

THE ASSESSMENT OF HISTOMORPHOLOGICAL IN-UTERO EXPOSURE TO VARIED DOSES OF CARBAMAZEPINE ON THE DEVELOPMENT OF VENTRICULAR SYSTEM IN THE ALBINO RAT FETUSES (*RATTUS NORVEGICUS*).

Ann Mwangi¹, Joseph Kweri², Caroline Ndung'u³

^{1,2} Department of Human Anatomy, School of Medicine (SOMED), College of Health Sciences (COHES) Jomo Kenyatta University of Agriculture and Technology (JKUAT) Kenya.

³ Department of Human Anatomy, School of Medicine (SOMED), College of Health Sciences (COHES) Egerton University.

IJASR 2021

VOLUME 4

ISSUE 5 SEPTEMBER – OCTBER

ISSN: 2581-7876

Abstract: The in-utero exposure to carbamazepine, an anticonvulsant medicine has been shown to perturb the normal morphogenesis of the fetal brain when used in management of maternal conditions. However, the anatomical histomorphological teratogenic effect on the ventricular system when exposed at different gestation periods and at different doses is not well elucidated. The broad objective of this study was therefore to evaluate the histomorphological teratogenic effects of exposure to various doses of carbamazepine on the ventricular system when exposed at different gestation periods. In conducting the study, a static group experimental study design was adopted. The animal experimentation was carried out at Small Animal Facility for Research and Innovation (SAFARI) animal house while tissue processing for histology and stereological analysis was done in the department of human anatomy. A Sample size of 30 albino rat dams (*Rattus norvegicus*) weighing between 200-250grams were used in the study as determined by use of the resource equation method. These 30 Albino rats were divided into two broad study groups of 3 rats control and 27 rats experimental. To evaluate the teratogenic histological effects of carbamazepine on differing doses, the 27 rats in the experimental group were further subdivided into three study groups of 9 rats as follows; (i) Low carbamazepine group [LCG-20.7mg/kg/bw] (ii) medium carbamazepine [MCG-72.3mg/kg/bw], (iii) High carbamazepine group [HCG-124mg/kg/bw]. To further evaluate the teratogenic effects of carbamazepine on differing gestation periods, the 9 rats in each of the three dose categories were further subdivided into three groups of 3 rats according to trimesters as follows; (i) Trimester I-(3rats); (ii) trimester II-(3rats) and (iii) trimester III-(3rats) respectively. The findings of the study showed that there was dilatation of ventricular system as well as disaggregation of choroid plexus in the lateral ventricle of the fetal brain. In conclusion, carbamazepine is teratogenic to the developing fetal brains and its teratogenicity is time and dose dependent. The study recommends that carbamazepine should not be used during pregnancy and particularly during 1st and 2nd trimesters.

Keywords: Carbamazepine, Anticonvulsant, Teratogenic, Histomorphology, Corpus callosum

INTRODUCTION

In-utero exposure to carbamazepine perturbs the normal morphogenesis and cyto-differentiation of fetal organs when applied in management of maternal conditions during pregnancy, Matlow, J., & Koren, G. (2012); Włodarczyk *et al.*, (2012), the specific histomorphological perturbation on the ventricular system when various doses of carbamazepine are administered at different gestation periods has not been studied. The morphogenetic perturbations to the developing neuronal tissues in the fetus are believed to be caused by its inhibitory mode of action due to accumulation of its principal metabolite (carbamazepine 10 epoxide) in maternal blood plasma, creating a negative osmotic gradient. Coupled with its low molecular weight of 236.27g/mol enhances it to cross the maternal blood placenta barrier, accumulate in fetal brain tissue, interfering with the neuro-developmental events including; cell proliferation and migration, synaptogenesis, axonal sprouting, gliogenesis, neurogenesis, synaptogenesis, myelination among others that leads to physiological apoptotic cell death of the fetal brain tissue and oxidative stress (Ikonomidou *et al.*, 2010). As such, these intrauterine disturbances to the developing neuronal tissues in the fetus may cause permanent structural damage to the brain that may manifest in form of some of the behavioural mental conditions seen in adulthood like mild mental retardation, cyclic maniac depressive disorders, suicidal ideation among others, whose causes are yet to be established (Bath & Scharfman, 2013; Fujimura *et al.*,

2017).The present study therefore aim at evaluating histomorphological teratogenic effects of in-utero exposure to various doses of carbamazepine on fibres of corpus callosum when administered at different gestation periods.

