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## Teratogenic Effects of the Anti-Epileptic Drug (Levetiracetam) on Albino Rat Fetuses during Pregnancy and Lactation.

Abd El Wahab El Ghareeb<sup>1\*</sup>, Hamida Hamdi<sup>1,2</sup>, and Mohammed Eleyan<sup>3</sup>.

<sup>1</sup>Zoology department, Faculty of Science, Cairo University, Egypt.

<sup>2</sup>Biology department, Faculty of Science, Taif University, Saudi Arabia.

<sup>3</sup>Biotechnology department, Faculty of Science, Islamic University of Gaza.

### ABSTRACT

The use of the older generation antiepileptic drugs (AEDs) during pregnancy and lactation is known to be associated with increased risk of birth defects in the offspring. Much less has been known about newer generation AEDs to which Levetiracetam (LEV) belongs. LEV is a broad spectrum antiepileptic drug which is currently licensed worldwide. The aim of this study was to evaluate the teratogenic effects of LEV on fetuses of albino rats. Pregnant albino rats (*Rattus norvegicus*) were administered daily oral doses of 300mg/kg or 600mg/kg from the 5<sup>th</sup> day of gestation till the end of lactation. The animals were sacrificed at the end of gestation and during lactation. Fetuses were removed from the uterus and evaluated for mortality rate, growth parameters, morphological and skeletal malformations as well as histological study of brain, liver and kidney. The data revealed that fetal weights were significantly reduced in most study groups. Resorption rates were significantly increased with increasing LEV doses. It was found that mild degenerative changes were observed in the liver, kidney as well as the brain following LEV administration. Levetiracetam pretreatment caused a non-significant effect on the level of lipid peroxidation. No significant correlation was noted between GSH levels and the anticonvulsant effects of Levetiracetam. Thus administration of LVE during pregnancy and lactation should only be considered if the expected benefit to the mother is greater than any possible risk to the fetus.

**Keywords:** antiepileptic drugs, teratogenicity, gestation, lactation

*\*Corresponding author*

## INTRODUCTION

According to the World Health Organization (WHO) epilepsy is defined as a chronic neurological condition characterized by recurrent unprovoked epileptic seizures (WHO, 2012). A seizure is typically an episode of sudden onset manifesting as a disturbance of consciousness, behavior, emotion, motor, sensory or autonomic function precipitated by excessive electrical discharges in the grey matter of the brain (Chadwick D.,2001).This definition incorporates a broad range of seizure types and epilepsy disorders, all of which share abnormal electrical activity within the brain as their fundamental basis.Epilepsy is one of the most common neurological conditions, affecting an estimated 50 million people worldwide (Sander J and Shorvon S, 1996; WHO, 2001). Epilepsy affects all age groups, varies widely in its nature and severity, and is highest at the extremes of the age range (Moran N *et al.*, 2000).In people with epilepsy, the association between age, social class and healthcare resource utilisation was less pronounced than in people without epilepsy. This observation suggests that epilepsy abolishes the protective influence of young age and elevated social class on healthcare consumption (Gaitatzis A *et al.*, 2002).

Numerous epidemiological studies have shown that the offspring of epileptic mothers have a two to three fold higher risk of congenital malformations than the general population (Dansky and Finnell 1991). Each year 40000 infants are exposed to anticonvulsant drugs in *utero* worldwide, with the estimated birth of 1500-2000 infants having congenital malformations as a consequence of intra-uterine exposure to AEDs. Most studies indicate that the antiepileptic therapy rather than the maternal disease or convulsions is the major cause of malformations detected at birth. One example is an illustrative experimental study by (Finnell and Chernoff, 1982). In that study, they used an inbred strain of mice having a genetically determined spontaneous seizure disorder known as quaking (qk). The qk/qk mice had several seizures a day throughout gestation, yet produced normal healthy pups. Upon treatment with AEDs, the seizure frequency diminished as the malformation rate increased, showing that the AEDs were the cause of teratogenicity. Another example is a recent clinical study by Holmes *et al.* (2000), showing no increase in malformations in offspring of mothers with epilepsy, who were not treated with AEDs. Although there is a risk of birth defects with AED therapy during pregnancy, the deleterious effects on the fetus of an uncontrolled seizure during pregnancy and at labour outweigh the risk of malformations for most epileptic women. About 30% of women with treated epilepsy will have an increase in seizure frequency during pregnancy (Schmidt *et al.*, 1983). Although, the majority of children born to women with epilepsy are normal, they are at increased risk for malformations (Perucca E, 2005). AEDs have the potential to affect fetal development throughout pregnancy. However, pregnant women should not stop their medication. The reasons are due to the frequency and severity of their underlying epileptic disorder and also the fact that avoidance of using any AED in women of childbearing age is not a reasonable or safe option for many patients with significant epilepsy(Barrett C and Richens A,2003).Having seizures during pregnancy by affecting the mother's cardiovascular status can cause a risk to both the mother and the fetus (Minkoff H,1985).Therefore, understanding the ways of preventing AED-related abnormalities is an important factor in the care of epileptic women and their offspring. This goal will be reached by examining the differential effects and mechanisms of AED teratogenesis.

Congenital malformations are anatomical or structural anomalies which take place during embryogenesis. Several medicines and certain chemicals may cause malformations or permanent defects which may lead to death by passing to the fetal circulation, which is called teratogenesis (Brendel R *et al.*, 1989). Development of embryo is affected by teratogens mostly during the process of organogenesis, which is recognized as the time period from the occurrence of the neural plaque to closure of the plate. Several studies show that the antiepileptic drug therapy rather than the maternal disease or convulsions is the major cause of malformations identified at birth. Annerggers and colleagues found that the rates of malformation in the offspring of mothers with epilepsy treated with AEDs are higher than in the children of mothers with no AED treatment (Annerggers JF *et al.* 1978; Jick SS and Terris BZ, 1997). In addition, mean plasma AED concentrations are higher in mothers with malformed infants than mothers with healthy children (Dansky LV *et al.*, 1987). There is a higher risk in children of mothers with polytherapy compared to monotherapy and occasionally a clear relationship between daily dose and risk of malformations has been documented (Morrow Jet *et al.*, 2006). Selected drugs are thought to be associated with specific malformations (Battino D and Tomson T, 2007).

