International Journal of Research and Development in Pharmacy and Life Sciences
Available online at http//www.ijrdpl.com
October - November, 2016, Vol. 5, No.6, pp 2388-2396
ISSN (P): 2393-932X, ISSN (E): 2278-0238

Research Article

STUDY ON EFFECTS OF CARBAMAZEPINE AND PHENYTOIN ON DEVELOPMENT OF CHICK EMBRYO

Anil Kumar Reddy*[1], Fulzele R R[2], UjwalGajbe[2], Kondam Ambareesha[3]

1. Department of Anatomy, ANIIMS, Port Blair, Andaman and Nicobar Islands, India
2. Department of Anatomy, J.N.M.C, Sawangi (Meghe), Wardha, Maharashtra, India
3. Assistant Professor, Department of Physiology, MNR Medical College& Hospital, Sangareddy, Telangana, India-502001

***Corresponding author's Email:** kumarlucky48@gmail.com

(Received: July 28, 2016; **Accepted:** September 11, 2016**)**

ABSTRACT

The knowledge about the Teratogenic drugs is crucial while prescribing medications to the pregnant women. Treating epileptic pregnant women is always challenge as most of antiepileptic drugs are potent teratogens and the increase of seizures and alterations in drug clearance further complicates the medication advice to pregnant women with seizure disorder. The present experimental study used fresh fertilized white leg horn chicken eggs to know the effects of carbamazepine and phenytoin administration during critical period of organogenesis i.e. on 5th day of incubation. Control eggs were injected distilled water and the study group were injected by various doses of drug concentration in distilled water. Chick embryos were collected on 19th day of incubation, first gross malformation were observed and recorded. Liver tissue has been collected from chick embryos for histological study. High mortality rate observed in chick embryos treated with higher dose of drug concentration. Hepatic damage, loss of normal architecture of liver, vacuolations, dilated central vein, inflammatory infiltration with significant portal inflammation observed. The mortality rate with more number of gross malformations recorded in phenytoin administered group than carbamazepine.

Keywords: Antiepiletptic drugs, pregnant women, Teratogenicity, chick embryo, central vein.

INTRODUCTION

Embryogenesis is an intricate process that can easily be disrupted by means of teratogenic agents. As we know that most of the Antiepileptic drugs (AED) drugs are teratogenic agents such as phenytoin and carbamazepine[1]. The use of AED is the first line of treatment to treat the epilepsy patients, but treating epileptic pregnant women's is a risk process[2]. Adverse effects from phenytoin and carbamazepine medications are reported in several experimental studies; the range of teratogenicity are dose related. Phenytoin and Carbamazepine are the most frequently prescribed AED.

Phenytoin (diphenylhydantoin sodium, dilantin) is an antiepileptic drug useful to treat partial seizures and generalized tonic-clonic seizures. Phenytoin is believed to protect against seizures by causing voltage-dependent block of voltage-gated sodium channels[3]. It is marked with trade name DILANTIN, FENTOIN-ER, EPSOLIN in India. Commonly prescribed doses are 100 mg twice daily, 400 mg/day

©SRDE Group, All Rights Reserved.

maximum dose, children 5–8 mg/kg/day. The primary site of action appears to be the motor cortex where spread of seizure activity is inhibited[4]. Phenytoin reduces the maximal of brain stem centres responsible for the tonic phase of tonic-clonic (grand mal) seizures. Phenytoin acts to dampen the unwanted, runaway brain activity seen in seizure by reducing electrical conductance among brain, it lacks the sedation effects associated with phenobarbital. It has a basic action to block Na^+ and Ca^{2+} channels and also used for other clinical cases like treatment of various CNS disorders[4],[5]. The majority of the dose (up to 90%) is metabolized to 5-(4'-hydroxyphenyl)-5-phenylhydantoin (p-HPPH). This metabolite undergoes further glucuronidation and is excreted into the urine. CYP2C19 CYP2C9 catalyse the aforementioned reaction[3].

Nowadays, Phenytoin is less frequently preferred in women with epilepsy due to increase of incidences reported in major malformations and also phenytoin clearance is more in pregnancy associated with a fall in plasma concentrations and possible loss of seizure control[6]. The pharmacokinetic changes of earlypregnancy and postpartum occur more slowly with phenytoin than other AED[4],[6].

In humans, pregnancy registry indicate that increase rate of infants whose epileptic mothers were under prolonged phenytoin treatment during pregnancy presented with FetalHydantoin Syndrome (FHS) with characteristic physical features craniofacial anomalies which include nasal bridge, cleft lip, cleft palate and microcephaly[6],[7].

The reports associated with results of carbamazepine usage in pregnant women indicated higher risk of structural birth defects including spina bifida[8]. However, nopregnancy register has yet shown any statistically significant increase in riskrelative to the total population.Modest pharmacokinetic changes occur during late pregnancy, but dose changes are not usually required. Carbamazepine is compatible withbreastfeeding in the full-term infant[9].