MATERIALS AND METHODS

Study site/Location

All experiments that included breeding, handling, weighing, carbamazepine administration and measurements of fetal parameters as well as the fetal brain was done at the Small Animal Facility for Research and Innovation (SAFARI) situated in Jomo Kenyatta University of Agriculture and Technology (JKUAT). Histological procedures were carried out in Human Anatomy labs.

Study Design

A static group laboratory based experimental study design was adopted

Description of Albino rats used in the study

Female albino dams used in the study were of the 3rd series breed and weighed between 200-250g.They were used because of the following known scientific facts; (i)They have a large litter size,(ii)Low incidence of spontaneously occurring congenital defects, (iii)a relatively short gestational span,(iv)low cost of maintaining the animals and, (v)considerable amount of the reproductive data on the rat is already available (Bailey *et al.*, 2014; Pritchett & Corning, 2016).

Acquisition and feeding of the dams

The albino rats were purchased from the Small animal facility for research and innovation (SAFARI) animal house, located in Jomo Kenyatta University of Agriculture and Technology (JKUAT) main campus. They were fed on a standard diet as determined by American institute of nutrition (2011) that included rodent pellets from UNGA meals limited (Thika), and water *adlibitum*. They were kept in spacious polycarbonate plastic cages in the animal house as determined by (Allen *et al.*, 2016).

Sample size calculation

In calculation of the sample size, resource equation was applied to get 30 albino rats determined by (Arifin *et al.*, 2017). The formula states that the measured value 'E' which is the degree of freedom of analysis of variance (ANOVA) based on a decided sample size value ('E') should lie between 10 and 20 animals according to this equation. Therefore, a value less than 10 necessitates adding more animals which increases the chance of getting significant results while a value more than 20 has been shown to increase the cost of the study without increasing the significance of the results. Therefore, total number of groups=10 while the total number of animal sis 30. $E = \text{Total number of Animals} - \text{Total number of groups}$. E is therefore is 30-10 which is 20

Grouping of animals

After confirmation of pregnancy, the rats were assigned into two broad study groups of 3 rats in control group and 27 rats in experimental group. The 27 rats in the experimental group were further divided into three sub-groups of 3 rats each assigned according to the dose administered as low (LCG), Medium (MCG) and High carbamazepine group (HCG). Each of the subgroups of the LCG, MCG and HCG were further subdivided into smaller sub-groups according to the time of administration as first (TM₁), second(TM₂) and third(TM₃) trimesters comprising of 3 rats each.

Determination and acquisition of carbamazepine

A simple guide for conversion of human to animal dosages was used as determined by (Nair & Jacob, 2016) formula as follows; The correction factor (Km) is estimated by dividing the average body weight (kg) of species to its body surface area (m²). For example, the average human body weight is 60 kg, and the body surface area is 1.62 m².

Therefore, the Km factor for human is calculated by dividing 60 by 1.62, which is 37. The Km factor values of a rat is used to estimate the HED as: $HED\text{ mg / kg} = \text{Rat dose mg / kg} \times \text{Animal K} / \text{Human K}$ Eq. As the Km factor for each species is constant, the Km ratio is used to simplify calculations. Hence, Equation is modified as: $HED\text{ mg / kg} = \text{Animal dose mg / kg} \times \text{K ratio}$ Eq. The Km ratio values are already provided and are obtained by dividing human Km factor by animal Km factor or vice versa. Carbamazepine tablets from Novartis Farma Pharmaceuticals, batch number TL787 were obtained from a local chemist in Thika and were used to make the reconstitutions and administration was done using an oral gavage needle gauge 16.

