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Teratogenic Effect of Carbamazepine Administration in Pregnant Rats

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ABSTRACT

Background: Carbamazepine "CBZ" (Tegretol) is an anticonvulsant and mood stabilizing drug used primarily in the treatment of epilepsy and bipolar disorder, as well as trigeminal neuralgia. It has been assigned to pregnancy category D by the U.S Food and Drug Administration (FDA). CBZ can cause fetal harm when administered to pregnant women. Epidemiological data suggested that there may be an association between the use of CBZ during pregnancy and congenital malformations, specifically spina bifida and developmental disorders. The possible malformation-specific risks with CBZ use during pregnancy need to be considered, so the present work was conducted to evaluate the genotoxicity of two doses of CBZ (3.6 mg and 10.8 mg/ 100g body weight/ day) in pregnant female rats and their fetuses. Chromosomal aberration in bone marrow cells and histopathological examination of liver and kidney of pregnant rats were also determined. **Materials and Methods:** Fourty five pregnant Sprague Dawley rats were randomly divided into the groups. The first was administered oral doses of distilled water and served as control. The other two groups were administered oral doses of CBZ suspended in distilled water equivalent to 3.6 mg and 10.8 mg/100g body weight/day respectively for 15 day from the 6^{th} day to the 20^{th} day of gestation. Females were sacrificed on the 20^{th} day of gestation. **Results:** Administration of CBZ 3.6 mg and 10.8 mg /100g body weight to pregnant rats from the 6^{th} till the 20^{th}day of gestation. Decreased fetal body weight, crown-rump length, increased resorbed and dead fetuses were observed compared to the control ones. Moreover, CBZ-high dose group(10.8mg/100g) causedmalformations that could be described as severe growth retardation. At the same time, bone marrow metaphases of CBZ-treated pregnant rats revealed structural chromosomal aberrations. Whereas, histopathological examination of liver and kidney of pregnant rats treated with both doses of CBZshowed cellular alterations.**Conclusion**:It has been found that usage of antiepileptic CBZ during gestational period may create risk, associated with maternal toxicity, hepato- and nephrotoxicity and chromosomal aberrations in pregnant rats, with intrauterine growth retardation which was manifested by low body weight, length reduction and malformations. These alterations were dose dependent. The benefits of taking CBZ must be weighed against the potential risks to boththe developing fetus and the mother.

Keywords: Carbamazepine, pregnant rats, fetus malformations, chromosomal aberration, histopathology of liver and kidney.

INTRODUCTION

Carbamazepine (Tegretol) is an anti-convulsant and mood stabilizing drug. It is used primarily in the treatment of epilepsy and bipolar disorder, as well as trigeminal neuralgia. It is also used for a variety of indications, including attention-defect hyperactivity disorder, schizophrenia, phantom limb syndrome, paroxymal extreme pain disorder, neuromytonia, intermittent explosive disorder and post-traumatic stress disorder[(1)].

Carbamazepine (CBZ) is an imino-dibenzyl derivative, structurally similar to the tricyclic antidepressants. It is available as tablets of 100, 200, and 400mg, and as a suspension of 100 mg/5ml. It is chemically unrelated to other anticonvulsants or other drugs used to control the pain of trigeminal neuralgia. The mechanism of action of CBZ has been partially elucidated. It stabilizes hyperexcited nerve membranes, inhibits repetitive neuronal discharges and reduces synaptic propagation of excitatory impulses. It is conceivable that prevention of repetitive firing of sodium-dependent action potentials in depolarized neurons via useand voltage dependent blockade of sodium channels may be its main mechanism of action. Whereas, reduction of glutamate release and stabilization of neuronal membranes may account mainly for its antiepileptic effects .It is extensively metabolized in the liver via the epoxide- diol

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pathway, to the active metabolite carbamazepine-10,11-epoxide. Time to peak serum concentration is 4-8 hours [2].

Carbamazepine can cause fetal harm when administered to a pregnant woman. Epidemiological data suggested that there may be an association between the use of CBZ during pregnancy and congenital mal-formations, specifically spina bifidaand developmental disorders. This medication crosses the placenta. In pregnant female exposed to CBZ during the first trimester, several human studies have shown 1% risk for neural tube defects. The general population's risk for having a baby with a neural tube defect, such as spina bifida (an opening in the spine or brain), is 0.1% (1/1000 births). Therefore, taking this drug in the first trimester of pregnancy will increase the risk for having a baby with a neural tube defect [3,4,5]**.** In addition, some studies have reported 2 to 3% increased risk for major birth defects, such as heart defects and cleft lip, in epileptic women who were takingCBZ. Also, an increased frequency of growth retardation and small head size were detected [6,7,8]**.** Another study reported that CBZ increases the risk of neural tube defects; but, it does not increase the risk of other mal-formations, and it is also not associated with an increased risk of developmental delay[9]**.** Another studysuggested that children exposed to CBZ have reduced verbal ability compared to the control children [10].

Aim of the work

The present work was conducted to evaluate the genotoxicity of two therapeutic doses of CBZ (3.6 mg and 10.8 mg/ 100g body weight) on pregnant female rats and their fetuses. Fetal congenital malformations were assessed.Chromosomal aberration in bone marrow cells and histopathological examination of the liver and kidney tissues of pregnant rats were also determined.

Materials and Methods

Drug

Carbamazepine /Tegretol, is 200 mg tablets obtained from Novartis Pharma, S.A.E., Cairo, Egypt. It is 5H-dibenz[b,f]azepine-5-carbox-amide, with molecular formula $C_{15}H_{12}N_2O$ and molecular weight 236.27. It is an odorless,white powder that is practically insoluble in water and soluble in ethanol and in acetone [2]**.**

Animals

Adult Sprague Dawley male and female albino rats were used in this experiment, with average weight 150- 200 gm. They were taken from the animal house of El Nasr Pharmaceutical Chemicals Co. Animals were caged separately, males in cages and females in others. All rats were housed in a quite non-stressful environment for one week before beginning the present study. They were offered normal rat chows and water *ad libitum*.