Carbamazepine (CBZ) drug was synthesized by Schindler in 1960. First it was used as a drug to treat the trigeminal neuralgia, but in 1965 onwards it has been uses as an anticonvulsant and antiepileptic drug in UK. CBZ also used for mood-stabilizing drug, bipolar disorder and in trigeminal neuralgia. It is marked under the brand name called Tegretol or Equetro.The primary usage of carbamazepine for the treatment of epileptic seizures, neuropathic pain, bipolar disorder, as well as trigeminal neuralgia[10]. It is also used for variety of indications such as hyperactivity disorder, schizophrenia, phantom limb syndrome, complex regional pain syndrome, neuromyotonia, borderline personality disorder, post-traumatic stress disorder. The usage of CBZ during pregnancy causes mainly neural tube defects like spina bifida and neurodevelopmental problems. The intrauterine exposure of CBZ can also cause birth defects that includes cleft palate, fingernail hypoplasia, microcephaly, cardiovascular and urinary tract anomalies[9],[10]. The association between maternal use of carbamazepine and its role in formation of major malformations is not very well understood. Oxcarbazepine, a derivative of carbamazepine, reportedly has fewer and less serious side-effects.

Several authors have been done experiments on chick embryo to know the teratogenic effects of AED. The selected AED for study are known teratogenic effects on developing foetus, however experimental studies always have advantages over pregnancy registry to know the critical period of exposure, and dose related effects, lethal dose and mechanism of teratogenicity. The current experimental study aimed to investigate teratogenic effects of carbamazepine and phenytoin by injecting into developing chick embryo. Thereby, it was also aimed to determine potential results with its use during pregnancy.

MATERIALS AND METHODS:

The study was approved by the Institutional Ethical Committee, which has duly authorized by CPCSEA for animal experiments.The experimental study was carried in department of anatomy, J N Medical College, Sawangi, Wardha. Three Hundred and Twenty (320) fresh fertilized eggs of white leghorn chicken were incubated at 37 ± 1^0C (60-80% humidity). The eggs with cracked shell, unfertile eggs, double yolks, longs stored eggs, decomposed eggs were excluded from the study. Only fresh fertilized pathogen free white leghorn chicken eggs are used.

Two most commonly used AED are selected for the study i.e. Phenytoin and Carbamazepine. In the first set of experiment group, Eggs were treated with Dilantin and second set of experiment group were treated with Carbamazepine. In each set of experiment, eggs were divided into four groups

©SRDE Group, All Rights Reserved.

i.e. Group A, B, C & D. 40 eggs were allotted to each group, eggs were injected on 5th day of incubation.

First set of experiment group were treated with Carbamazepine. After washing with 70% alcohol, eggs were labelled as C1, C2, C3 and C4. Each group contains 40 eggs. C1 group injected with 10 µL distilled water, subsequent groups C2, C3 and C4 were given single injection with Carbamazepine concentration 0.9mg/egg, 2.1mg/egg, 4.2mg/egg in 10 µL distilled water.

Second set of experiment group; eggs were removed from the incubation, washed with 70% alcohol and properly labelled on the outer shell according to group wise as P1, P2, P3 and P4. Each group having 40 eggs. The control eggs i.e. P1 group were treated with 10 µL distilled water, Subsequently P2, P3, P4 was treated with single dose injection of Phenytoin concentration 0.8mg/egg, 1.6mg/egg, 3.2mg/egg in 10 µL distilled water.

Method of Injection:

The eggs were injected to the air cell cavity present at the blunt or round end of the egg by making small window (hole) of 4mm with forceps without breaking the shell. The fluid was injected by using sterile 28-gaugeneedle, the hole were sealed quickly after the injection with paraffin wax or sterilized plaster. Eggs were turned twice a day by a mechanical device, temperature was carefully monitored throughout the period of incubation.

Viscera Collection:

The eggs are opened at 19th day of incubation by breaking the egg shell, chick embryos were collected and examined for viability, gross malformations. The viability of the embryos was assessed by the heartbeat. The chick embryos were dissected, liver tissue was taken for morphological and histological examination. The collected viscera were fixed in 10% buffered formalin (pH 7.2) and dehydrated through a series of ethanol solutions, embedded in paraffin wax and paraffin blocks were prepared. Sections of 5µm thicknesses were cut by using a rotary microtome. Sections are stained with Haematoxyline-Eosin (H&E) and then examined under light microscope.

RESULTS:

Out of 320 eggs, 160 eggs were allotted for each drug for experiment. Carbamazepine was used in the first experiment and the second experiment group were treated with Phenytoin. The chick embryos were collected on 19th day of incubation just before completion of hatching period (21days) and examined for viability and other gross malformations of control (P1, C1), and drug injected groups are given in the table 1.

Histopathological Observations:

The morphological characteristics and the incidences of teratogenic effects on the liver of chick embryos in the control and experimental groups were compared with each other.