Administration of carbamazepine

All rats in first trimester (TM₁) group in Low, Medium and High dose categories received carbamazepine from gestation day GD₁-GD₂₀ while the rats in second trimester (TM₂) group in Low, Medium and High dose categories received carbamazepine from gestation day GD₇-GD₂₀. Rats in third trimester (TM₃) group in Low, Medium and High dose categories received carbamazepine from gestation day GD₁₄-GD₂₀

Determination of fetal growth parameters

Fetal growth parameters that included fetal and organ weights, crown-rump lengths, head circumference, head lengths and bi-parietal diameters were taken on the day of delivery and recorded. This was obtained by use of a digital weighing scale Vernier caliper.

Procedure for harvesting the fetal brains

After the Fetuses were removed from the maternal uterine horns, they were euthanised by use of concentrated carbon dioxide. Then the following procedure was followed to harvest their brains; (i) Fetuses were mounted onto the dissection board using mounting pins -dorsal side facing the board, (ii) using a pair of scissors and forceps lateral borders along the lower margin of the temporal bone was opened and the skull cap removed, (iii) Using a magnifying glass, the whole fetal brain was identified, (iv) To avoid damaging the fetal brain, the meninges was opened along the superior sagittal sinus retracted up carefully since the brain lies within the meninges, (iv) The entire brain was excised/ scooped at the level of foramen magnum, (v) Each brain was examined for general external features and obvious congenital malformations (vi) Brain weights were taken by use of a digital weighing scale and their weights to body weight ratio were calculated (vii) The brains were immersed in the formaldehyde, to proceed with processing either for light or histostreology for 12 hours

Tissue preparation for light microscopy

In preparation of tissues for light microscopy, the following procedure was followed; (i) The brains were fixed in Zenkers' solution for 24 hours, (ii) They were dehydrated in an ascending concentration of alcohol (50%, 60%, 70%, 80%, 90%, 95% and 100% (absolute) each for one hour, (iii) They were cleared by immersion with cedar wood oil for 12 hours, (iv) They were then infiltrated with paraplast wax for 12 hours at 56°C, (v) The brain tissue was then orientated in the longitudinal axis (frontal to occipital lobe), (vi) They were then embedded in paraffin wax on the wooden blocks, (vii) Excess wax was trimmed-off till the entire length of the brain tissue was exposed, (viii) 5µm thick longitudinal sections were cut from head to tail regions with Leitzsledge rotary microtome, (ix) The cut sections were floated in water at 37°C to spread the tissue, (x) The sections were stuck onto glass slides using egg albumin, applied as thin film with a micro-dropper, (xi) The slides were then dried in an oven at 37°C for 24 hours, (xii) Blinding was done by coding all the slides by the research assistant in absence of the researcher (xiii) They were stained with different stains including: -Haematoxylin and Eosin (H&E), based on the cellular structures that needed to be studied.

Histomorphological analysis

20-25 slides were chosen using systematic uniform random sampling guided by the co-efficient of error calculations. The slides were observed under a BP Olympus microscope at different magnifications and photomicrographs of the precentral gyri of the frontal lobe were taken using a 32 megapixel digital camera. They were then exported to the computer screen where Adobe fireworks software was used for labelling, and were analysed through observations.

Ethical Approval

All procedures for animal handling, feeding, humane sacrificing and harvesting of organs were performed as per laid down protocols, with approval from Animal Ethics Committee Jomo Kenyatta University of Science and Technology as well as the laid down protocols and regulations by International Animal Research Institute (IARI) of USA as outlined by (Gomez *et al.*, 2010).The study went through the legal and administrative requirements as required by JKUAT and the laws of Kenya, (See document attached in the appendices; REF: JKU/2/4/896A).