Female rats were mated in the proportion of 2 females for every male over night. Each morning a vaginal smear was taken to check for the presence of sperms or plug in the vagina. Zero day of pregnancy was considered to be the day on which sperms or plug was found in the vagina.

Fourty five pregnant rats were randomly divided into three groups. The first was administered oral doses of distilled water and servedas the control group. The other two groups were administered oral doses of CBZ suspended in distilled water equivalent to 3.6 mg and 10.8 mg/100g body weight/day respectively for 15 day from the 6^{th} to the 20^{th} day of gestation. These doses represent minimal and maximal therapeutic doses in humans, and were calculated for rats according to **Paget and Barnes** [11]. All groups, except for colchicine, were sacrificed after 4 hours from last drug administration.

Teratogenic study

Females were sacrificed on day 20^{th} of gestation and fetuses were counted and weighed; the number of alive, resorbed and dead fetuses were recorded. Fetuses were examined externally to investigate any abnormalities. Also, they were stained with Alizarin red- S according to the method of **Weesner and Parry** [12] and their skeletons were examined.

Cytogenetic study

At the 20^{th} day of gestation, some pregnant female rats were injected with colchicine (4 mg/kg body weight) and sacrificed 2 hours later. The femur bones were quickly separated. Chromosomes of bone marrow cells were prepared according to the modified method of **Luck and Tice** [13]. Chromosomal aberration assay was performed by screening fifty well metaphases per animal for scoring different types of aberrations [14].

Histopathological examination

At the end of the experimental period, all pregnant rats were sacrificed and theirlivers and kidneys were picked out, rinsed in phosphate buffer (pH 7.5) and fixed in phosphate-buffered formalin. Sections were prepared and stained with hematoxylin and eosin [15].

Statistical analysis

Data of the different groups were compared using Student T-test. Differences at $P \leq 0.05$ were considered significant.

RESULTS

In the present work, the teratogenic effect of CBZ (Tegretol) at low and high doses (3.6 mg & 10.8 mg/100 g body weight) on the pregnant rats from the 6th up to the 20th day of gestation were evaluated. These effects were manifested in the number of implantation sites, resorption sites, live and dead fetuses, fetal body weight and crown-rump length of fetuses.

It was found that during this stage, the mean number of implantation sites decreased gradually as the dose of the drug increased (Table 1).

In the same group of animals, the percentage rates of still-live fetuses was 74.65 % and 60.87 % respectively, while the control one was 98.82 % . This means that, the percentage rates of the still-live fetuses and implantation sites among the treated groups, inversely proportional to the drug dose (Table 1). It was also recorded that the percentage rates of late dead fetuses maternally treated with low and highdoses of CBZ from the 6th up to 20th day of gestation were 11.27 % and 18.84 % respectively, (Table 1). This percentage rate was increased by the increase of dose. But the early dead fetuses showed increase in the percentage of resorbed bodies after exposure to both doses of CBZ. These percentage rates were 14.08 % and 20.29 % respectively, while the control one was 1.18 % (Table 1). This means that, the percentage rate of resorption was increased by the increase of dose.

Carbamazepine was also found to import its toxicity to fetuses maternally treated with both doses of the drug. This toxicity was manifested in a remarkable drop in the body weight. The low and high doses showed statistically highly significant difference ($P \leq 0.01$) M 2.62 ± 0.04 g and M 1.52 ± 0.07 g respectively, when compared to the control one, M 3.66 ± 0.13 g (Table 2& Fig. 1).

In the present study, the most common phenomenon of this stage is a fetal growth retardation induced by CBZ was measured by the crown-rump length. The mean of the crown-rump lengths in both doses were M 2.01± 0.08 mm and M 1.04± 0.06 mm respectively. It was statistically highly significant ($P \leq 0.01$) when compared to the control group, which recorded M 3.09± 0.07 mm (Table 3).

Ossification of the skeletal system:

The skeletal system of the maternally treated fetuses on the 20thday of gestation with low and high doses of CBZ was studied compared to the control group. It was noticed that, the skeleton in these fetuses showed abnormality manifested in various forms such as: incomplete ossification, absence of central and lateral disc and scoliosis of vertebral column.

Ossification of the skull:

The treated pregnant rats with 3.6 and 10.8 mg/100 g body weight of therapeutic doses ofCBZ from the 6th up to the 20th day of gestation revealed that incomplete ossification in premaxilla , maxilla , nasal , frontal, parietal , plate, interparietal and supraoccipital plate were observed as compared to the control (Fig.2A, 3A& 4A). It was recorded that,the percentage rates of complete ossification of these bones were 30.11 % and 12.59 % respectively while the normal group was 97.31%(Table 4) .

In addition, less ossification of mandible, basioccipital and supraoccipital was recorded when the fetuses were treated with both doses of CBZ as shown in Fig. 3 and 4. The percentage rates of incomplete ossification in both previous doses were 69.89 % and 87.41 %while the control one was 02.69% (Table 4). It was found that, the percentage rates of sternebrae in different doses were 48.71 % and 56.29 % (fused) while the normal group was 00.42% and 30.56% and 34.18 % (absence), while the control one was 00.26% as shown in Table 4.

Ossification of ribs and vertebrae:

The present study revealed that, the normal pattern of axial skeleton or vertebral column are 32 to 34 vertebrae, which include 7 cervical, 13 thoracic, 6 lumber, 4 sacral and 2 to 4 caudal vertebrae. Most of these vertebrae and central disc were clearly observed with a deeply stain of Alizarin red "S" on the normal development (Fig. 2B).

As shown in Figs. (3B & 4B) fetuses from mothers treated with 3.6 and 10.8 mg/ 100 g body weight of CBZ as a therapeutic doses, their developmental pattern of vertebrae, central disc and scoliosis were delayed in ossification.