The majority of studies on fetal malformations and AEDs, have been made in patients treated with the five leading AEDs - phenytoin (DPH), carbamazepine (CBZ), phenobarbital (PB), primidone (PR), and sodium valproate (VPA) (Shorvon SD. 1990; Yerby MS. 1991; Shuster EA. 1996; Jans D and Fuchs V. 1964; Lindhout D and Omtzigt JGC. 1992; Nakane Yet *et al.*, 1980; Samrén EB *et al.*, 1997; Yerby MS *et al.*, 1992; Rosa F. 1991; Friis ML *et al.*, 1986; Annerggers JF *et al.*, 1978). The newer AEDs (e.g. gabapentin, lamotrigine, oxcarbazepine, tiagabine, topiramate and vigabatrin) are recommended for use in those patients who do not respond to, or cannot tolerate, the older AEDs (e.g. carbamazepine, phenytoin, sodium valproate) (National Institute for Clinical Excellence). Licensed in the UK in 2000, levetiracetam is the most recent of the AEDs to be introduced and is indicated as adjunctive therapy for the treatment of partial-onset seizures with or without secondary generalization (UCB Pharmaceuticals Ltd, 2004).

Our study aimed to evaluate the teratogenic effect the levetiracetam as an antiepileptic drug during gestation and lactation periods. In 2006, levetiracetam was granted approval as adjunctive therapy for myoclonic seizures in patients 12 years of age or older (Rossetti AO, Bromfield EB. 2005; Garcia C and Rubio G. 2009). While it is primarily used for epilepsy, other off-label uses for levetiracetam include bi-polar disorder, migraine prophylaxis, neuropathic pain, postherpetic neuralgia, and myoclonus (Farooq MU *et al.*, 2009). While it is primarily used for epilepsy, other off-label uses for levetiracetam include bi-polar disorder, migraine prophylaxis, neuropathic pain, postherpetic neuralgia, and myoclonus (Farooq MU *et al.*, 2009). The mechanism of action of levetiracetam does not resemble those of the other AEDs currently available for clinical use (UCB, 2004). For example, levetiracetam does not interact with the conventional AED targets implicated in the modulation of inhibitory and excitatory neurotransmission (UCB, 2004; Klitgaard H, 2001). Instead, experimental evidence suggests that levetiracetam acts selectively against abnormal patterns of neuronal activity. Levetiracetam is further distinguished from other AEDs by its antiepileptogenic profile, as demonstrated by its ability to inhibit the development of kindling in rodents (Kupferberg H, 2001). This study will add to the medical

literature on Levetiracetam exposure during pregnancy and Lactation and will provide insight to women and healthcare professionals about the risks to the fetus.

## MATERIALS AND METHODS

### Experimental animals

The present experimental study is carried out on the albino rat (*Rattus norvegicus*). The standard guidelines of The Institutional Animal Care and Use Committee (IACUC) were implemented in handling the animals.

Females of 11-13 weeks old were selected for the present study and the first day of gestation was determined by the presence of sperms in the vaginal smear (McClain and Becker, 1975). A daily record of the weight of the pregnant females was made throughout the whole gestation period. The percentages of abortion were calculated in each group; abortion was determined by the presence of blood drops and sudden drop in the weight of the pregnant females.

### Experimental strategy

Levetiracetam (LEV) was purchased from UCB Pharmaceutical Sector (Chemin du Foriest, Belgium), a range of doses used to determine the dose which induces teratogenic effect.

### Experimental design

**Route of administration:** Oral.

**Time of administration:** Scheduled from the 5<sup>th</sup> day, daily during both gestation and lactation.

### Experimental groups

**Group (A):** Control group received *distilled water* from 5<sup>th</sup> day of gestation to 21 day of lactation.

**Group (B):** Treated group 300mg/kg of *Levetiracetam* from 5<sup>th</sup> day of gestation to 21 day of lactation.

**Group (C):** Treated group 600mg/kg of *Levetiracetam* from 5<sup>th</sup> day of gestation to 21 day of lactation.

### Developmental observations

On the 20<sup>th</sup> day of gestation, all pregnant rats of groups (A, B and C) were sacrificed and total implantation sites, fetal mortality rate (resorbed or still birth) and living fetuses were recorded. On the 7<sup>th</sup>, 14<sup>th</sup> and 21<sup>st</sup> day of lactation respectively the neonates of groups

(D, E and F) were sacrificed. Fetal body weight, body length, tail length and external malformation were recorded.

### **Examination of the external features**

The fetuses were examined for the occurrence of any malformation especially in the limbs, the head and neck area.

### **Sample Preparation**

On the 20<sup>th</sup> day of gestation, all pregnant rats of groups (A, B, and C) were sacrificed by decapitation. On the 7<sup>th</sup>, 14<sup>th</sup> and 21<sup>st</sup> day of lactation respectively, the neonates of treated groups (A, B and C) were sacrificed by decapitation. Parts of the liver, kidney and brain tissues of fetuses of pregnant of different groups were fixed for histological preparation.

### **Skeletal examination**

Fetuses were preserved in 95% ethyl alcohol and were stained with double staining of fetal skeletons for cartilage (Alcian blue) and bone (Alizarin red) solution.

### **Oxidative stress investigation**

A 0.2gm of organ tissue was homogenized in 2ml of phosphate buffer. The homogenate was centrifuged and the clear supernatant was kept in deep freezer at -40°C for oxidative stress studies.

### **Determination of lipid peroxidation**

Lipid peroxidation was determined according to the procedure of Satoh, (1978) and Ohkawa, *et al.*, 1979.

### **Determination of Glutathione reduced**

Glutathione content was determined according to the procedure of Beutler *et al.* (1963).

### **Statistical analysis**

All the values were presented as means ( $\mu$ )  $\pm$  standard errors of the means (S.E.M) comparison between more than two different groups was carried out using the one-way analysis of variance (ANOVA) followed by Turkey-Kramer's multiple comparison test (Armitage and Berry, 1987), where  $P < 0.05$  was considered significant. GraphPad Software InStat (version 2) was used to carry out the statistical tests.