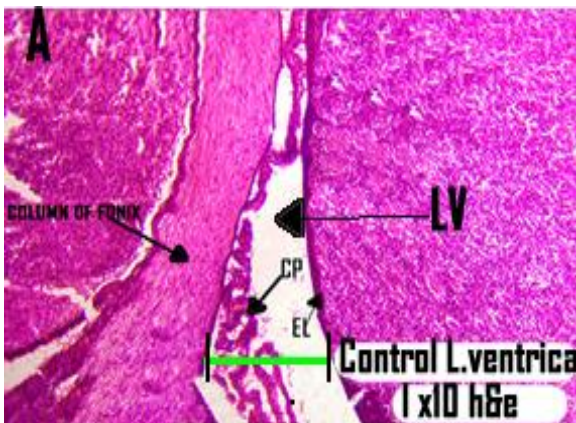
RESULTS

Histological examination of the fetal brain sections under different magnifications revealed normal histological appearance and organization of the ventricular system. However, in carbamazepine treatment groups, there were varying presentations including; dilatation of lateral ventricles as well as disaggregation of choroid plexus. These effects were observed to be time and dose dependent as they were more prominent in medium and high carbamazepine dose groups when the medicine was administered during the first and second trimesters. Trimester three had no significant outcomes except when administered on high doses. The results were as illustrated below;

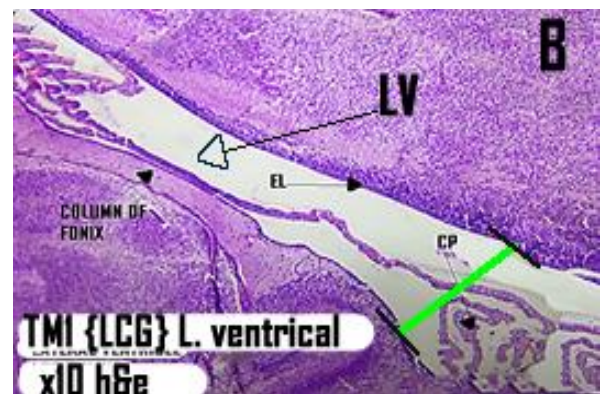
Influence of carbamazepine on the development of the ventricular system

The prenatal exposure to carbamazepine was histologically seen to perturb the brain development, ranging from a significant dilatation of the lateral ventricles to disaggregation of the choroid plexus. This was observed to be dependent the dose and time dependant in that when carbamazepine was administered in trimester one (TM₁) and two(TM₂) the pattern on the effects in the lateral ventricle and the disaggregation of choroid plexus were seen to depict similar morphological appearance in both the TM1 and TM2 photomicrographs across all the carbamazepine treated groups (LCG,MCG and HCG) when compared with the control figures 1, and figure 2 respectively. However, when the carbamazepine was administered in the third trimester (TM₃), there was no marked significant difference in the morphological appearance ofthe lateral ventricle and the disaggregation of choroid plexus between the LCG and the MCG as compared with the control. It is only in the HCG that shown slight disturbances in thelateral ventricle and the disaggregation of choroid plexus compared with the control, figure 3 D.

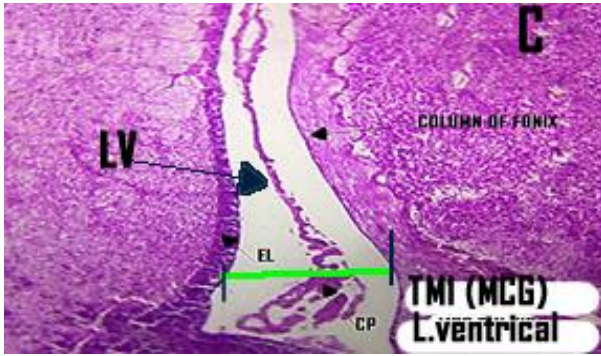
From these observations it was clear that carbamazepine teratogenic effect on the development ofthe lateral ventricle and the choroid plexus was at high dosages (MCG and HCG). These two critical teratogenic doses of carbamazepine exacted their morphogenetic inhibitory effects to the differentiation of the corpus callosal commissural fibres in the fist and in the second trimester (TM₁ and TM₂).



