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Chromosomes abnormalities caused byTegretol drug on the albino rat

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Abstract:
The present work was performed to illustrate the harmful effects of different doses of Tegretol which has active ingredient (**Carbamazepine; CBZ**) on the genetic of the adult albino rat and on the prenatal foetuses of rat at 20th day of gestation. Tegretol (CBZ) noticeably, increased chromosomal aberrations and decreased the mitotic index.

Keywords: Tegretol, teratogenicity, adult rat and 20-day-old Albino rat foetuses.

1. 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 [7].

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 **[23, 20]**. 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 **[8].** 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 **[25**].

It has been found that the effect of **(CBZ)** on chromosomes of human was studied to detect its mutagenic potential. Analysis of chromosome breakage sister chromatid exchanges and cell cycle trials were performed in peripheral lymphocyte cultures. In vivo trials failed to reveal any significant increase of chromosome aberrations or sister chromatid exchanges or any decelerating of the cell cycle. Significant dose dependent increase in chromosome aberrations but not in sister chromatid exchanges were observed in the invitro analyses [**4**]**.**

[14] had anlayzed the leukocyte chromosomes of the epileptic pateints on mono-therapy with (CBZ) and diphenyl-hydantoin (DPH), they found a respectable elevation of exchange-type aberrations as compared to control . In the (CBZ)administered groups mostly chromatid translocations and in the DPH group exclusively dicentric chromosomes. Also, sister-chromatid exchange and chromosome aberrations were studied in peripheral lymphocytes of epileptic children administered in mono-therapy with (VPA). The frequencies of sister chromatid exchanges in the VPA-administered epileptic child were significantly more than the control group while the rates of chromosome aberrations were higher but not significantly different from the control **[12**].

[**21**]examined the geno-toxic and cyto-toxic potential of chronic oral administration of antiepileptic phenobarbital in mice through several end points like chromosomal aberrations, induction of micro-nuclei, mitotic index of bone marrow cells . The results revealed that **carbamazepine (CBZ)** caused the genotoxic effect under the in vitro conditions and the cytotoxic effects were defined by a decrease in cytokinesis-block proliferation index of all concentrations **[1**].

2. 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 recent dosesare(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): which received (1/150 ofLD50; 5 mg/kg). Males and females were administered with tegretol (CBZ) (l) for seven days before mating, after that they do copulation with each other.Pregnant females continued receiving Tegretol (CBZ) at 20th day of gestation.

We brought Virgin males and females of age 8-12 weeks and About 200g weight were supplied **by Theodor Bilharz Research Institute** (Giza,Egypt) .Rats were adapted for 7 days with a 12:12-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 **[18]**. 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 20thday of her gestation, the uteri were obtained by caesarean sections**. For Cytogenetic investigation:-*A-Chromosomal preparation:***Rats were scarified on the day following the last day of injection .Bone marrow cells were collected to study the chromosomal aberrations and mitotic indices. Metaphase spread was prepared which has been modified according to **[16].**

3. Results

Cytogenetic effects of Tegretolwith the active ingredient Carbamazepine(CBZ) were observed in the chromosomes of bone marrow cells of rats and represented by standard aberrations in control rat Fig. (1)**.** It was founded that Tegretol(CBZ) in a long term of administrating with different doses caused a decrease in frequency of mitosis in bone marrow cells of female rats and their foetuses when administered for a long periods(27 day)for females &'21day' maternally to the foetuses. But in males it couldn't affect as the period of administering the drug (7 days) was not enough to produce an effect on bone marrow. It was noted that, the number of undivided cells were statistically highly significant as compared to control group various forms of chromosomal aberrations were found among the same group of animals such as ring shaped, Deletion, end to end association, stickiness and centromeric attenuation. Not all of these abnormalities were highlysignificant as comparedwith the control group.The data obtained in the present study are presented in Figs (1-8)

I-Chromosomal aberrations: The chromosome result obtained from 5 rats, 50 metaphase spread fields which examined for the chromosomal aberration.There are many kinds of chromosome aberrations, they can be clustered in two basic groups, structure and numerical aberrations but not both types are observed in bone marrow cells of rats administered with Tegretol(CBZ).The Rattusnorvigicus have 21 pairs of chromosome which exhibited normal pattern in control group rats Fig(5).

a-Structural aberration: The significantly founded chromosome structure aberrations Include end to end association seen at Figs (1,2&6)of foetuses ,deletion in Figs (3,7) and centromeric attenuation Figs (4,8).