## RESULTS

### Effects of *Levetiracetam* on albino rat fetuses during gestation

#### Fetal mortality

It was found that the mortality rate in the group treated with 300mg/kg *Levetiracetam* did not exceed 0.6% but it reached 10.8% in the other groups.

#### Growth retardation

The morphological examination of fetuses showed that *Levetiracetam* caused growth retardation represented by decrease in fetal body weight, body length and tail length (Table 1).

Table 1: The body weight, body length and tail length of fetuses on the 20<sup>th</sup> day of gestation

Groups of fetuses during gestation	Average body wt. of fetuses	Average body length of fetuses	Average body tail length of fetuses
Group A	3.6009 <sup>a</sup> ± 0.0082	5.0365 <sup>a</sup> ± 0.01882	1.505 <sup>a</sup> ± 0.005
Group B	2.6775 <sup>b</sup> ± 0.061	3.765 <sup>b</sup> ± 0.06	1.223 <sup>b</sup> ± 0.0149
Group C	2.3162 <sup>c</sup> ± 0.087	3.542 <sup>c</sup> ± 0.0859	1.178 <sup>c</sup> ± 0.018

Data are represented as mean ± standard error

Means with the same letter in the same parameter are not significantly different

F-probability expresses the effect between groups, where P<0.05 is very highly significant.

#### Morphological malformations

The malformations found in fetuses maternally treated with *Levetiracetam* were hematoma, anomalies of limbs as well as visceral hernia. The effect *Levetiracetam* on the percentage of hematoma in fetuses on the 20<sup>th</sup> day of gestation is shown in Table (2) and Fig. (1).

External anomalies were mainly anomalies of limbs indicated by oligodactyly (Table 3).

Table 2: Effect of *Levetiracetam* on the percentage of hematoma in fetuses on the 20<sup>th</sup> day of gestation

Groups	No. of examind fetuses	No. of hematoma	Percentage of hematoma (%)
Group A	196	0	0
Group B	193	89	46.113%
Group C	157	91	57.961%



**Fig.1.**Albino Rat fetuses from the different studied groups.

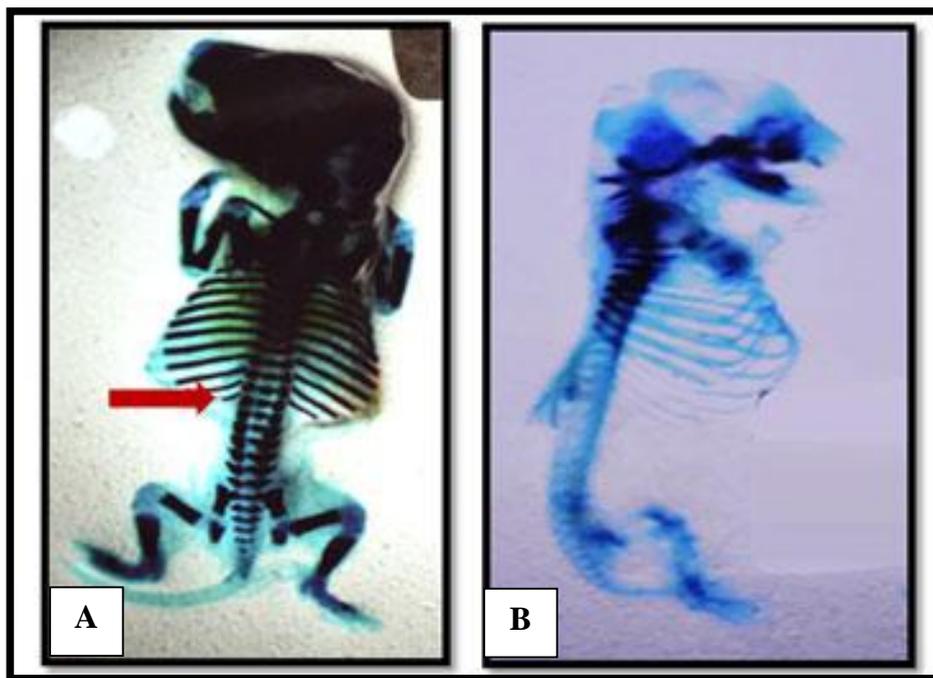
A. Fetus from control group revealing normal external characteristics on 20<sup>th</sup> day of gestation.  
 C, D,E and G. fetuses from group treated with 600mg/kg LEV showed oligodactyly,deformed embryo, and hematoma at the hind limb, hematoma at the neck.  
 B and F. fetuses from group treated with 300mg/kg LEV showeddeformed embryo with short neck **growth retardation.**

**Table 3: Effect of *Levetiracetam* on external anomalies in the fetuses on 20<sup>th</sup> day of gestation.**

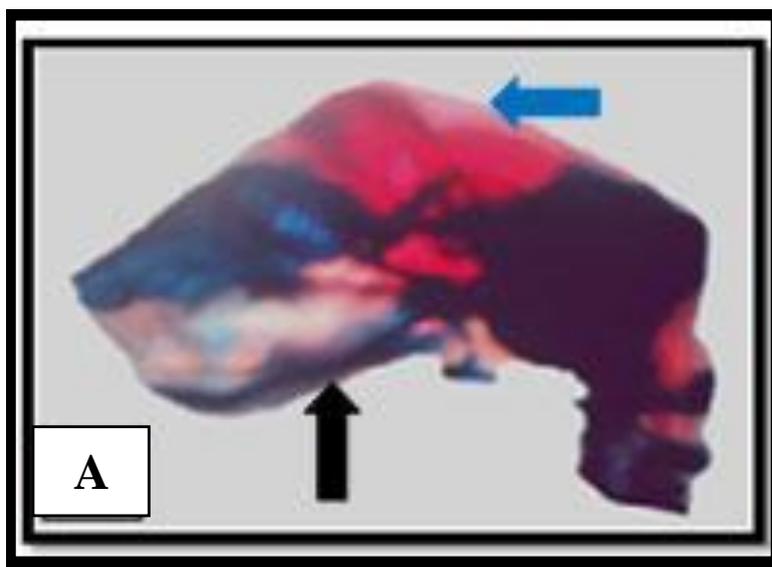
Groups during gestation	No. of fetuses examined	Anomalies of limbs	
		Absence of digits	
		No.	%
Group A	196	-	-
Group B	193	-	-
Group C	157	3	1.91%