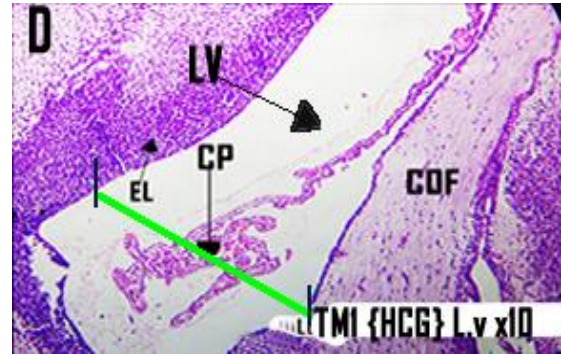
A: control: Normal thickness of lateral ventricle (as indicated by green line), well-organized choroid plexus (CP) (mag x10)



B: LCG: showing an enlarged lateral ventricle at TM₁ (LCG), shown by green line, with clustered mal-developed and disjointed choroid plexus (CP) mag x10

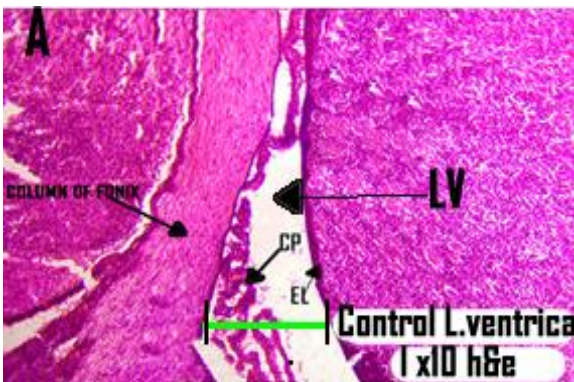


C: MCG: showing further hypertrophy of the lateral ventricle as shown by green line at TM_1 declustered and further mal-developed choroid plexus (CP) (mag x10).

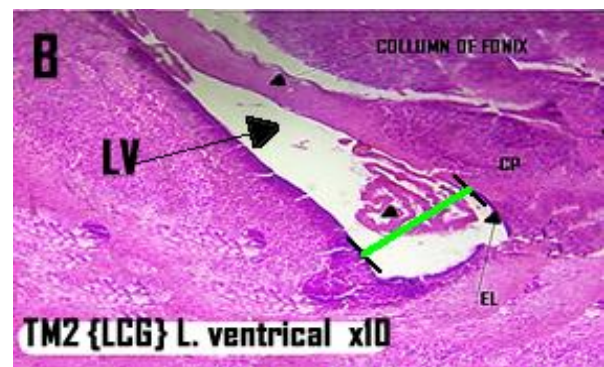


D: HCG: showing the most enlarged lateral ventricle as shown by the green line at TM_1 (HCG) and the *most de-clustered* mal-developed choroid plexus (CP) (mag x10).

Figure 1: The TM_1 comparative thickness and organization of the ventricular system in: (a) the control; (b) LCG; (c) MCG; and (d) HCG



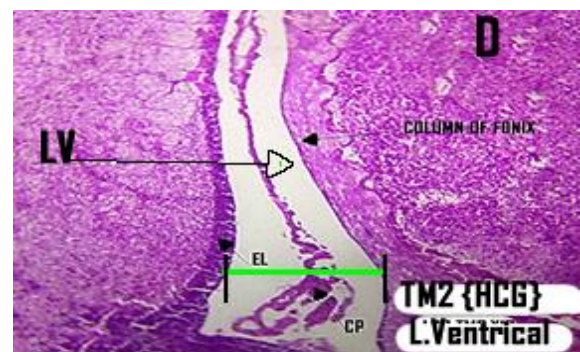
A: Control: showing the normal thickness of lateral ventricle (as indicated by green line), well-organized choroid plexus (CP) as well as a network of ependymal cells of the Control fetal brain (mag x10)



B: LCG Enlarged lateral ventricle at TM_2 (LCG) showing clustered mal-developed choroid plexus (CP) and disjointed ependymal layer fetal brain MAG x10H&E)