The percentage rates of the skeletal abnormalities in the treated fetuses ribs were 25.91 % and 27.32 % (irregular shape), while the normal group was 01.67 %, 32.27 % and 41.14% (missing) , while the control one was 00.00 %, 20.46 % and 21.65 % (incomplete ossification), while the normal group was 01.23 %, in the central disc 31.52 % and 33.52 % (absence), while the control one was 00.00 % and 59.43 % and 61.13 % (scoliosis), while the normal group was 03.65 % of vertebral column respectively, (Table 4).

Ossification of the fore and hind limbs:

Carbamazepine (Tegretol) was found to cause various kinds of skeletal malformation in rat fetuses on the 20th day of gestation . This was manifested in the inhibition of ossification in the most of pectroral and pelvic girdle with both doses 3.6 and 10.8 mg/ 100 g body weight.

The absence of ossification was recorded in the fore limbs especially in radius, ulna and metacarpals bones. The same result was also observed in the hind limbs in tibia, fibula and metatarsal bones. However, the incomplete ossification was observed in humerus in the fore limbs and femur in hind limbs, compared to the normal one,(Figs.2C, 3C& 4C).So the most common phenomena of skeletal abnormalities which was induced by the drug are absence of ossification centers deletion and incomplete ossification in the bones of both fore and hind limbs.

In addition the above abnormalities, all the bones of the treated fetuses showed a clear reduction in their length and size when compared with the normal ones. The percentage rates of incomplete ossification of the fore limbs in both doses of CBZ were 49.53 % and 53.46 %, while the control group was 04.52 %. On the other hand, the hind limbs were 55.84 % and 62.37 %, while the normal one was 03.67 % respectively, (Table 4). It means that, the increase of the drug dosage lead to the increase in the percentage rate of skeletal abnormalities.

Cytogenetic Study:

Cytogenetic effects of CBZ were observed in the chromosomes of bone marrow cells of pregnant rats and represented by standard aberrations at the 20th day of gestation .It was found that CBZ at doses 3.6 mg and 10.8 mg /100 g body weight caused a marked decrease in frequency of mitosis in bone marrow cells of pregnant rats when treated from the 6th up to the 20th day of gestation. The mean number of mitotic index in both doses were M 30.2±1.16 and M 22.8±1.53 respectively when compared to the control one M 43.0± 1.87 , (Table 5).

It was observed that, the number of undivided cells were statistically highly significant ($P \leq 0.01$) when compared to the control. The mean numbers of undivided cells in both doses were M 72.20± 5.24 and M 90.80 ± 4.34 respectively when compared to the control group M 6.40 ± 0.93, (Table 6).

Moreover, various forms of chromosomal aberrations were found among the same group of animals such as ring shaped, centric fusion, end to end association, stickiness and centromeric attenuation. Frequency of these abnormalities were statistically highly significant ($P\leq 0.01$) when compared to the control group (Table 7 and Fig.5).

The most characteristic forms of these aberrations are centric fusion, end to end association and centromeric attenuation. The mean number of these aberration were M 10.00±1.48, M 16.40±1.81 and M 10.00 ±1.22 respectively at dose 3.6 mg/100g body weight, while M12.80±1.07, M18.60±2.27 and M13.60 ±1.08 respectively at dose 10.8 mg/100 g body weight, whilethe normal group were M 1.20±0.37,M 0.60± 0.40 and M 0.80±0.37 (Table 7).

Histopathological Examination:

In the present study some histopathological changes were realized in the liver and kidney tissues of pregnant rats treated with CBZ from the 6th up to the 20thdayof gestation when compared to the control group. Normal liver tissue consists of classical lobules which are prismatic in shape with a central vein in the middle of each lobule. Each lobule consists of cords of hepatocytes; the cords are separated by blood sinusoids which take the same radial direction in the hepatic cells (Fig.6).The hepatocytes are polygonal in shape; each cell has one or two vesicular round nuclei with a fine chromatin net work. There is another kind of cells called Kupffer cells; they are large branched cells hanged by their process to the

wall of the sinusoids. These cells have large oval nuclei (Fig.6).

Treatment with CBZ (Tegretol) at a daily dose of 3.6 mg /100g body weight on liver of the pregnant rats at the 20th day of gestation, showed number of pathological changes manifested in nuclear pyknosis in hepatocytes and intercellular hemorrhage (Figs.7A&B); while in the other dose (10.8mg/100g body weight) there were cellular vacuolization, congested blood vessels, collapsed stroma and enlargement of portal tract by accumulation of fibrous tissue (Figs.8A&B) as compared to the normal group.

Investigation of sections of kidney cortex of the control rat the renal tubules with various shapes, the glomeruli and interstitial connective tissue in between them. Each gromerulus is surrounded by Bowman's capsule (Fig.9).

Microscopical examination of the treated kidney of pregnant rats from the 6th up to the 20thday of gestation with both doses of CBZ (Tegretol) showed damage of the renal tubules with increased lymphocytes. Some glomeruli were lobulated or atrophied and surrounded with hyaline material (Figs.10A&B and figs.11A&B).

DISCUSSION

Approximately three to five births per thousand are to women with epilepsy [16]. Many antiepileptic drugs have well known teratogenic effects, and there are risks (theoretical and evidence based) for obstetrical complications, poor neonatal outcomes, congenital malformations and even cognitive effects on child later in life [17]. CBZ is a viable and important option forwomen with focal epilepsy during their childbearing years because ofits low cost, wide availability, relative safety for fetal outcomes and effectivenessduring pregnancy [18].

Matalon*et al.* [19] reported that maternal use of antiepileptic drugs during pregnancy has been associated with two to three-folds increase in the rate of major congenital anomalies in the fetus anda decrease in gestational age at delivery.

