have been performed on the 20^{th} day of gestation and the skeletal abnormalities noticed infoetuses of the Tegretol (CBZ)-administered females should be deduced with caution, and also studies are essential to complete this assessment. Even so, excessive oxidative stress is highly associated to embryo toxicity and embryonic stage are highly having a tendency to tissue and organ damage. Additionally**[Wells etal. ;2005, Gracyetal. ;2009, Calabrese etal.; 2010]**said ; gestation is a physiological condition with high metabolic demand and , in the happening of abnormalities in pregnancy, oxidative imbalance may happen, and extra free radicals can promote injury to the foetus, which has an antioxidant defense system. It is necessary to point up that the main intracellular antioxidant component; glutathione only reaches its maximum production at the end of the gestation period. The current study experimented the force of Tegretol (CBZ), low and high doses, on foetal growth and organogenesis (from $1^{st}$to the 20^{th}gestational days), it was found that all doses caused foetal mortality and growth restriction , malformation in the component of skeletal system according to the dose.

The presented study had also revealed that parentally administration of Tegretolseverely harms liver and disrupted the structure of hepatic parenchyma. Also, resulted in extensive hemorrhage accompanied with the presence of hemosiderin pigment within the hepatic tissue, dilation and congestion in the central vein, induced lymphatic &hydropic degeneration and pyknosis of nuclei which was associated with necrosis of hepatocytes. This result is in agreement with **[Rang etal;2012]** has no effect on organs color of treated animals except for the liver which changed from dark brown to the light brown according to dose indicating that CBZ is hepatotoxic.

[Björnsson& Olsson, 2005; Björnsson;2008] said; Serious (CBZ)-associated hepatotoxicity become visible in the following two shapes : (1) a hypersensitive reaction as the form of granulomatous hepatitis that presents with fever & abnormal liver functions (2) acute hepatitis and hepatocellular necrosis with inflammation. The last form is the suitable form for our study. Additionally, the founding of a specific auto-antibody directed against a human liver

microsomal-protein in a patient who had severe hepatotoxicity with (CBZ) has been reported **[Pirmohamedet al.; 1992].**Upon these reports, the liver injury associated with (CBZ) is thought to have an immune-allergic basis. Dislike another antiepileptic drug, oxcarbazepine (OXC), is not seem to be involved with idiosyncratic hepato-toxicity in humans, and there have been only some cases reports showing mild or temporary liver injury **[Ahmed and Siddiqi; 2006, Björnsson; 2008].**

In histopathological studies, significant hepatic necrosis & loss of hepato- cytes, especially around the central vein, were found in the foetuses administered (CBZ) maternally, and these effects were same to acetaminophen -induced liver injury **[Antoineet al; 2009].**Because Cy-ps are generally expressed around the central vein of the liver, this observation suggested that Cy-ps may be occupied in (CBZ)-induced liver injury.

On other hand Tegretol (CBZ) in our study not only caused hepatotoxicity but also caused damage for the foetus's kidney .The current study displayed that Tegretol produced adverse injuries in renal tissues of parentally administeredfoetuses. The histopathological examination revealed severe deterioration in glomerulus, hemorrhage, congestion in the inter-tubular capillaries, pyknosis of nuclei, glomerulus atrophy, cytoplasm vacuolation, hydropic degeneration, cloudy swelling, fragmentation, homology and necrosis of renal tubule cells. The foetuses kidneys were looked affected in all animals parentally administered with Tegretol (CBZ) drug; Another effect is separation of epithelial lining cells from the basement membrane of renal tubules and the collecting ducts may be result of the toxicity of drug and this result is in harmony with **[Van de Water etal.;1994]** that found separation of the epithelial lining from the basement membrane due to tubules epithelial lining cells death & this may resulting from damage of the cells significance to loss cytoskeleton and destruction of plasma membrane. This result prove the findings of previous studies **[Robbins etal.;1994]** that stated the congestion is a result of acute inflammation causing blood flow alters within the vessels and arise relax and extend of blood vessels then cause accumulation of blood inside vessels. In addition**,[Garveretal.;1976]** said that the congestion caused by the concentration of the drug in critical sits of cells, which causes higher levels of red blood serum than simple plasma drug levels do .