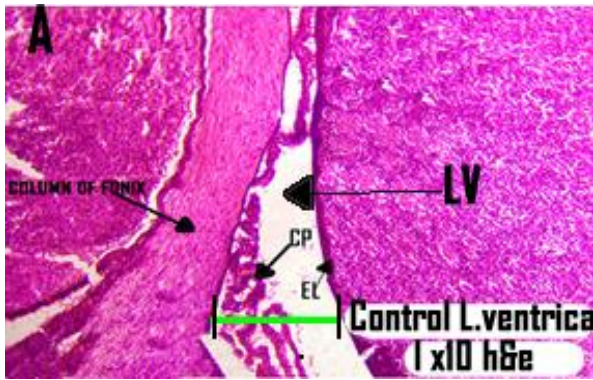


C: MCG: showing marked enlargement of the lateral ventricle at TM_2 (MCG) showing clustered further mal-developed choroid plexus (CP) and disjointed ependymal layer.

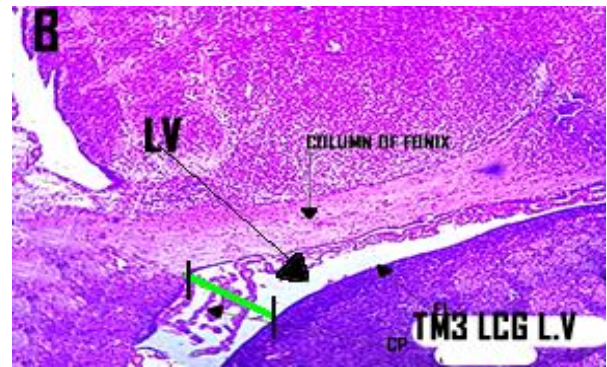


D: HCG: showing the highest enlargement of the lateral ventricle at TM_2 (HCG) showing *most* clustered mal-developed choroid plexus (CP) and disjointed ependymal layer.

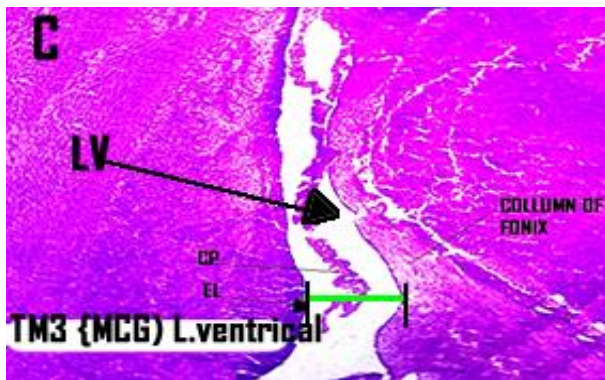
Figure 2: The TM_2 comparative thickness and organization of the ventricular system in: (a) the control; (b) LCG; (c) MCG; and (d) HCG



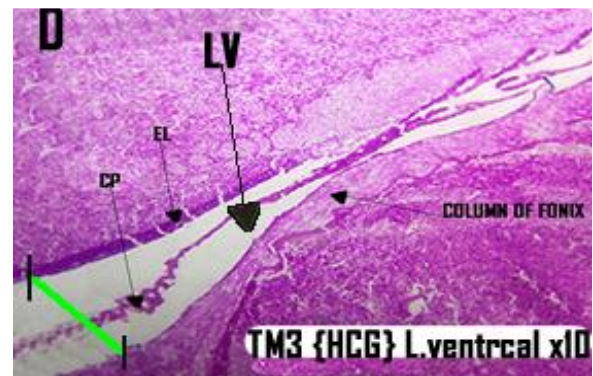
A: Control: Normal thickness of lateral ventricle (as indicated by green line), well-organized choroid plexus (CP) as well as a network of ependymal cells of the Control fetal brain (mag x40)



B:LCG: Photomicrograph B: disaggregated choroid plexus in lateral ventricle at TM₃ (LCG) showing clustered mal-developed choroid plexus (CP) and disjointed ependymal layer



C: MCG: More disaggregated choroid plexus in the lateral ventricle at TM₃ (MCG) showing clustered further mal-developed choroid plexus (CP) and disjointed ependymal layer.