Carbamazepine is a commonly prescribed anticonvulsant that is originally thought to be ideal for use in pregnancy as initial reports showed no teratogenic risks above baseline. **Jones *et al.*** [20] described a pattern of malformations similar to those seen in fetal hydantoin syndrome. CBZ is metabolized into oxidative intermediates (epoxides). Clearance of these metabolites relies on epoxide hydroxolase enzyme activity. The clearance ofCBZ and CBZ-10,11-epoxide shows minimal change during pregnancy and there is evidence of increased free drug. Free CBZ more readily crosses the placental barrier and is more clinically relevant for fetal exposure.Infants with a reduced hydroxolase enzyme activity would likely be at an increased risk for this pattern of malformations if their mother used carbamezapine during pregnancy [18,21,22,23].

There is a feature relationshipbetween CBZ use during pregnancy, developmental defects and fetal CBZ syndromeaccording to clinical and epidemiological studies [24,25,26,27].

Regarding the present resultsthattest the impact of CBZ, low and high therapeutic doses, on fetal growth and organogenesis (from 6-20 gestational days), it was found that both doses caused fetal growth restriction inversely proportion to the dose, incomplete ossification of the skull, ribs and hind limbs, without ossification in maxilla,beside scoliosis of vertebral column.

In the present study, decreased fetal body weight and crown-rump length, increased resorption and malformations were seen in both CBZ treated groups compared to the control ones. Moreover, CBZ-high dose group (10.8mg/100g) caused malformation that could be described as severe growth retardation. **Suchestonet *al.*** [28] realized that toxic effects of highCBZ dose caused intrauterine growth retardation which was manifested by low body weight and length reduction and they also reported that decreased mice length may be due to retarded ossification of the long bones and there was a relationship between decreased long bone ossification centers and fetal weight reduction. This is consistent with the present results for intrauterine and natal growth retardation in both doses of CBZ.

Moreover, **Artamaet *al.*** [29] reported that prenatal exposure to CBZ may induce postnatal onset of severe growth retardation, suggesting disturbance of growth hormone-insulin like growth factor-I axis. According to **Gerenuttiet *al.*** [30] and **Gerenuttiet *al.*** [31]perinatal toxicity of any drug depends on reproductive performance of mother and drug dose. Corpus luteum plays an important role forreproductive performance by secretion of progesterone and 20-hydroxy

progesterone, which maintains fetal growth**.** High dose of CBZ usually affects corpusluteum and then reproductive performance may lead to growth retardation. **Pérez *et al.*** [32] explained that growth retardation of mice in the first day of delivery depends on anti-proliferative effects of CBZ and showed that these effects may be due to an increase in mitotic index and persistent block at the boundary of metaphase andanaphase associated with cell proliferation inhibition. Further, **Elshama*et al*.** [33]reported that there was a significant decrease in weight of different organs and length of upper and lower limbs of mice in the first day of delivery in high CBZ-dose group. Teratogenic effect of CBZ represented as growth retardation and neuro-developmental toxicity depending on its overdose degree. These explanations also support the results of the present work.

In the present results, examination ofbone marrow metaphases of CBZ-treated pregnant rats revealed structural and numerical chromosomal aberrations. These chromosomal aberrations were centromeric attenuation, centric fusion, ring shaped,end to end association,gap, breaks and stickiness. These results are comparable to those seen after phenytoin and CBZ exposure in mice, rats and humans. Studies carried out in a batch consisting of six non-inbred female rats received phenytoin orally in a dose of 25 mg/100 gbody weight daily. In parallel a further sixfemale rats of the same batch were treated with primidone 0.06 mg/100 g body weightdaily. The treatment was started on the first day of gestation and lasted five days. The animals were killed the day after the last dose. The phenytoin -treated animals had an increased number of abnormal bone metaphases (stickiness, inter-chromatid bridges and gaps). Fifty metaphases were counted ineach case. Further, the phenytoin-treatedanimalsand those treated with primidone showed abnormal metaphases compared to the control animals [34].Moreover, it has been reported that the effect of CBZ on human chromosomes was studied to determine its mutagenic potential. Analysis of chromosome breakage, sister chromatid exchanges (SCE) and cell cycle studies were performed in peripheral lymphocyte cultures. *In vivo* studies failed to detect any significant increase of chromosome aberrations or SCE or any slowing of the cell cycle. A significant dosedependent increase in chromosome aberrations but not in SCE was observed in the *in vitro* analyses[35]**. Herha and Obe** [36] anlayzed the leukocyte chromosomes of epileptic pateints on monotherapy with carbamazepine (CBZ) and diphenylhydantoin (DPH), they found a significant elevation of exchange-type aberrations as compared to the control group. In the CBZ treated group predominantly chromatid translocations and in the DPH group exclusively dicentric chromosomes. Also, sister-chromatid exchange (SCE) and chromosome aberrations have been studied in peripheral lymphocytes of epileptic children treated in monotherapy with valproic acid (VPA). The frequencies of SCE in the VPA-treated epileptic children were significantly higher than the control group, while the rates of chromosome aberrations were slightly higher but not significantly different from the control one[37]. Furthermore, **Biswas*et al*.** [38]examined the genotoxic and cytotoxic potential of chronic oral administration of antiepilepticphenobarbital (PB), in mice, through several endpoints such as chromosomal aberrations, induction of micronuclei, mitotic index of bone marrow cells etc. The results, when compared to the control, showed that PB has both genotoxicand cytotoxic potential in apparently increasing intensity at longer periods of chronic feeding in mice. In another study, the genotoxicity of the antiepileptic drug, CBZ, was tested using cytokinesis-block micronucleus assay. *In vitro* analysis was performed in human blood lymphocytes at five different concentrations of CBZ. The results indicated that CBZ caused the genotoxic effect under *in vitro* conditions and the cytotoxic effects were revealed by a decrease in the cytokinesis-block proliferation index at all concentrations [39].