Table 1: Percentage of viability and gross deformities seen in Phenytoin and Carbamazepine treated groups.

S. No	Deformities	Experiment - I				Experiment - II			
		C1	C2	C3	C4	P1	P2	P3	P4
1	Mortality	2.5%	12.5%	25%	95%	5%	17.5%	30%	100%
2	Stunted growth	5%	5%	95%	100%	2.5%	Generalised decrease	95%	---
3	Beak deformity (Short beak)	Nil	2.5%	40%	100%	Nil	5%	55%	---
4	Feathers	Abundant	Normal	Scanty	Scanty	Normal	Scanty	Scanty	---
5	Wings	Normal	Normal	Relatively shorter	shorter	Normal	shorter	shorter	---
6	Ectopiacordis	Nil	Nil	70%	100%	Nil	Nil	75%	---
7	Visceroptosis	Nil	Nil	Nil	50%	Nil	Nil	15%	---

©SRDE Group, All Rights Reserved.

First experiment: Total 160 eggs were divided into four groups, 40 eggs were allotted to each group and the eggs are treated with various concentration of Carbamazepine started with therapeutic dose based on weight.

Group C1:

The control group in which the eggs were administered with single injection of 10 µL distilled water. On histological examination of liver sections showed normal histological architecture with central vein, radiating hepatic cords are observed. Small sinusoids with well-developedbig size hepatocytes were seen. Maturing blood cells are seen inside the vessels (Fig. 2.1 & 2.2).

Group C2:

The Histological examination of the livertreated with 0.9mgconcentration of Carbamazepine per egg revealed mild disturbances in architecture of liver with numerous small tiny vacuoles (hydropic degeneration), these vacuoles have indefinite borders. The major portion of the section showed disturbed hepatic card arrangements, stagnant blood cells and sinusoids were also widened (Fig 3).

Group C3:

This group injected with single injection of 2.1mg/eggconcentration of Carbamazepine. On observation of liver sections showed enlarged central vein,periportal inflammation,large number of vacuoles with disturbed endothelial lining. Some of the sections showed existence of inflammatory leucocytes infiltration in the portal area composed mainly of lymphocytes (Fig 4.1 & 4.2).

Group C4:

This group injected with higher dose of Carbamazepine as single injection of 4.2mg/egg. Only 2 (5%) chick embryos were viable out of 40. Most of the embryos were dead at early stages of development. Sections of liver tissue showed severe hepatic damage with complete loss of normal architecture, enlarged hepatocytes, infiltration of inflammatory cells seen in higher quantity (Fig 5).

Second experiment: 40 eggs are allotted to each group out of 160 eggs. Chick embryos are treated with various concentration of Phenytoin started with therapeutic dose based on weight.

Group P1:

The chick embryos of control group were given single injection of 10 µL distilled water at 5th day of incubation. On microscopic examination of sections of liver showed central vein, regular pattern of radiating large hepatic cells with small sinusoids. The sections are similar to the C1 group in the first experiment (Fig 2.a, 2.b).

Group P2:

The Histological examination of the livertreated with single injection of 0.8mg/egg Phenytoin diluted in 10 µL distilled water. The higher degree of sections shows loss of normal architecture, marked loss of the uniformity and regularity of the hepatic cords. In some of the sections hepatocytes lost their hexagonal shape and vacuolations in their cytoplasm with pyknosis of nuclei (Fig 6).

Group P3:

The increased concentration of phenytoin (1.6mg/egg) administered chick embryos showed adverse effect than previous groups. Complete loss of normal architecture with necrotic hepatocytes are seen throughout the sections. Increased amount of reticular fibres with lymphocytic infiltration observed in several sections. The cytoplasm shows large vacuoles (Vacuolar degeneration) with indefinite borders. In some sections, we found there was focal necrosis of the hepatic cells and infiltration of inflammatory cells in portal area. The degenerated cells shows slight cytoplasmic mass forming a narrow peripheral rim. Hepatocytes were highly damaged exhibiting marked hydropic degeneration, characterized by extensive vacuolization of the cytoplasm (Fig 7.1 & 7.2).

Group P4:

The chick embryos administered with single injection of lethal dose of Phenytoin (3.2mg/egg) at 5th day of incubation. At the time of opening of eggs, it was found that all the embryos were dead during early stages of development.

DISCUSSION:

The early rapid development of large size chick embryo, its accessibility for visualization, experimental manipulation makes this model best for biological research and it has significant similarities with the human embryo at the molecular, cellular and anatomical levels[11].

The greatest problem is pregnant women with epilepsy in all over the world and on which several studies are being conducted to understand the teratogenic effect of antiepileptic drugs on embryo[12]. The frequency of seizures increases about 20% in pregnant women and allows to a

©SRDE Group, All Rights Reserved.