**Skeletal examination**

Fetuses to mothers maternally treated with 600mg/kg Levetiracetam from the 5<sup>th</sup> day to the 20<sup>th</sup> day of gestation showed shortness of the 13<sup>th</sup> rib, non-ossification of fore and hind limbs. Metacarpals and phalanges are completely none ossified.Fused ribs, lack of ossification of caudal vertebrae, metatarsals and curved ilium while in the group maternally treated with 600mg/kg Levetiracetamthere was lack of ossification of caudal vertebrae and hind limb, shortness of the 13<sup>th</sup> rib and incomplete ossification of the skeleton as shown in Figure (2 A&B). Mandibular hypoplasia as well as incomplete ossification of roof of the skull (Fig.3).



**Fig. 2.**A photograph of skeletal system of fetus on the 20<sup>th</sup> day of gestation maternally treated with 600mg/kg Levetiracetam from 5<sup>th</sup> day to 20<sup>th</sup> day of gestation; A, shortness of the 13<sup>th</sup> rib (red arrow) and B, incomplete ossification.

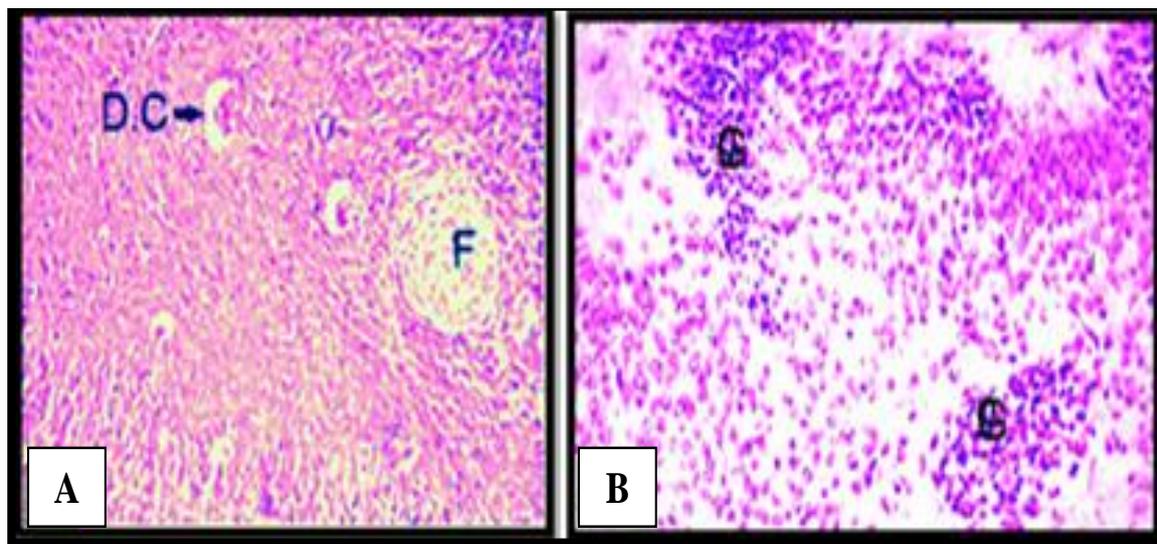


**Fig. 3.**A photograph of skull of fetus on the 20<sup>th</sup> day of gestation maternally treated with 600mg/kg Levetiracetam showing mandibular hypoplasia (arrow) as well as incomplete ossification of roof of the skull (blue arrow).

**Histological studies**

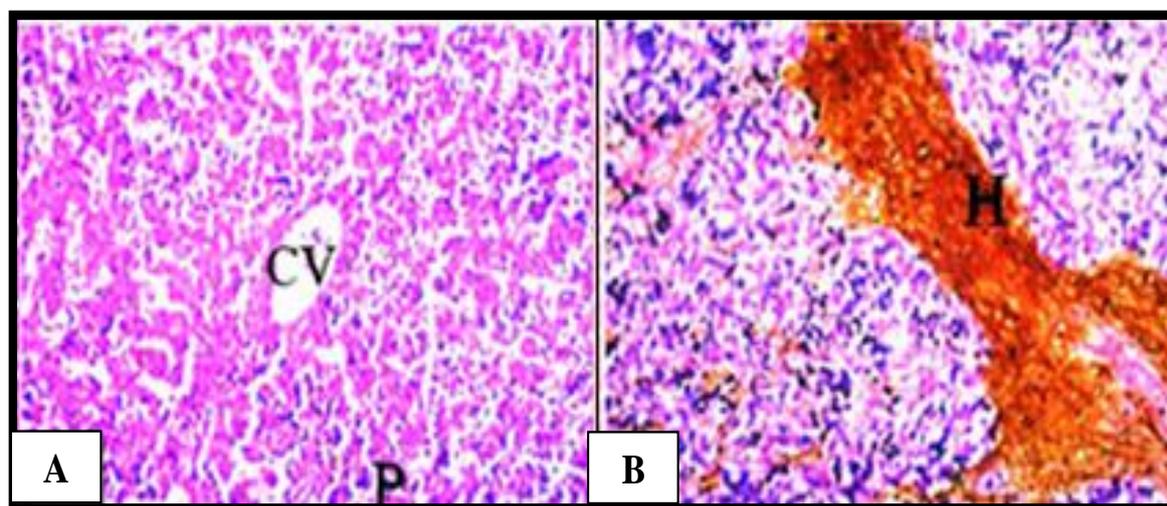
Examination of serial transverse sections of the brain, liver and kidney of albino rat fetuses maternally treated with Levetiracetam on the 20<sup>th</sup> day of gestation showed some histological changes.

**In the brain**, the group of fetuses maternally treated with 600mg/kg Levetiracetam showed the presence of fibrosis, degenerated cell and focal gliosis (figs. 4A&B).