Multiple physiologic changes during pregnancy influence drug disposition, including increased volume of distribution, increased renal elimination, altered hepatic enzyme activity and a decline in plasma protein concentrations. The majority of antiepileptic drugs are characterized by significant increases in clearance during pregnancy.Physiological changes during pregnancy are commonly considered to be underlying factors[40,41]**.**

Histopathological examination of liver tissues of pregnant rats treated with daily dose 3.6 mg/100g body weight of CBZ at day 20th of gestation showed sinusodial dilatation, cellular vacuolization, hemorrhage, fibroblastic cells ; whereas daily dose 10.8 mg/100g of CBZ at

day20th of gestation showed hemorrhage, fibroblastic cells, increased proliferation in the elongated walls of bile ducts and intercellular vacuoles. Moreover, kidney of pregnant rat treated with daily dose 3.6 mg/100g of CBZ at day 20th of gestation showed dilatation of Bowman's capsules, dilatation of proximal tubules and shrinkage of some glomeruli while others were lobulated, while high daily dose 10.8mg/100g of CBZ at day 20th of gestation showed dilatation of Bowman's spaces, degeneration of distal convoluted tubules with numerous haemorrhagic areas.

The present results are in agreement with the work of **El-Sayyad*et al.*[42]**who revealed that the applied antiepleptics, lamotrigine and valporate, caused marked pathological alterations in maternal tissues, including hepatotoxicity, characterized by disruption of the normal integrity of hepatic lobules with prominent dilatation of blood sinusoids. A slight perivascular leukocytic cell infiltration was remarked with sloughing and degeneration of endothelial cells lining of the blood vessel and nephrotoxicity where the glomeruli showed a marked increase of cellularity, lobulated and massive hyaline degeneration of renal tubules. Most of them showed degeneration of their epithelial lining cells with missing of their tubular Lumina. The interstitial tissues exhibited accumulation of hyaline casts. Different pathological alterations of glomeruli were also observed post treatment with lamotrigine and valporate. **Akta *et al.*[43]**observed irregularities on the basal lamina in kidneys of mother rats treated with valproic acid.

CONCLUSION

It has been found that the use of antiepileptic CBZ during gestational period may create risk, associated with maternal hepatotoxicity, and nephrotoxicity and chromosomal aberrations in pregnant rats, with intrauterine growth retardation which was manifested by low body weight, length reduction and malformations. These alterations were dose dependent.The risk associated with in utero antiepileptic CBZ exposure are of considerable importance, as this medication is used off label. This expansion of clinical applications has increased the rate of exposure. So, the prescription of CBZ must be balancedto maintain therapeutic objectives for the mother but, at the same time, to minimize fetal drug exposure.

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Table 1- Effect of low and high doses of Carbamazepine on rat fetuses at the 20th day of gestation.

Fetus	Implantation Sites	Still Live		Dead		Resorbed	
Dose		**M.**	**%**	**M.**	**%**	**M.**	**%**
Control	8.5	8.4	98.82	0.0	00.00	0.1	1.18
Low dose	7.1	5.3	74.65	0.8	11.27	1.0	14.08
High dose	6.9	4.2	60.87	1.3	18.84	1.4	20.29

M. = mean. % = Percentage.

Table 2- Effect of low and high doses of Carbamazepine on body weight (w/g) of rat fetuses at the 20th day of gestation.

Statistical analysis	Control	Treatment Low dose	Treatment High dose
Range	3.15- 4.26	2.48- 2.80	1.20 -1.87
M.± S.E.	3.66± 0.13	2.62± 0.04**	1.52± 0.07**

The results expressed as M± S.E. of ten rat fetuses.
M.= mean . S. E. = standard error . * * = highly significant (P≤ 0.01).

Fig. 1- Photograph of rat fetuses, control and treated with Carbamazepine from the 6th to the 20th day of gestation showing: A - Normal fetus. B -Treated fetus with the low dose. C- Treated fetus with high dose. (Bouin's solution x1.5).

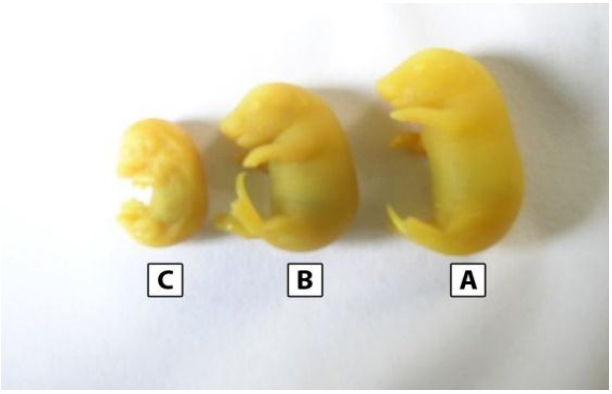


Table 3- Effect of low and high doses of Carbamazepine on crown-rump length (L/mm) of rat fetuses at the 20th day of gestation.

Statistical analysis	Control	Treatment Low dose	Treatment High dose
Range	2.80-3.50	1.60-2.40	0.80-1.30
M.± S. E.	3.09±0.07	2.01±0.08**	1.04±0.06**

The results expressed as M± S.E. of ten rat fetuses.
M.= mean . S. E. = standard error . * * = highly significant (P≤ 0.01).

Table 4-Teratogenic effect of low and high doses of Carbamazepine on the skeletal system of rat fetuses at the 20th day of gestation.

Skeletal defects	Percentage of examined bone fetuses %		
	Control	Low dose	High dose
Skull			
Incomplete ossification	02.69	69.89	87.41
Complete ossification	97.31	30.11	12.59
Sternebrae			
Fused	00.42	48.71	56.29
Absence	00.26	30.56	34.18
Normal	99.32	20.73	09.53
Ribs			
Irregular shape	01.67	25.91	27.32
Missing	00.00	32.27	41.14
Incomplete ossification	01.23	20.46	21.65
Complete ossification	97.10	21.36	09.89
Vertebrae centra			
Absence	00.00	31.52	33.52
Scoliosis	03.65	59.43	61.13
Normal	96.35	09.05	05.35
Fore Limbs			
Incomplete ossification	04.52	49.53	53.46
Complete ossification	95.48	50.47	46.54
Hind limbs			
Incomplete ossification	03.67	55.84	62.37
Complete ossification	96.33	44.16	37.63

% = Percentage.