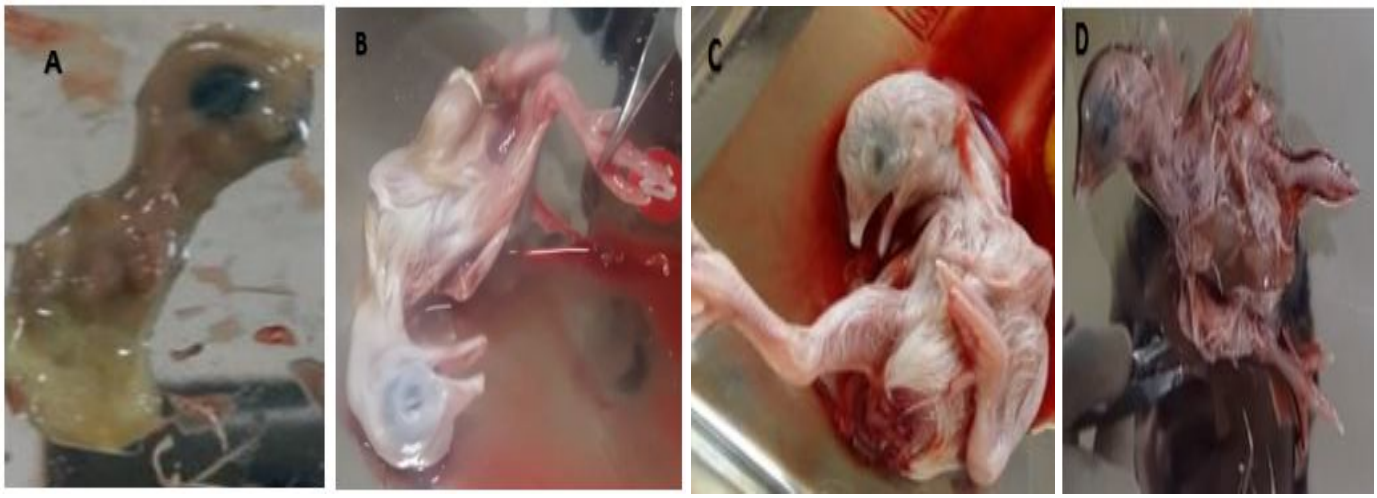

Fig 1: Chick embryo showing Gross anomalies

A – Dead embryo at early stages, B – Stunted Growth, C – Visceroptosis, D – Ectopia Cordis.

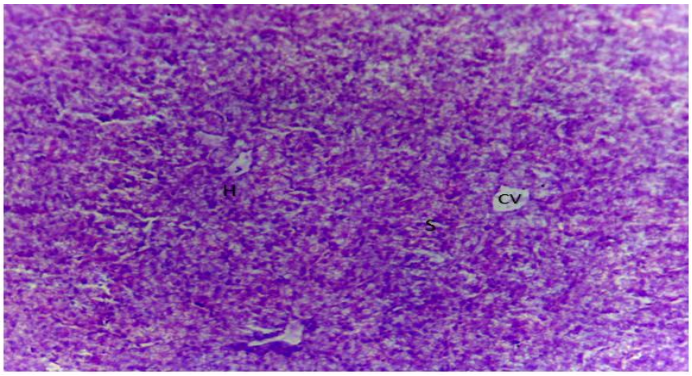

Fig 2: Histological Section of Liver of control (C1 & P1) chick embryo showing normal architecture with Central vein (CV), Small Sinusoids (S), Hepatocytes (H).

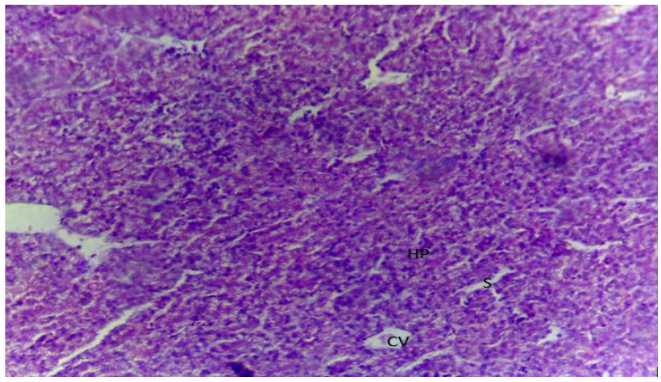

Fig 3: Histological Section of Liver of Group C2 Showing Mild disturbances in architecture of liver, Central vein (CV), numerous vacuolations (V), and Disturbed hepatic cord (HP) arrangements.