D: HCG: showing ventricular hypertrophy in the lateral ventricle at TM₃ (HCG) showing most clustered mal-developed choroid plexus (CP) and disjointed ependymal layer.

Figure 3: The TM₁ comparative thickness and organization of the ventricular system in: (a) the control; (b) LCG; (c) MCG; and (d) HCG

DISCUSSION

The current study established that *in-utero* exposure to carbamazepine is teratogenic to the fetal brain in a dose and time dependent manner. For instance, it was established that the lateral ventricular dilatation as well as differentiation of cells of the choroid plexus differed according to the dose of carbamazepine exposure as well as with the time of exposure. In the various treatment groups (LCG, MCG, HCG), effects were observed to be more when treatment was done in trimester one (TM₁) and trimester two (TM₂); (Figure 1 and Fig 2). The study also established that when the treatment was done at trimester three (TM₃), there was no marked significance difference in thickness of the fibers of the splenium of corpus callosum both between the carbamazepine treated groups against the control (Fig 3), except when high carbamazepine dosages were administered. These findings were in tandem with findings from a previous study on histomorphological effects of lamotrigine on fetal brain that revealed that in the treated group, the lateral ventricles were dilated and the plexiform layer of the cerebral cortex was relatively less differentiated (Sah *et al.*, 2013). This was also attributed to several histopathological alterations including pyknotic and degenerated neurons, fibrin deposition (fibrosis), disorganization of the cerebral cortex, dilated and enlarged blood vessels and dilated ventricles as described in a previous study findings (Werler *et al.*, 2011; El-gaafarawi *et al.*, 2015).

CONCLUSION

In conclusion of the study has established carbamazepine use during pregnancy is teratogenic to the ventricular system, and interferes with the differentiation of the choroid plexus the brain of the developing fetus particularly when administered during the first and second trimester regardless of the dosage as indicated by the histological features. When administered in trimester three the effects are not significant except when administered on high doses. The most vulnerable window period for carbamazepine teratogenicity in addition established to be the first trimester while the most critical dose was 124g/kg/bw.

ACKNOWLEDGEMENTS AND RECOMMENDATIONS

Recommendations

The study recommends that; use carbamazepine during pregnancy should be avoided by all means as it has been shown to be teratogenic to the developing fibres of corpus callosum as well as the ventricular system particularly in trimester TM1 and TM2 by seeking appropriate alternatives that are safer to the fetus. Should expectant mothers be on chronic use of carbamazepine and the drug cannot be withdrawn because of associated withdrawal side effects to the mother, the doses should be adjusted to the minimal effective dosages that would confer the maximum maternal benefits and reduce the teratogenic risks to the developing fetal brain. Further studies be carried out in non-human apes that have close pyrogenetic relations to humans, to ascertain its teratogenicity in relation to doses.

Acknowledgements; Author is grateful to DR. Kweri J.Kariuki, Chairman Department in Human Anatomy, Jomo Kenyatta University of Agriculture & Technology for his support, patience and inspiration at each stage of this study.