Table 5- Effect of low and high doses of Carbamazepine on the mitotic index (no. cells/1000 meta.) in the bone marrow of pregnant rats.

Dose	No. of dividing cells /1000 cells					M. ± S. E.
	I	II	III	IV	V	
Control	49	38	42	45	41	43.0± 1.87
Low dose	31	27	34	29	30	30.2±1.16**
High dose	24	22	26	28	19	22.8±1.53**

The results expressed as Mean ± S.E. of five pregnant rats.
M.= mean . S. E. = standard error . * * = highly significant (P≤ 0.01).

Table 6- Effect of low and high doses of Carbamazepine on the No. of undivided cells (no. cells/50 meta.) in the bone marrow of pregnant rats.

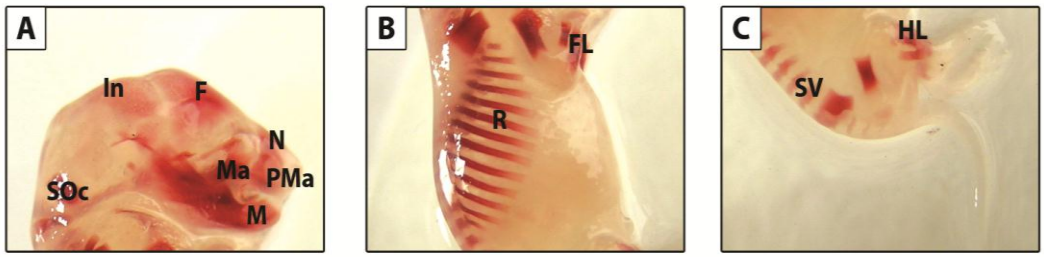
Dose	No. of cells /50 metaphase stage					M. ± S. E.
	I	II	III	IV	V	
Control	6	4	8	9	5	6.40±0.93
Low dose	55	73	87	69	77	72.20±5.24**
High dose	95	101	79	82	97	90.80± 4.34**

The results expressed as Mean ± S.E. of five pregnant rats.
M.= mean . S. E. = standard error. * * = highly significant (P≤ 0.01).

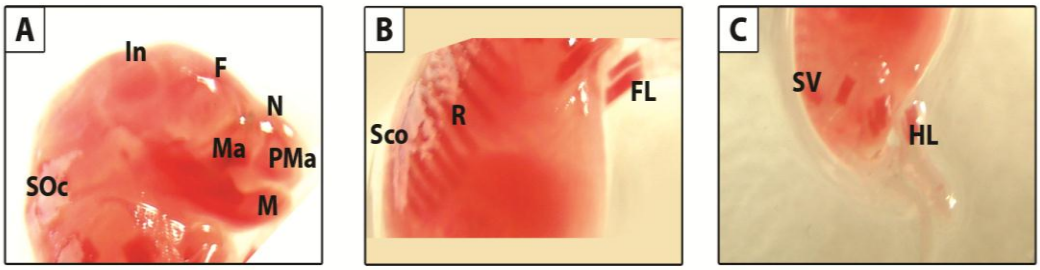
Table 7- Chromosomal aberrations in bone marrow cells of pregnant rats treated with low and high doses of Carbamazepine at the 20th day of gestation.

Treatment	Control	Low dose	High dose
Ring Shaped	0.40±0.25	07.40±1.29**	08.40±1.17**
Centric fusion	1.20±0.37	10.00±1.48**	12.80±1.07**
End to end	0.60±0.40	16.40±1.81**	18.60±2.27**
Stickiness	0.60±0.25	10.60±1.44**	13.00±1.58**
Centromeric Attenuation	0.80±0.37	10.00±1.22**	13.60±1.08**

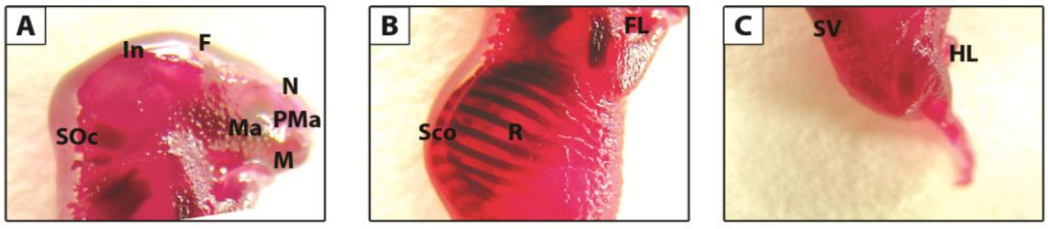
The results expressed as M ± S.E. of five pregnant rats.
M. = mean. S. E. = standard error. * * = highly significant (P≤ 0.01).