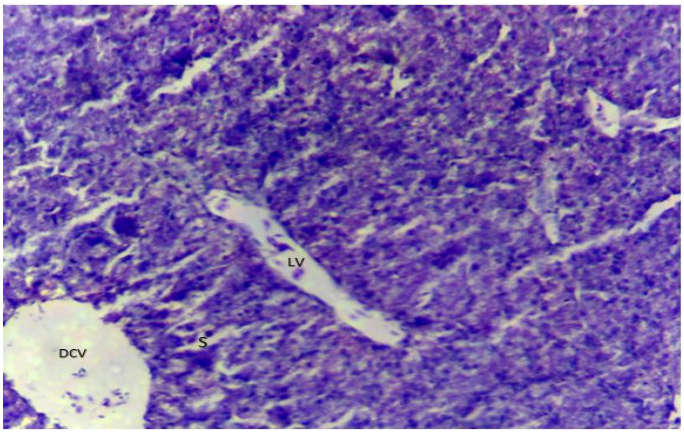

Fig 4.1: Histological Section of Liver of Group C3 Showing Dilated central vein (DCV), Large Vacuoles (LV), enlarged sinusoids (S) with disturbed endothelial lining.

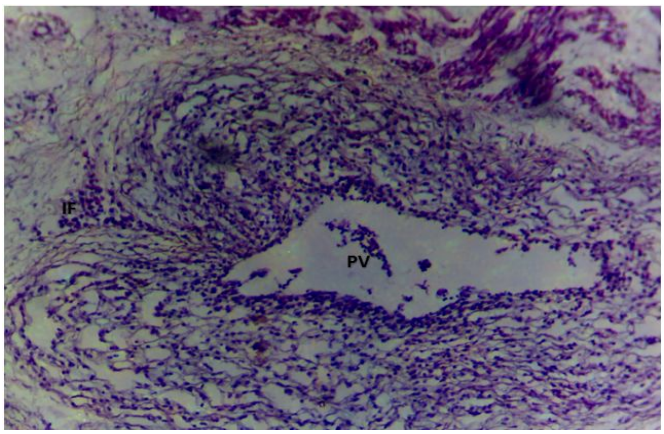

Fig 4.2: Histological Section of Liver of Group C3 Showing Portal vein (PV), infiltration of lymphocytes and neutrophils (IF)

©SRDE Group, All Rights Reserved.

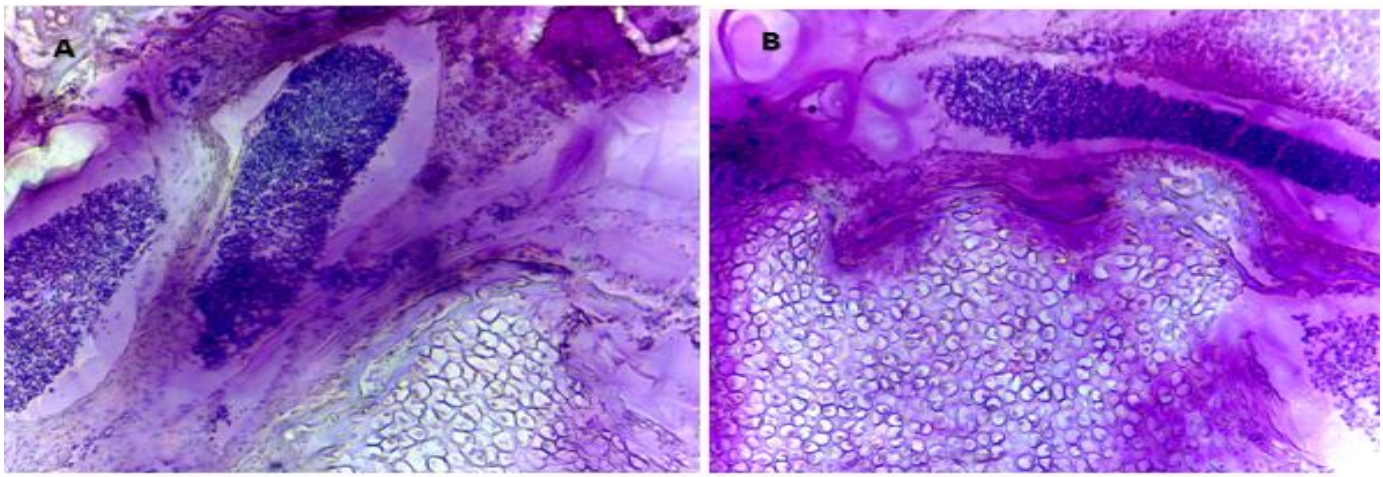

Fig 5: Section of Liver treated with higher dose of (4.2mg/egg) carbamazepine showing severe hepatic damage with complete loss of architecture of liver.