***End to end association:** Fig.(1,6) shows the mean value of End to end association chromosome in the administered groups .There is a highly significant difference between control group and Tegretol (CBZ)administered groups where the mean value for the control was(4.6±2.07d) but the administered were (52.4±3.21a);(40.8±2.28b); (21.4±2.41c) and (7.2±1.92d) for G1;G2;G3&G4 respectively.This aberration is the only type that appeared in foetuses which parentally administered with Tegretol (CBZ) the mean value of this aberration listed in Fig. (2)..*a, b & c: There is no significant difference (P>0.05) between any two means, within the same column have the same superscript letter.The mean difference is significant at the 0.05 level Vs Control, p< 0.01.

***Chromatid deletions:** The data in Figs (3,7) represent the mean value of chromosome deletion in 50 field well spread metaphase rats' bone marrow cells. There was a highly significant increase in the mean value of Tegretol (CBZ) administered rats than of the control group, where; the mean value of chromosome deletion is (44.6±2.41a); (33.8±1.64b);(20.6±1.52c) and(9.2±2.39d) in G1;G2;G3 &G4 respectively While the mean value of control group (3.4±1.14e).

***Centromeric attenuation:**There is increase in the mean value of centromeric attenuation in rat chromosome in Tegretol (CBZ) groups compared to control group as,the average of centromeric attenuation in administered groups and Control group is showed as follow at control group (2.6±1.52e); (21.0±1.58a) at G1; (16.0±1.58b)at G2 ;(9.6±1.14c) at G3 and (6.4±1.14d) at G4 respectively as appeared in Fig. (4,8).

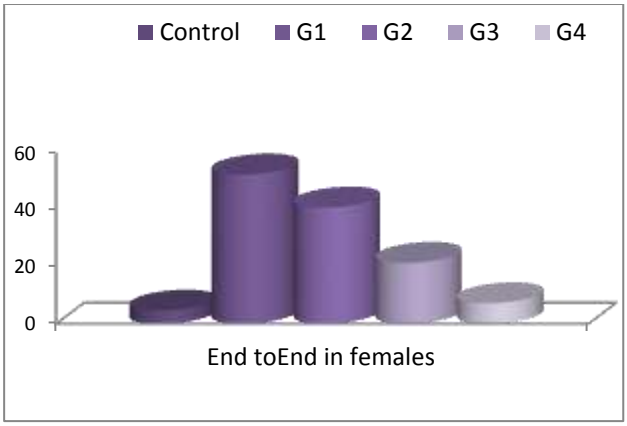

Fig. (1) Histogram showing; mean values of end to end aberration (E.TO E.) In Female rats obtained from control and Tegretol (CBZ)-administered groups. G1: (1/8 LD50 98mg/kg), G2: (1/50 LD50; 16 mg/kg), G3: (1/100 LD50; 8 mg/kg), G4 (1/150 LD50; 5mg/kg.

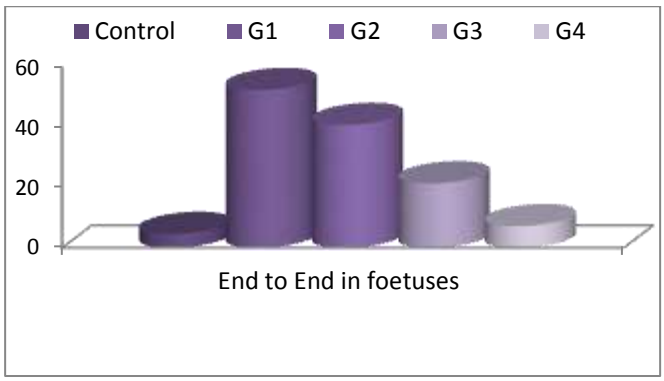

Fig. (2) Histogram showing; mean values of end to end aberration (E.TO E.) In foetuses rats obtained from control and Tegretol (CBZ)-administered groups. G1: (1/8 LD50 98mg/kg), G2: (1/50 LD50; 16 mg/kg), G3: (1/100 LD50; 8 mg/kg), G4 (1/150 LD50; 5mg/kg).