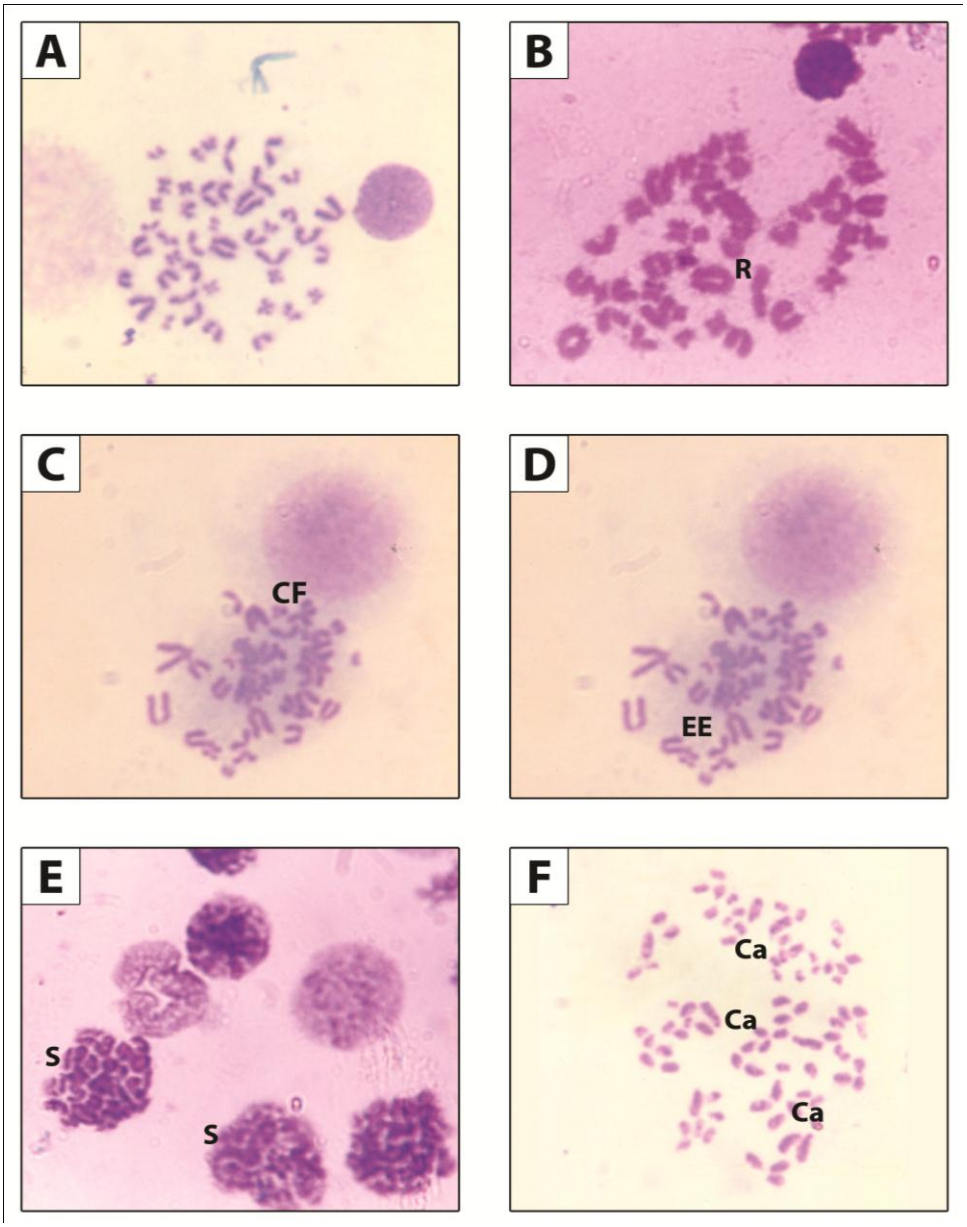
Figs.2- Photographs of control rat fetus at the 20th gestation day, showing: A- skull bones: mandible (M), maxilla (Ma), premaxilla (PMa), nasal(N) , frontal (F), interparietal (In) & supraoccipital (SOc). B- normal ribs (R) and fore limbs (FL). C- normal bones in the sacral vertebrae (SV) & hind limbs (HL). (Alizarin red x10)



Figs.3- Photographs of the 20th gestation day rat fetus treated with low dose of carbamazepine, showing: A- incomplete ossification of the skull bones and no ossification in maxilla (Ma).
B- incomplete ossification of the fore limbs (FL) , ribs(R) & scoliosis (Sco) of vertebral column.
C- incomplete ossification of the sacral vertebrae(SV) & hind limbs(HL). (Alizarin red x10)



Figs.4-Photographs of the 20th gestation day rat fetus treated with high dose of carbamazepine, showing: A- incomplete ossification of the skull bones: frontal (F), interparietal (In) & supraoccipital (SOc) & no ossification in mandible (M), maxilla (Ma), premaxilla (PMa) and nasal (N). B- Incomplete ossification of the fore limbs (FL), ribs (R) and scoliosis (Sco) of vertebral column. C- Incomplete ossification of the sacral vertebrae (SV) & hind limbs (HL). (Alizarin red x10)



Figs.5- Photomicrographs ofbone marrow cells metaphase spread of normal and treated pregnant rats with both doses of carbamazepine on the 6th to the 20th day of gestation, showing: A-normal chromosomal pattern. B-ring shaped (R). C-centric fusion (CF). D-end to end association (EE). E- stickiness (S). F- centromeric attenuation (Ca). (Gimsa stain X 1250).

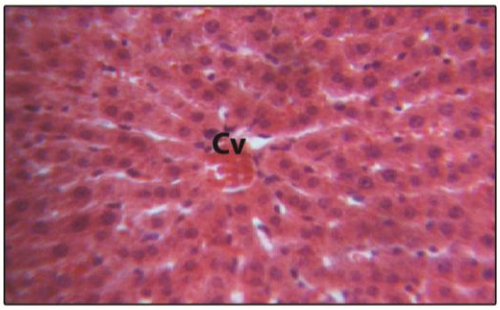
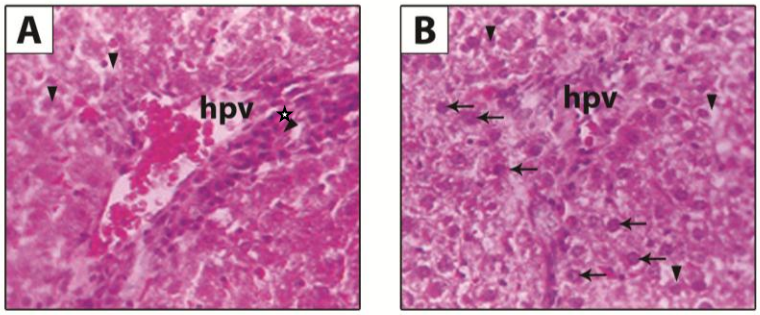