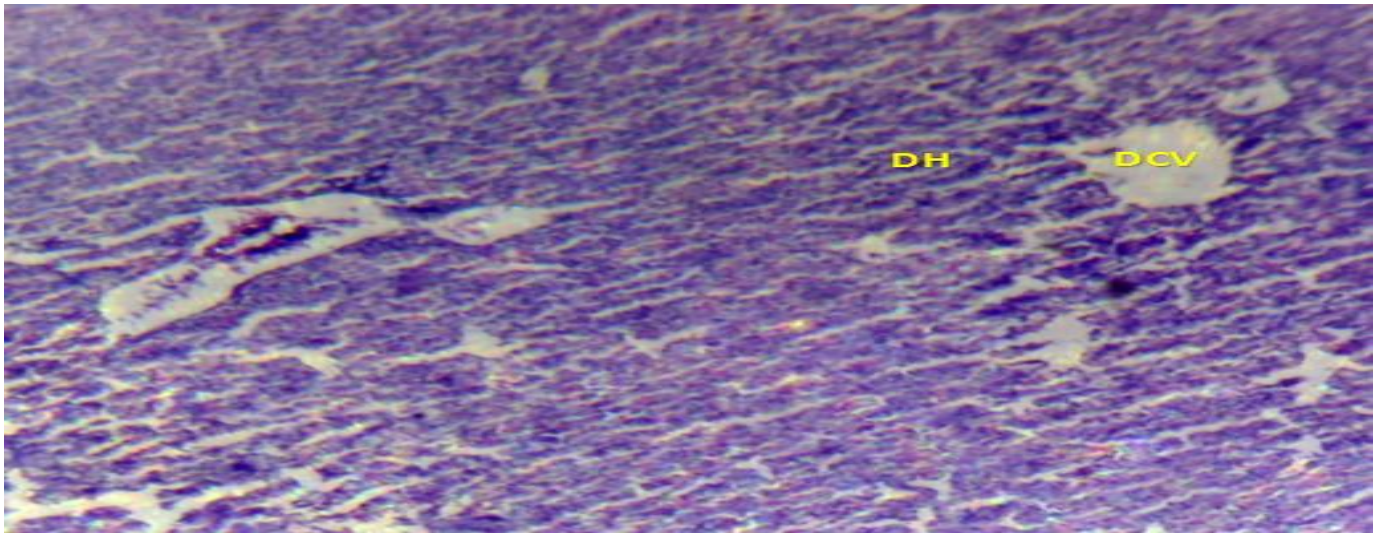

Fig 6: Histological Section of Liver of Group P2 Showing marked loss of uniformity and regularity of hepatic cords, dilated central vein (CV), Degenerating hepatocytes (DH), Nuclei pyknosis (py).

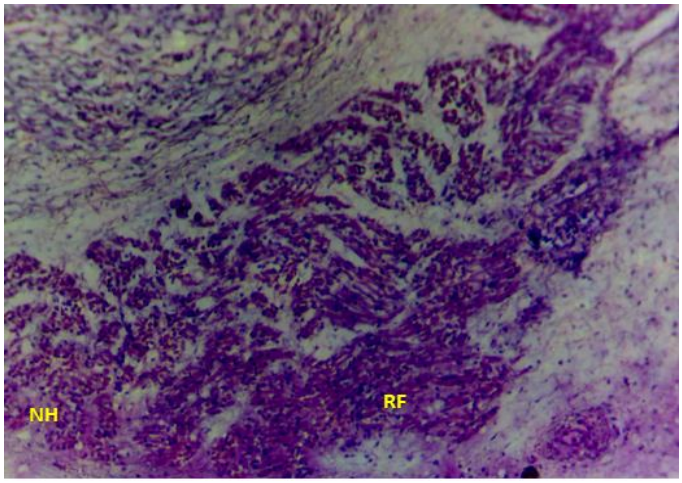

Fig 7.1: Histological Section of Liver of Group P3 Showing increased reticular fibres (RF), Necrotic hepatocytes (NH), Lymphocytic infiltrate.

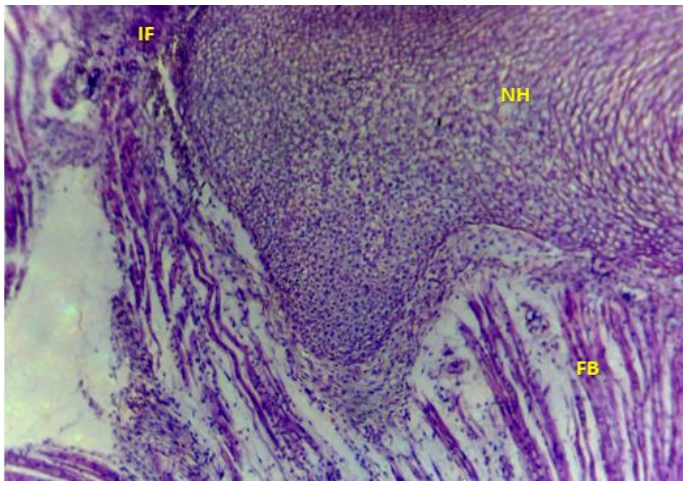

Fig 7.2: Histological Section of Liver of Group P3 Showing toxic changes with complete loss of normal architecture, Necrotic hepatocytes (NH), Fibrosis and Inflammatory changes, Fibrous bands (FB) with Lymphocytic infiltration (IF).