El-Gaafarawi&Aboel-Magd; 2015regarded depletion in tissue total glycogen Content of rat kidney. degeneration of renal tubules, inflammatory infiltration, congestion of blood vessels and the glomeruli have been fragmented and atrophied . The major histopathological defects in kidneys appeared as following: spreading out of Bowman's space which characterized by enlargement of Bowman's space. Same as **[Frazier etal.;2012]** that recommended the enlargement of Bowman's space may be according to the hydrostatic pressure which increased inside Bowman's capsule owing to glomerular hyper-filtration or, as a consequence of shrinkage of the capillary tufts caused by atrophy. Glomerular atrophy, this is also in agreement with **[Frazier etal.;2012]** may be caused by chronic degenerative alterations in the glomeruli and subsequent hemodynamic alterations leading to shrinkage and reduction of one or more glomerular capillary tufts, in general match with enlargement of Bowman's space. These results are also in harmony with **[Okada et al.; 2005, Elshamaetal. 2013]** who confirmed that the atrophy of glomeruli and degeneration of renal tubules causes a retard renal growth. The Congestion and hemorrhage noticed in the kidneys of foetuses among (the epithelial lining of the renal tubules, collecting ducts) and (among the glomerular cells); this may be a result of drug toxicity leading to epithelial lining cells death in tubules & glomerulus and these results agreed with**.[Lamyaa Al-Ibrahimi;2015]** that noticed glomerular shrinkage or atrophy as a result of glomerular cell death or inflammations of the glomerular epithelial cells causing glomerular cell death.

In the present work a damage effect of Tegretol (CBZ) on the lung of foetuses under experiment ,as it caused oedema, corrugation of the bronchiolar wall, degeneration, necrosis, hemorrhage, pyknosis of nuclei in the lining epithelium of respiratory bronchiole and air spaces, dilation and severe congestion of blood vessels. The epithelial cells of respiratory and terminal bronchioles detached in their lumen.

The mechanism of lung injury by the long term use of (CBZ) has been assumed to be an immune mediated hyper-sensitivity, according to the in vitro lymphocyte stimulation studies **[Houwerzijlet al.; 1977]**. The mechanisms of acute (CBZ) - toxicity conversely are unknown.In acute processes, three phases can be well-known in sequence: the so-called initial, exudative & proliferative stages. **[Kitson&Wauchob; 1988]** described pulmonaryoedema after a massive overdose of (CBZ). They recommended that, in massive overdose, the anti-diuretic effect of the drug may be sufficient to cause pulmonary oedema, a hypothesis supported by their low protein levels in blood. Although pulmonary toxicity is un-common; broncho spasm, pulmonary oedema , interstitial pneumonitis, bronchiolitis

obliteransorganising pneumonia, and pulmonary nodules have all been attributed **[Milesi-Lecatet al;1997 , Wilschutet al;1997]** The procedure of lung damage is believed to be an immune-mediated hyper-sensitivity reaction**.[MauriHellwegetal.; 1995]**.This may partly clarify the infrequency of the condition in lung transplantation, as this patient populace are on high dose immuno suppressant manner , in this way a combination of cyclosporin, azathioprine &prednisolone.CBZ ;induced hyper sensitivity condition has been reported in associate to re-activation of viral infection, a common trouble in transplantation**[Aiharaet al.;2003]**

Histological examination through lung biopsy showed intra luminal fibrosis of the distal air spaces with foamy alveolar macrophages, signifying Bronchiolitis ObliteransOrganising Pneumonia (BOOP). Later than stopping of (CBZ) administration; all the abnormalities in gamma-globulins gradually improved without any medication. BOOP may be a result of various causes such as; drugs, acute respiratory infections, radiation treatment or emerge idiopathically. **[Cordier; 2000, Cazzatoet al.; 2000].** The BOOP, was result of repeated respiratory infections that caused by (CBZ)-induced hypogamma- globulinemia.

Conclusions:-

This work showed that Tegretol which has the active ingredient (Carbamazepine; CBZ) causes high percentage of foetal mortality in all treated groups. As well as causes growth retardation for the foetuses. Haematoma; dark red patches scattered on the different parts of the body of foetuses at 20^{th} day of gestation parerntally treated with Tegretol. This investigation indicated that most elements of foetuses skeleton showed more or less, complete ossificationTegretol (CBZ)causes histological alterations in foetal liver, kidneys and lungs structures of the foetuses at 20^{th} of gestationin all groups.

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