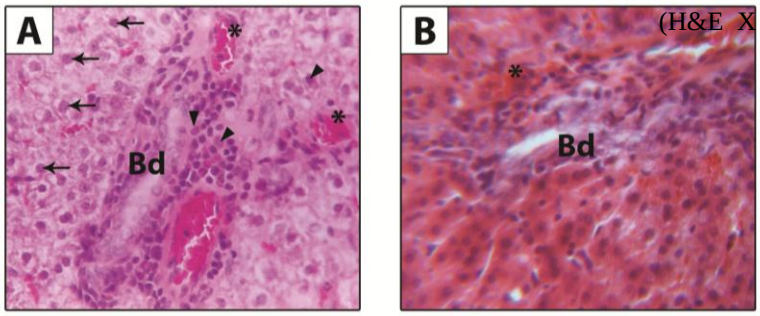
Fig. 6-Section of normal liver of pregnant rat shows normal hepatic cells radiating from the central vein (Cv).

(H& E X 312).



Figs.7A&B-Sections of liver of the pregnant rat treated with daily dose of Carbamazepine 3.6 mg/100g at day 20th of gestation show: vacuolated hepatocytes (▲), hypertrophied nuclei of them (→) highly dilated and congested hepatic portal vein (hpv), lymphocytic infiltration in the portal area (✡)

(H&E X 312).



Figs.8A& B- Sections of liver of the pregnant rat treated with daily dose of Carbamazepine10.8 mg/100g at day 20th of gestation show: vacuolated hepatocytes with signs of pyknosis in their nuclei(→), highly congested hepatic portal vein (hpv) which contains haemolysed blood cells , increased proliferation in highly elongated wall of the bile duct (Bd) , congested blood sinusoids and numerous haemorrhagic areas (*).

(H& E X 312).

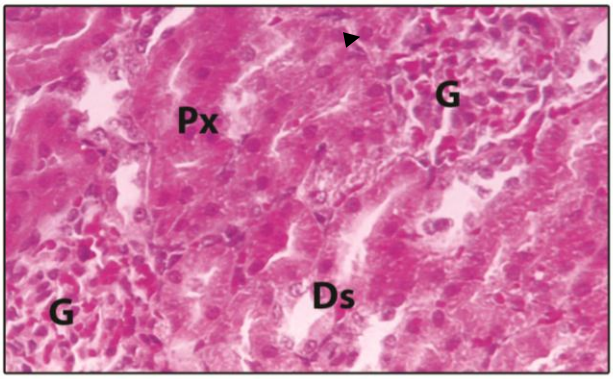
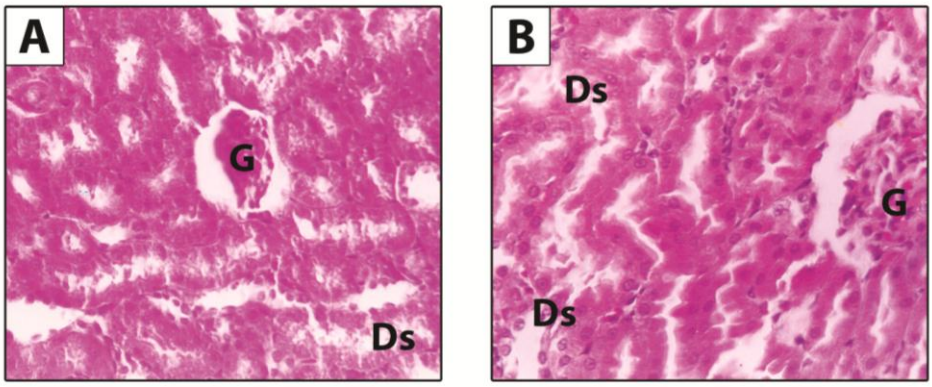


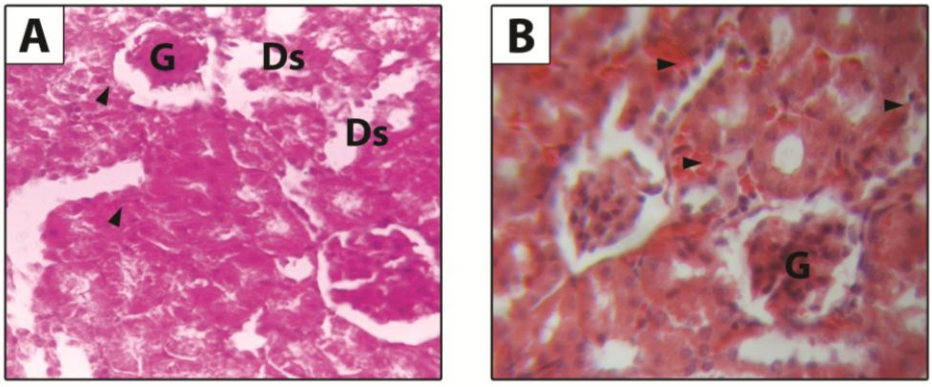
Fig.9- Section of kidney of control pregnant rat shows normal glomerulus(G) , proximal (Px) and distal convoluted tubules(Ds).

(H&E X 312).



Figs.10A& B-Sections of kidney of pregnant rat treated with daily dose of Carbamazepine 3.6 mg/100g at day 20th of gestation show: widened Bowman's space, atrophied glomerulus (G), destructed cuboidal cells of the distal convoluted tubules(Ds).

(H&EX 312).



Figs.11A& B-Sections of kidney of pregnant rat treated with daily dose of Carbamazepine 10.8mg/100g at day 20th of gestation show: lobulated and atrophied glomeruli (G), numerous haemorrhagic areas(▲) in between the convoluted tubules & highly destructed cuboidal cells(Ds).

(H&EX 312).