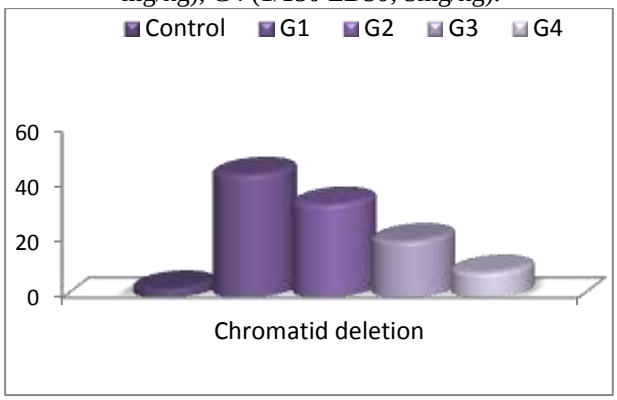

Fig. (3) Histogram showing; mean values of chromatid deletion aberration (D.) in Female rats obtained from control and Tegretol (CBZ)-administered groups. G1: (1/8 LD50 98mg/kg), G2: (1/50 LD50; 16 mg/kg), G3: (1/100 LD50; 8 mg/kg), G4 (1/150 LD50; 5mg/kg).

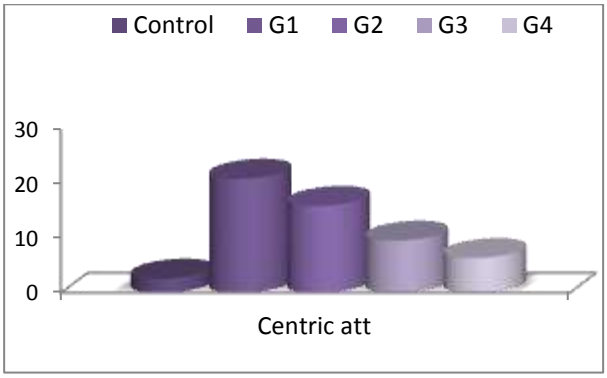

Fig. (4) Histogram showing; mean values of centric attenuation (c.att.) in Female rats obtained from control and Tegretol (CBZ)-administered groups. G1: (1/8 LD50 98mg/kg), G2: (1/50 LD50; 16 mg/kg), G3: (1/100 LD50; 8 mg/kg), G4 (1/150 LD50; 5mg/kg).

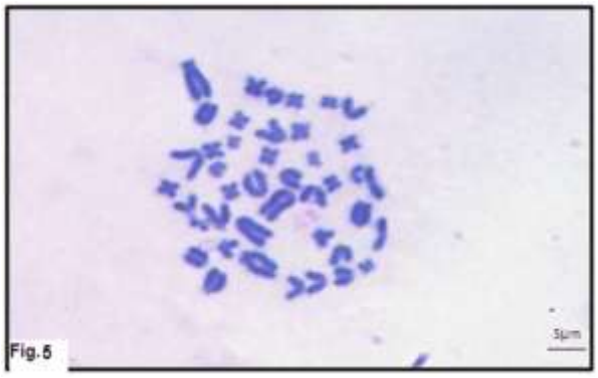

Fig. (5) Metaphase spread of chromosomes from bone marrow cells of normal rats.

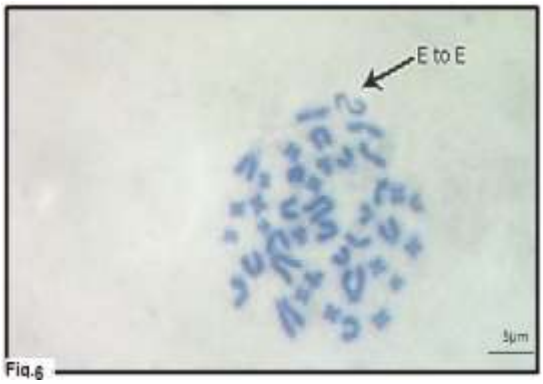

Fig. (6) Metaphase spread of chromosomes from bone marrow cells prepared from Tegretol (CBZ)-administered groups showing; end to end (EE). G1 (1/8 LD50; 98 mg/kg), G2: (1/50 LD50; 16 mg/kg), G3: (1/100 LD50; 8 mg/kg ,G4: (1/150 LD50; 5 mg/kg).