©SRDE Group, All Rights Reserved.

change in serum concentration and clearance of the antiepileptic drug in pregnancy[4]. The step-up necessity of antiepileptic drug usage in pregnant women to use higher doses or in combination with other antiepileptic drugs. The higher concentration of antiepileptic drugs in serum is associated with high risk. Many studies have been confirmed that the long-term usage of antiepileptic drug increases the risk of major malformations about 2-3 times. In addition, the studies performed on animals and humans concluded that they have behavioural teratogenicity as well as causing anatomic malformation because of cognitive disorders[13].

No antiepileptic drug is ideal to use in pregnancy as they are not completely safe due to the risk of fetal abnormality is increased. Ideally a plan for managing the woman's epilepsyduring pregnancy should be prepared before conception. Proper medical advice should be taken for cessation or alteration of antiepileptic drug treatment for sudden unexpected pregnancy[11], [13]. The smallest effective dose ofa drug with a low risk of teratogenicity shouldbe used. Doses may need to adjust as the pharmacokinetics of some drugs change during pregnancy. Apart from the teratogenic effects of phenytoin usage, it has been associated with drug induced gingival enlargement, Toxic epidermal necrolysis, hypertrichosis, rash, exfoliative dermatitis, pruritis, hirsutism, and coarsening of facial features. Phenytoin is also associated with induction of reversible IgA deficiency. Phenytoin elimination kinetics show mixed-order behaviour at therapeuticconcentrations. A small increase in dose may lead to a large increase in drug concentration as elimination becomes saturated and it takes more than 2 weeks to reach steady state. Carbamazepine increases the risk of developing lupus by 88%, may cause inappropriate antidiuretic syndrome, involved in cognitive anomalies. It has potential drug interactions; precaution should be taken in combining other medicine with it, including other antiepileptics. Eventually lower levels are seen when carbamazepine administered with Phenobarbital, Phenytoin and some of the antiepileptic drugs are more rapidly metabolised with carbamazepine for example Phenytoin[9], [12]. When single dose of carbamazepine administered, its plasma half-life is about 30 hours, but it is a strong inducer of hepatic enzymes.

In a study Temiz et al,[13]reported that phenytoin caused less teratogenic effect than expected when given in therapeutic concentration to chick embryo but higher dose of phenytoin showed teratogenic effect on neural tube development. Review on literature says that the use of valproic acid causesanatomic defects on fetus in first three months pregnancy and behavioural defects in last three months of pregnancy and also the use of phenobarbital increases the risk of formation of anatomic and behavioural defects. The use of carbamazepine and lamotrigine causes formation of cleft lip and palate, but the overall risks are less. It has been reported that teratogenic effect risk is low in case levetiracetam used in pregnancy. On other side, levetiracetam use during pregnancy has caused growth retardation, fetal skeletal system anomalies and increase of incidence of fetalmoratlity.

M Singh et al, [14]reported thatdilantin or other hydantoin anticonvulsant agents may interfere directly or indirectly the embryogenesis of developing chick embryos.The study was conducted to know the effect of dilantin on thoraco-abdominal organs of developing chick embryos said that 55% embryos were found to be viable and 45% were dead, while in control only 10% embryos were dead. Of treated viable embryos, total visceral defects was 68%, out of which, visceroptosis 29%, thin anterior abdominal wall 28%, ectopiacardis 10% and dextrocardia 1 % were found. Histological examination of liver sections displayed granular degeneration hepatic cells with loss of cytoarchitectural pattern. Well noticed dilatation of central vein observed with discontinuous lining epithelium. All observation made in the reported by M singh et al. are in similar to the results obtained in the present study.

Tureci E [15]done experiment on chick embryo by administering leviteracetam and valproicacic. Leviteracetam caused severe developmental abnormalities but the results of valproic acid showed more frequent developmental abnormalities than levetiracetam and the risk increased still further when both drugs were administered in combination. The administration of dilantin(3mg/egg) on 4th day of incubation in the developing chick embryo showed significant reduction in the weight of the brain and the size of cerebellum reported by shah GL.

©SRDE Group, All Rights Reserved.

The results of histochemical studies of cerebellum presents degeneration of purkinje cells, narrow molecular layer, oedematous infiltration seen between granular and molecular layer.

Study by Singh M et al, [16]Single injection of phenytoin 3mg/egg was injected into the yolk sac of white leghorn chick embryos followed by detailed teratological study. At the end of experiment on 19th day, the surviving embryos showed generalized decrease in body weight, growth retardation, wide range of malformations divided into limb, craniofacial, abdominal and ocular defects. Skeletal defects are also observed in some of the embryos including hypoplasia of digital phalanges and nails and shortened wings.

The current experimental study conducted on developing chick embryo to know the dose related teratogenic effects of administration of carbamazepine and phenytoin. Liver tissues were collected for histological examination from chick embryos. The primary site for the drug metabolism is Liver, it synthesizes many metabolising enzymes. The cytochrome p450 is the most crucial family of metabolising enzymes synthesised by liver. CYP enzyme mechanism can be influenced by many substances by induction or inhibition leading to impaired liver function. Researches have been studied on teratogenic effects of various antiepileptic drugs on chick embryo including carbamazepine and Phenytoin. Limited data available for experimental studies on dose related effects started with therapeutic index dose to high dose of Carbamazepine and phenytoin administered in the period of organogenesis. The observations of present study facilitates to compare the results obtained by both drugs used for the experiment, which will help the clinicians to choose the drug for pregnant women.