**Fig. 4.** A photomicrograph of brain of fetus on the 20<sup>th</sup> day of gestation maternally treated with 600mg/kg Levetiracetam from 5<sup>th</sup> day to 20<sup>th</sup> day of gestation; A, fibrosis (F) as well as degenerated cell (D.C). H&E 40x and B, focal gliosis (G). H&E 40x.

**In the liver**, the liver of fetuses maternally treated with 300mg/kg Levetiracetam showed focal pigmentation in hepatic parenchyma (Fig. 5A). The liver of fetuses maternally treated with 600mg/kg Levetiracetam showed congestion in blood sinusoids with focal hemorrhage in hepatic parenchyma (Fig. 5B).



**Fig. 5.** A photomicrograph of a section of liver of fetus on the 20<sup>th</sup> day of gestation maternally treated with 300mg/kg Levetiracetam from 5<sup>th</sup> day to 20<sup>th</sup> day; where A, enlarged portion of focal pigmentation in hepatic parenchyma (F.P). H&E 80x and B, congestion in blood sinusoids with focal hemorrhage (H) in hepatic parenchyma. H&E 40x.

**In the kidney**, the fetuses maternally treated with 600mg/kg Levetiracetam showed congested blood vessels and focal hemorrhages in between the tubules (Fig. 6).

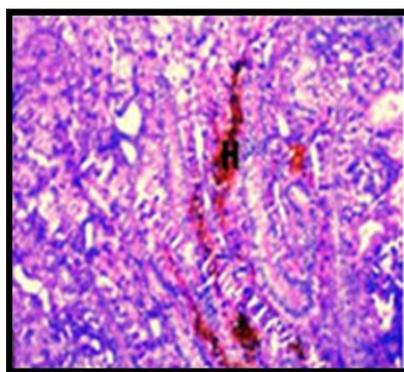


Fig. 6.A photomicrograph of the fetuses maternally treated with 600mg/kg Levetiracetam.

**Oxidative stress investigations during gestation**

**Malondialdehyde**

The treated rat fetuses on the 20<sup>th</sup> day of gestation indicated a slightly increase in brain and liver lipid peroxidation content throughout the experiment compared to control fetuses.

**Glutathione reduced (GSH) content**

Administration Levetiracetam induced a slightly decrease in glutathione content as demonstrated in Table (4).

**Table 4: Effect of Levetiracetam on the GSH content and MDA activity in albino rat fetuses during gestation and lactation.**

Cerebrum		
Parameter	Group	Gestation
GSH	Control	0.0737 <sup>a</sup> ± 0.0004
	Treated (B)	0.0699 <sup>a</sup> ± 0.0014
	Treated (C)	0.0697 <sup>c</sup> ± 0.027
MDA	Control	37.667 <sup>a</sup> ± 0.018
	Treated (B)	37.701 <sup>a</sup> ± 0.024
	Treated (C)	37.707 <sup>a</sup> ± 0.026
Cerebellum		
Parameter	Group	Gestation
GSH	Control	0.0540 <sup>a</sup> ± 0.0008
	Treated (B)	0.0479 <sup>a</sup> ± 0.0181
	Treated (C)	0.0466 <sup>a</sup> ± 0.0016
MDA	Control	34.825 <sup>a</sup> ± 0.007
	Treated (B)	34.851 <sup>a</sup> ± 0.015
	Treated (C)	35.034 <sup>a</sup> ± 0.168
Liver		
Parameter	Group	Gestation
GSH	Control	0.1515 <sup>a</sup> ± 0.0007
	Treated (B)	0.1462 <sup>a</sup> ± 0.0016
	Treated (C)	0.1432 <sup>a</sup> ± 0.0025

MDA	Control	22.418 <sup>a</sup> ± 0.0038
	Treated (B)	23.075 <sup>a</sup> ± 0.2025
	Treated (C)	23.2 <sup>a</sup> ± 0.1006

Data are expressed as mean ± Standard error (N=6)

Means with the same letter in the same parameter are not significantly different

### Effects of Levetiracetam on albino rat neonates during lactation

#### Growth retardation

The morphological examination of neonates maternally treated with Levetiracetam showed that growth retardation represented by decrease in body weight, body length and tail length according to control neonates on the 7<sup>th</sup>, 14<sup>th</sup> and 21<sup>st</sup> day of lactation (Table 5).

**Table 6: The body weight, body length and tail length of neonates on 7<sup>th</sup>, 14<sup>th</sup> and 21<sup>st</sup> days of lactation.**

Groups During lactation	Average body wt. of neonates			Average body length of neonates			Average tail length of neonates		
	7 <sup>th</sup> day	14 <sup>th</sup> Day	21 <sup>st</sup> day	7 <sup>th</sup> day	14 <sup>th</sup> day	21 <sup>st</sup> Day	7 <sup>th</sup> day	14 <sup>th</sup> day	21 <sup>st</sup> Day
Group D	12.168 <sup>a</sup> ± 0.181	23.366 <sup>a</sup> ± 0.539	40.169 <sup>a</sup> ± 1.014	6.669 <sup>a</sup> ± 0.124	10.719 <sup>a</sup> ± 0.172	11.775 <sup>a</sup> ± 0.367	2.573 <sup>a</sup> ± 0.103	6.475 <sup>a</sup> ± 0.088	7.457 <sup>a</sup> ± 0.149
Group E	10.499 <sup>b</sup> ± 0.355	21.168 <sup>b</sup> ± 0.70445	32.298 <sup>b</sup> ± 0.624	6.454 <sup>b</sup> ± 0.161	10.005 <sup>b</sup> ± 0.3463	11.618 <sup>b</sup> ± 0.802	2.418 <sup>b</sup> ± 0.125	6.392 <sup>b</sup> ± 0.114	7.171 <sup>b</sup> ± 0.1405
Group F	9.238 <sup>c</sup> ± 0.861	18.034 <sup>c</sup> ± 1.006	29.288 <sup>c</sup> ± 1.356	6.095 <sup>c</sup> ± 0.653	8.891 <sup>c</sup> ± 0.458	10.754 <sup>c</sup> ± 0.501	2.260 <sup>c</sup> ± 0.163	6.300 <sup>c</sup> ± 0.152	7.217 <sup>c</sup> ± 0.138