Conflict of interest; the authors declare that they have no competing interests

REFERENCES

- Allen, K., Hamada, H., Yamada, T., Minakamil, H., Yoshikawa, H. (2010). Impact of planning of pregnancy in Women with epilepsy on seizure control during pregnancy and on maternal and neonatal outcomes. *Seizure -European Journal of Epilepsy* 23(2), 112-116
- Agarwal, B.N., Agarwal, N.K., Mediratta, P.K., Sharma K.K. (2010). Effect of lamotrigine, oxcarbazepine and topiramate on cognitive functions and oxidative stress in PTZ kindled mice. *European Journal of Epilepsy*, 20(3), 257-262
- Ahmed, R.G. (2017). Antiepileptic drugs and developmental neuroendocrine dysfunction: Every why has A Wherefore? *Arch Med*, 9 (6) 2 -14
- Arifin, W. N., & Zahiruddin, W. M. (2017). Sample Size Calculation in Animal Studies Using Resource Equation Approach. *The Malaysian journal of medical sciences*, 24(5), 101-105.
- Bath, K. G., & Scharfman, H. E. (2013). Impact of early life exposure to antiepileptic drugs on neurobehavioral outcomes based on laboratory animal and clinical research. *Epilepsy & behavior: E&B*, 26(3), 427-439.
- Bailey, F., Orlow, S.J., Lamoreux, M.L. (2014) The Tyr (albino) locus of the laboratory mouse. *Mamm Genome* 15: 749-758.
- Belghuis, B., Stapleton, C., Hulst, J., De Haan, G., Lindhout D., Demurtas, R. (2017). Major malformations in infants exposed to antiepileptic drugs in utero, with emphasis on carbamazepine and valproic acid: a nation-wide, population-based register study. *Acta Paediatrica*. 93:174-176.
- El-gaafarawi and Magdy Abouel-magd, M. (2015). Teratogenic Effect of Carbamazepine Administration in Pregnant Rats. *The Egyptian Journal of Hospital Medicine* 59, (2), 244-57.
- Fujimura, K., Mitsuhashi, T., Takahashi, T., (2017). Adverse Effects of Prenatal and Early Postnatal Exposure to Antiepileptic Drugs: Validation from Clinical and Basic Researches. *Official journal of the Japanese society of Neurology*, 39 (8), 635-643.
- Gedzelman, E. & Meador, K. J. (2012). Antiepileptic drugs in women with epilepsy during pregnancy. *Therapeutic advances in drug safety*, 3(2), 71-87

11. Gomez, H.J., Cirillo, V.J & Irvin, J, D. (2010). Carbamazepine: A review of Human pharmacology. *Drugs*, 30, 13-24.
12. Hill, D. S., Wlodarczyk, B. J., Palacios, A. M., Finnell, R. H. (2011). Teratogenic effects of antiepileptic drugs. *Expert review of neurotherapeutics*, 10(6), 943–959
13. Ikonomidou, C., Imare., Malake, J. (2010). Prenatal effects of antiepileptic drugs. *Epilepsy currents*, 10(2), 42- 46.
14. Matlow, J., & Koren, G. (2012). Is carbamazepine safe to take during pregnancy?. *Canadian family physician Medecin de famille canadien*, 58(2), 163–164.
15. National Research Council (US) Committee for the Update of the Guide for the Care and Use of Laboratory Animals. Guide for the Care and Use of Laboratory Animals. 8th edition. Washington (DC): National Academies Press (US); 2011. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK54050/> doi: 10.17226/12910
16. Nair, A. B. & Jacob, S. (2016). A simple practice guide for dose conversion between animals and human. *Journal of basic and clinical pharmacy*, 7(2), 27–31.
17. Nie, Q., Su, B., & Wei, J. (2016). Neurological teratogenic effects of antiepileptic drugs during pregnancy. *Experimental and therapeutic medicine*, 12(4), 2400–2404. <https://doi.org/10.3892/etm.2016.3628>
18. Pritchett & Corning, (2016) Variation in the hooded pattern of rats, and a new allele of hooded. *Genetics* 36: 254–266.
19. Sah, N., Pandit, R. K., Dhungel, S. (2013). Effect of Lamotrigine on fetal rat brain morphology. *Janaki Medical College Journal of Medical Sciences*, 1 (1):26-29
20. Werler, M. M., Ahrens, K. A., Bosco, J. L., Mitchell, A. A., Anderka, M. T., Gilboa, S. M., Holmes, L. B., & National Birth Defects Prevention Study (2011). Use of antiepileptic medications in pregnancy in relation to risks of birth defects. *Annals of epidemiology*, 21(11), 842–850.
21. Wlodarczyk BJ, Palacios AM, George TM, Finnell RH. Antiepileptic drugs and Pregnancy outcomes. *Am J Med Genet A* 2012;158A: 2071-9