CONCLUSION:

The hepatic damage with deleterious effects which had been reported in current study reveals exposure to high dose of Phenytoin and carbamazepine is not safe. The histological observations of liver exposed to both the drugs greatly increases the risk of tissue damage at cyto-architectural level. Hence, it may be concluded that phenytoin and carbamazepine administration may interfere directly or indirectly the embryogenesis of chick embryos. The adverse effects caused by the higher dose of phenytoin is more than carbamazepine. Further research is required to explore the teratogenic effects of administration of combination of carbamazepine and phenytoin. It is summarized that single administration of phenytoin and carbamazepine beyond therapeutic dose during critical embryogenesis produces congenital anomalies. Therefore care should be taken while prescribing the choice and dose of drug to epileptic pregnant women.

Acknowledgement: All the authors are thankful to Jawaharlal Nehru Medical College for providing necessary equipment and chemicals for the study and for their constant encouragement to complete the study.

REFERENCES

1. Perucca, P; Gilliam, FG (September 2012). "Adverse effects of antiepileptic drugs." Lancet neurology 11 (9): 792–802.
2. Tomson T, Battino D, Bonizzoni E, et al. Dose-dependent risk of malformations with antiepileptic drugs: an analysis of data from the EURAP epilepsy and pregnancy registry. Lancet Neurol. Jul 2011; 10(7):609- 617.
3. Massey K M, Teratogenic effects of diphenylhydantoin sodium, j oral /theraPharmacol, 2 (1966) 380.
4. Johnston M C, Sulik K K and Duley K H, Dipheynylhydantoin induced cleft lip and palate in A/J mice, J Dental Res, 57(1987) 326.
5. Elshore D J, Cleft palate in offsprings of female mice treated with phenytoin, Lancet, 2 (1969) 1074.
6. National Institute for Health and Clinical Excellence (January 2012). "Chapter 4: Guidance". The Epilepsies: The diagnosis and management of the epilepsies in adults and children in primary and secondary care. NationalClinical Guideline Centre. pp. 57–83.
7. Bittigau P, Sifringer M, Genz K, Reith E, Pospischil D, Govindarajalu S, Dzietko M, Pesditschek S, Mail, Dikranian K, Olney JW, Ikonomidou C: Antiepileptic drugs and apoptotic neurodegeneration in the developing brain. ProcNatlAcadSci U S A;99:15089–15094, 2002.
8. "TEGRETOL® (carbamazepine)". TGA Business Services. NOVARTIS Pharmaceuticals Australia Pty Limited. 17 June 2013. Retrieved 18 November 2013.
9. Hiremath GK, Kotagal P, Bingaman W, Hovinga C, Wyllie E, Morris H, et al., Risk factors for carbamazepine elevation and toxicity following epilepsy surgery. Seizure 2005; 14:312-317.
10. Sutcliffe AG, Jones RB, Woodruff G. Eye malformations associated with treatment with carbamazepine during pregnancy. Ophthalmic Genet 1998; 19:59-62.
11. Berman E., Chacon L., House D., Koch BA., Koch WE., Leal J., Lovtrup S., Mantiply E., Martin AH., Martrucci GI., Mild KH., Monahan JL., Sandtrom M., Shamsaifar

©SRDE Group, All Rights Reserved.

K., Tell R., Trillo MA., Ubeda A., Wagner P. Development of chicken embryos in a pulsed magnetic field. Bioelectromagnetics 1990; 11: 169- 187.

12. Waddington C H: Experiments on the development of chick and duck embryos, cultivated in vitro. Philosophical Transactions of the Royal Society of London Series B-Containing Papers of a Biological Character 1932, 221:179-230.
13. Temiz C, Temiz P, Demirel A, Sayın M, Umur AS, Ozer FD:Effect of sodium phenytoin concentration on neuraltube development in the early stages of chicken embryodevelopment. Journal of Clinical Neuroscience 16:307–311,2009.
14. M Singh et al., (2000). "Teratogenic effects of dilantin on thoraco-abdominal organs of developing chick embryos". Indian J Exp Biol. 2000 Oct;38(10):1026-30.
15. Tureci E et al., (2010). The effects of valproic acid and levetiracetam on chicken embryos. J ClinNeurosci. 2011 Jun;18(6):816-20.
16. Singh M, Shah GL.Teratogenic effects of phenytoin on chick embryos. 1989 Nov;40(5):453-8.
17. Shah GL et al. The teratogenic effect of dilantin on cerebellum in developing chick embryos. Journal of the Anatomical Society of India. 1988 Apr; 37(1): 1-8.

©SRDE Group, All Rights Reserved.