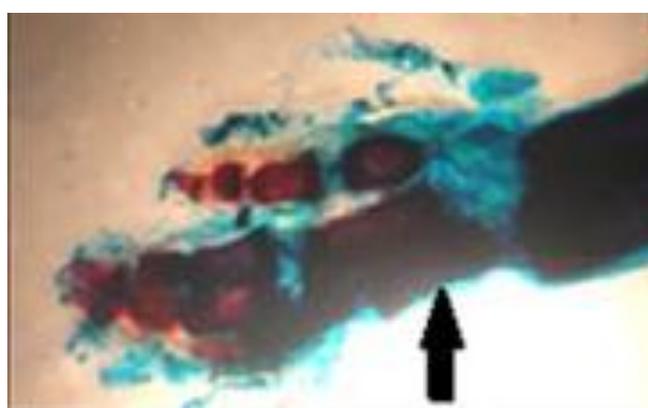
Data are expressed as mean ± Standard error (N=6)

Means with the same letter in the same parameter are not significantly different

F-probability expresses the effect between groups, where P<0.05 is very highly significant.

#### Skeletal examination

On 7<sup>th</sup> day of lactation, the group maternally treated with 300mg/kg Levetiracetam showed shortness of right 13<sup>th</sup> rib and absence of left 13<sup>th</sup> rib while the group maternally treated with 600mg/kg Levetiracetam showed fused metacarpus (Fig. 7).



**Fig. 7.A photomicrograph of the group maternally treated with 300mg/kg Levetiracetam**

### Histological studies

Examination of serial transverse sections of the brain, liver and kidney of albino rat neonates maternally treated with Levetiracetam on the 7<sup>th</sup>, 14<sup>th</sup>, and 21<sup>st</sup> days of lactation showed some histological changes.

**In the cerebrum**, the group maternally treated with 300mg/kg Levetiracetam on the 7<sup>th</sup> day of lactation showed pyknotic cell in the matrix of striatum in the cerebrum, degenerated cells and vacuoles (Fig. 8). The group maternally treated with 600mg/kg *Levetiracetam* on the 7<sup>th</sup> day of lactation showed vacuolization, degenerating cells and pyknotic nuclei in the matrix of striatum in the cerebrum, focal gliosis and degeneration neuron.

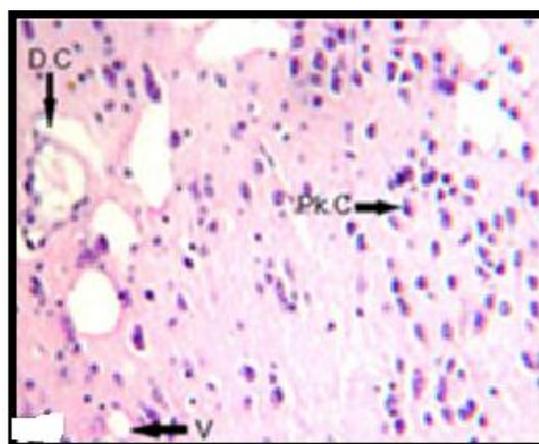


Fig.8. A photomicrograph of a section of brain section of neonates on the 7<sup>th</sup> day of lactation maternally treated with 300mg/kg Levetiracetam from 5<sup>th</sup> day of gestation to 7<sup>th</sup> day of lactation showing Pyknotic cell (Pk.C) in the matrix of striatum in the cerebrum, degenerated cell (D.C) and vacuole (v). H&E 40x.

The cerebellum of neonates maternally treated with 300mg/kg Levetiracetam on the 14<sup>th</sup> day of lactation showed neuron degeneration in purkinje cell layer. The group of neonates maternally treated with 600mg/kg Levetiracetam on the 14<sup>th</sup> day of lactation showed neuron degeneration in purkinje cell layer and vacuolization in medulla white matter (Fig. 9).

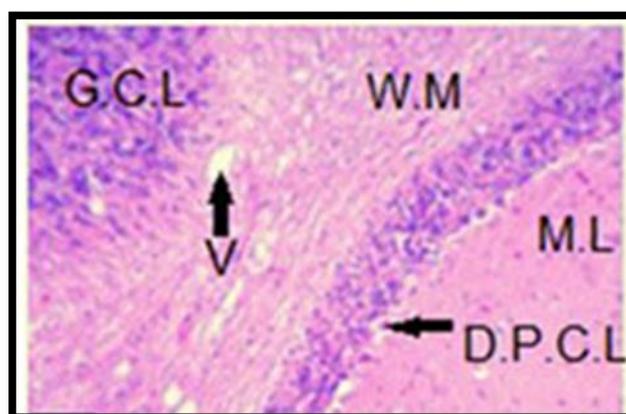


Fig. 9. A photomicrograph of a section of brain of neonates on the 14<sup>th</sup> day of lactation maternally treated with 600mg/kg Levetiracetam from 5<sup>th</sup> day of gestation to 14<sup>th</sup> day of lactation showing neuron degeneration purkinje cell layer (D.P.C.L) and vacuolization (V) in medulla white matter (W.M). H&E 40x.

The liver of neonates maternally treated with 300mg/kg Levetiracetam on the 7<sup>th</sup> day of lactation revealed histopathological changes which appeared as severe dilation and congestion in the central vein and portal veins. While those treated with 600mg/kg Levetiracetam on 7<sup>th</sup> day of lactation showed severe dilation and congestion in portal veins with inflammatory cell infiltration (Fig. 10).