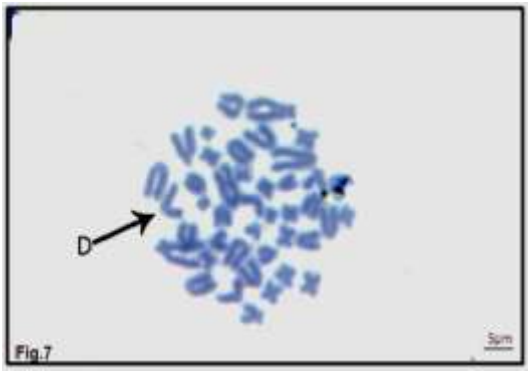

Fig. (7) Metaphase spread of chromosomes from bone marrow cells prepared from Tegretol (CBZ)-administered groups showing; deletion (D). G1 (1/8 LD50; 98 mg/kg), G2: (1/50 LD50; 16 mg/kg), G3: (1/100 LD50; 8 mg/kg ,G4: (1/150 LD50; 5 mg/kg)

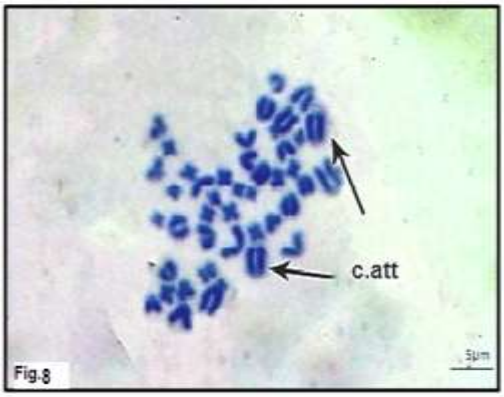

Fig. (8) Metaphase spread of chromosomes from bone marrow cells prepared from Tegretol (CBZ)-administered groups showing ; centric attenuation (C.att). G1 (1/8 LD50; 98 mg/kg), G2: (1/50 LD50; 16 mg/kg), G3: (1/100 LD50; 8 mg/kg ,G4: (1/150 LD50; 5 mg/kg)

4. Discussion

It had been found that 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 **[9]** .

It is necessary to point up that the main intracellular antioxidant element, glutathione, only reaches its maximum production at the end of gestation **[17, 24, 22].**The present study focused on cytogenic effect of Tegretol (CBZ) on the chromosome aberrationsresulting from administrating Tegretol (CBZ)orally.The present investigation of bone marrow at metaphase of Tegretol (CBZ)-administered rats revealed structural chromosomal aberrations. it has been regarded that the effect of (CBZ) on human chromosomes was studied to determine its mutagenic potential **[13]** . A single dose of carbamazepine to pregnant rats equilibrates across the placenta within 30 minutes after drug administration **[10, 11].** Although, there were no significant alterations in the proportion of external and visceral anomalies, which should describe the teratogenic effect. It has been said that (CBZ) makes a copy ofminor anomalies **[6].**Numerous of studies,

have been reported that men with epilepsy treated with (CBZ), had changed semen quality compared with controls **[15, 19].**This reveals the direct effects of (CBZ) in causing changes in semen in male with epilepsy. Abnormalities in sperm concentration, morphology and motility, which were observed in the current study, might play a clear role in causing reduced fertility in males with epilepsy**[15]** .The results are concordant with the inspection of reduced fertility between males of epilepsy investigated in previous studies **[3]**

Even so, the risk related to prenatal (CBZ) exposure is of significant importance, as this medication has a assortment of clinical applications. So, the prescription of (CBZ) must be observer to retain therapeutic effects for the mothers, at the same time, to reduce foetal drug exposure. Human trials are limited, and experimental animal models can be used in biological research, being of great value for medical science **[5, 2].** At the same time as recognition drugs and the other agents as potential teratogens, especial in cases of toxic effects are not as important or where malformations happen spontaneously and frequently in general .

5. Conclusions

This work showed that Tegretol which has the active ingredient (Carbamazepine; CBZ) causes high percentage of chromosomal abnormalities in all administered groups. As well as causes aberration in the chromosomes of the foetuses at 20^{th} day of gestation parentally administered with Tegretol (CBZ).

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