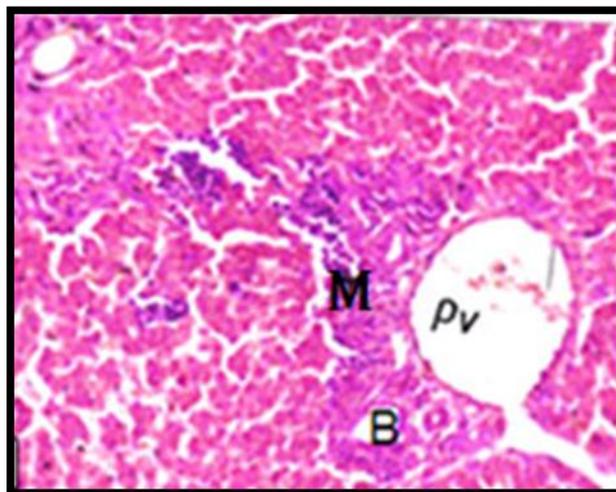


Fig. 10.A photomicrograph of a section of the liver of fetus on the 7<sup>th</sup> day of lactation maternally treated with 600mg/kg Levetiracetam from 5<sup>th</sup> day of gestation to 7<sup>th</sup> day of lactation showing severe dilation and congestion in portal veins (p.v) with inflammatory cell infiltration (m). H&E40x.

The group of neonates maternally treated with 300mg/kg Levetiracetam on the 14<sup>th</sup> day of lactation showed fibrosis in addition to that leucocytes with multiple dilated bile ductules and degenerated hepatocytes (Fig.11).

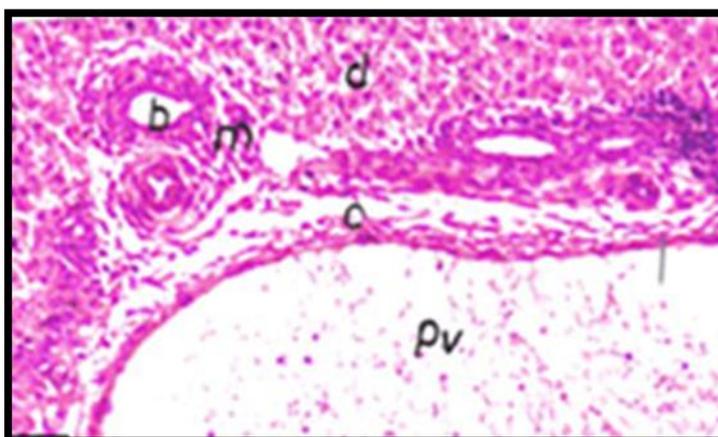


Fig.11.A photomicrograph of a section of the liver of neonates on the 14<sup>th</sup> day of lactation maternally treated with 600mg/kg Levetiracetam from 5<sup>th</sup> day of gestation to 14<sup>th</sup> day of lactation showing dilation in portal veins (p.v) with odema, few leucocytes infiltration in portal area (m), multiple dilated bile ductules (b) and degenerated hepatocytes (d). H&E 40x.

**In the kidney,** the kidney of neonates maternally treated with 300mg/kg Levetiracetam on the 7<sup>th</sup> day of lactation showed shrinking in glomeruli, detached cell of tubules, Pyknotic nuclei and \hydropic degeneration. The group of neonates maternally

treated with 600mg/kg Levetiracetam on the 7<sup>th</sup> day of lactation showed acute cellular swelling in the lining epithelium of the tubules at the cortex and glomerulus. The kidney of neonates maternally treated with 300mg/kg Levetiracetam on the 14<sup>th</sup> day of lactation showed hyperplasia and dysplasia in the lining epithelium of the tubules at the cortex (Fig. 12).

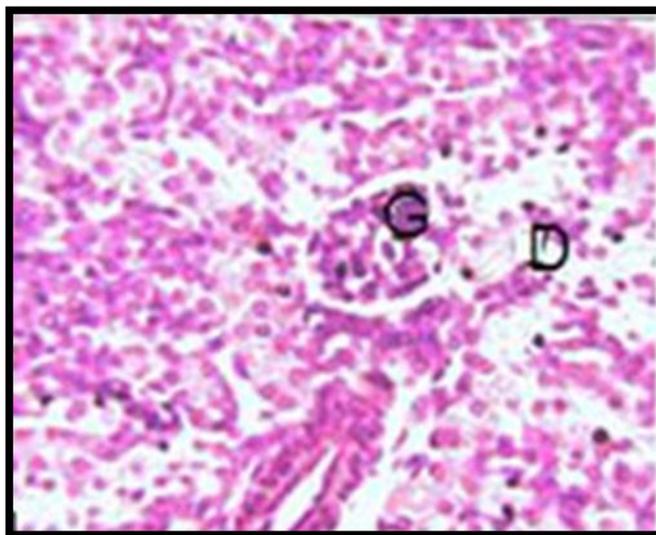


Fig.12.A photomicrograph of a section of the liver of neonates maternally treated with 300mg/kg Levetiracetam.

## Oxidative stress investigations during lactation

### Malondialdehyde

The treated rat neonates on the 7<sup>th</sup>, 14<sup>th</sup> and 21<sup>st</sup> day of lactation indicated a slightly increase in cerebrum, cerebellum and liver lipid peroxidation content throughout the experiment as compared to control neonates.

### Statistical analysis

Concerning one way (ANOVA) of lipid peroxide content, it was clearly established that general effect in-between groups was not significant (table 7).

### Glutathione reduced (GSH) content

The treated rat neonates on the 7<sup>th</sup>, 14<sup>th</sup> and 21<sup>st</sup> days of lactation indicated a slightly decrease in cerebrum, cerebellum and liver glutathione content throughout the experiment as compared to control neonates.

### Statistical analysis

Concerning one way (ANOVA) of glutathione content, it was clearly established that general effect in-between groups was not significant (Table 7).

**Table 7: Effect of Levetiracetam on the GSH content and MDA activity in albino rat fetuses during lactation.**

Cerebrum				
parameter	Group	D7	D14	D21
GSH	D	0.0477 <sup>a</sup> ± 0.0006	0.0542 <sup>a</sup> ± 0.05	0.0521 <sup>a</sup> ± 0.0007
	E	0.0456 <sup>a</sup> ± 0.05	0.0514 <sup>a</sup> ± 0.05	0.0508 <sup>a</sup> ± 0.0006
	F	0.0452 <sup>a</sup> ± 0.0003	0.0485 <sup>a</sup> ± 0.0115	0.0494 <sup>a</sup> ± 0.0005
MDA	D	24.427 <sup>a</sup> ± 0.0038	18.329 <sup>a</sup> ± 0.007	22.376 <sup>a</sup> ± 0.0023
	E	24.435 <sup>a</sup> ± 0.0025	18.331 <sup>b</sup> ± 0.004	22.389 <sup>a</sup> ± 0.0027
	F	24.439 <sup>a</sup> ± 0.0116	18.336 <sup>c</sup> ± 0.002	22.428 <sup>a</sup> ± 0.0169
Cerebellum				
parameter	Group	D7	D14	D21
GSH	D	0.0443 <sup>a</sup> ± 0.0003	0.0411 <sup>a</sup> ± 0.0002	0.0426 <sup>a</sup> ± 0.0002
	E	0.0436 <sup>a</sup> ± 0.0007	0.0427 <sup>a</sup> ± 0.05	0.0418 <sup>a</sup> ± 0.05
	F	0.0426 <sup>a</sup> ± 0.05	0.0421 <sup>a</sup> ± 0.0006	0.0416 <sup>a</sup> ± 0.0006
MDA	D	22.315 <sup>a</sup> ± 0.0033	13.842 <sup>a</sup> ± 0.003	14.219 <sup>a</sup> ± 0.0181
	E	23.321 <sup>a</sup> ± 0.0035	13.847 <sup>a</sup> ± 0.003	14.237 <sup>a</sup> ± 0.019
	F	23.546 <sup>a</sup> ± 0.0036	13.849 <sup>a</sup> ± 0.002	14.253 <sup>a</sup> ± 0.015
Liver				
parameter	Group	D7	D14	D21
GSH	D	0.0550 <sup>a</sup> ± 0.0002	0.0545 <sup>a</sup> ± 0.0002	0.0549 <sup>a</sup> ± 0.0003
	E	0.0546 <sup>a</sup> ± 0.0015	0.0534 <sup>a</sup> ± 0.0007	0.0527 <sup>a</sup> ± 0.0008
	F	0.0488 <sup>a</sup> ± 0.0026	0.0481 <sup>a</sup> ± 0.0015	0.0514 <sup>a</sup> ± 0.006
MDA	D	29.538 <sup>a</sup> ± 0.207	17.274 <sup>a</sup> ± 0.0034	45.365 <sup>a</sup> ± 0.001
	E	29.95a ± 0.0116	17.277 <sup>a</sup> ± 0.0202	45.804 <sup>b</sup> ± 0.009
	F	30.089 <sup>b</sup> ± 0.0054	17.353 <sup>b</sup> ± 0.0134	46.185 <sup>c</sup> ± 0.228

Data are expressed as mean ± S.D. (N=6)

Means with the same letter in the same parameter are not significantly different

F-probability expresses the effect between groups, where P<0.05 is significant.

### DISCUSSION

Maternal antiepileptic drug use is associated with an increased frequency of adverse pregnancy outcome including congenital malformations (Samr'en EB *et al.*, 1999). However, continuation of medication during pregnancy is often necessary to prevent seizures which would be harmful to mother and fetus.

Use of older generation antiepileptic drugs such as phenobarbital, phenytoin, valproate, and carbamazepine during pregnancy has been associated with an approximately 3-fold increased risk of birth defects (Meador K *et al.*, 2008). Recently, some new antiepileptic drugs (AEDs) have been launched, promising better antiepileptic properties and fewer side effects; however, the teratogenicity of this new generation of AEDs in humans is still unknown. The same holds true for levetiracetam (LEV), which has been registered for treatment of partial epilepsy and seems to be effective in idiopathic generalized epilepsy as well (Krauss GL *et al.*, 2003). It is used mostly as adjunctive therapy in doses of 1000–3000 mg/day. The drug is chemically unrelated to existing AEDs and precisely how it prevents seizures is unknown.

The present study was designed to evaluate the teratogenic effect of LEV on the growth and development of the fetuses of albino rats. Since selected doses (300mg/kg and 600mg/kg) were relatively low compared with toxic doses, we did not have any unexpected deaths until the end of the study, so all pregnant rats survived but fetal mortality was increased specially with the highest dose 600 mg/kg during the critical embryogenic period. In our study the rate of abortion in pregnant rats was increased in dose -dependent manner reaching the highest level in the dose 600 mg/kg Levetiracetam. In the present study, growth retardation of the fetuses and increased skeletal and internal organs variations were the major abnormalities observed in maternal Levetiracetam administration.

The morphological changes observed in the present work proved that Levetiracetam caused gross malformations, subcutaneous hematoma in different sites, abnormalities in limbs, specially paralysis and absence of digits. Our study showed that the hepatocellular lesions occurred in those rat fetuses treated with a higher dose of LEV. Severe dilation, congestion in central veins, portal veins, fibrosis, leucocytes infiltration in the portal area, multiple dilated bile ductules and degenerated hepatocytes detected in the liver sections. These findings are in agreement with the study of (Newsome *et al.*, 2007) who stated that levetiracetam was implicated in the pathogenesis of interstitial pneumonitis suggesting that long term use of levetiracetam could precipitate a diffuse interstitial pneumonitis-like reaction (El Khayat HA *et al.*, 2012)

In the current study, dose- and time-dependent degenerative changes were observed in the kidney. During 14 days after Levetiracetam administration, degenerative changes were detected in the two dose groups. Increased histopathological findings were observed in 18<sup>th</sup> days of administration such as focal hemorrhages in between the tubules and failure of junction between proximal and distal tubule. While in case of lactation our study revealed shrinking in glomeruli, acute cellular swelling in lining epithelium of the tubules at the cortex and Hydropic degeneration. Kathleen *et al* (2009), added Levetiracetam to the list of drugs causing interstitial nephritis in human. Levetiracetam pretreatment caused a non-significant effect on the level of lipid peroxidation. No significant correlation was noted between GSH levels and the anticonvulsant effects of Levetiracetam. Thus administration of LVE during pregnancy and lactation should only be considered if the expected benefit to the mother is greater than any possible risk to the fetus. To sum up the present study proved that care should be taken if Levetiracetam was administered with high dose during pregnancy and lactation for its effect were highly noticeable yet Levetiracetam itself is not discontinued during both periods.

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