MATERNAL PREGNANCY OUTCOME FOLLOWING PRENATAL EXPOSURE TO PHENYTOIN IN ALBINO RATS (RATTUS NORVEGICUS)

Caroline Sigei[1], Joseph Kweri[2], James Kanyoni[3], Ann Mwangi[4]Atanas Malik[5], Teresiah Musa[6], Rono Walter[7], Peris Macharia[8], Caroline Ndung'u[9], Shadrack Asena[10].

[1-8,10]Lecturers, Department of Human Anatomy, School of Medicine (SOMED),
College of Health Sciences (COHES)
Jomo Kenyatta University of Agriculture and Technology (JKUAT) Kenya.
[9]Lecturers, Department of Human Anatomy, School of Medicine (SOMED),
College of Health Sciences (COHES)
Egerton University.

IJASR 2021
VOLUME 4
ISSUE 3 MAY – JUNE **ISSN: 2581-7876**

Abstract: Although prenatal exposure to phenytoin has been shown to perturb the outcome of pregnancy when used prenatally, specific data on how varied doses of phenytoin affects maternal pregnancy outcome when exposed in different window periods is not well elucidated. This study therefore aimed to establish maternal pregnancy outcome following prenatal use of phenytoin varying doses when applied at different gestational trimesters in albino rats. In carrying out the study a total of 30 pregnant female albino rats weighing between 150-250grmas were randomly assigned into two broad study groups of 3 control and 27 experimental group. The 27animals in the experimental group were further subdivided into three study groups as follows [low phenytoin group(31mgs/kg/bw), medium (62mg/kg/bw) and high phenytoin group(124mg/kg/bw)] of nine (9) rats each. Each of the nine (9) rats in each of the low, medium and high phenytoin groups were further subdivided into three study sub- groups of 3 rats each as trimesters I (T1) trimester II (T2), and trimester III (T3) in order to determine the effects of phenytoin when exposed on different gestational periods. The control group received food and water adlibitum while experimental animals received food, water adlibitum and varying doses of phenytoin based on their study category and the exposure period every 8.am in the morning. All the pregnant rats were humanly sacrificed on the 20th day of gestation and the uterine walls opened along the antimesomentrial border, the fetuses were then harvested, all live fetuses and devoured fetuses counted, the resorbed glans were also counted and placental weights were taken. Data was then recorded in the tally sheets, entered into an excel data sheet and analyzed using statistical packages for social science version23 (SPSS VERSION23) for windows. The intergroup and intragroup comparisons were statistically analyzed usingone way analysis of variance (ANOVA) and all P-values less than 0.00 5 were taken to be statistically significant. There was statistically significant difference between the control group and the experimental group pregnancy outcome. The litter size and the placental weights of the control group were statistically different (high) compared to the experimental group (low) particularly when high phenytoin dose is administered during the first trimester. Consequently, resorbed endometrial glands as well as devoured fetuses between the control group were statistically different (low) compared to experimental group (high). This study found out that the maternal pregnancy outcome differs depending on the dose and time of prenatal phenytoin exposure as seen by reduced litter size and placental weights in the high dose group fetuses as well as trimester 1 group compared to low dose group fetuses and trimester 3 respectively, more clinical trials need to be conducted for proper and more accurate prenatal phenytoin dose adjustment

Keywords: Phenytoin, Anticonvulsant, Teratogenic, Pregnancy outcome.

1.1 Introduction

Prenatal exposure to phenytoin has been shown to cause various maternal pregnancy outcome through maternal physiologic and metabolic dis-functions including; alteration of folate metabolism, hemodynamic alterations, inhibition on potassium channels among others (Azarbayjan*et al.*, 2007; Galappatty*et al.*, 2018; Gelder*et al.*,2010.). Further, literature has attributed phenytoin prenatal perturbations to its hypoxic effects on the tissues and cardiac arrhythmia to the embryo leading to reactive oxygen species that are toxic which consequently affect proper development of embryonic and fetal structures (Bittigau et al., 2018; Dannielson*et al.*,2005; Katsiki*et al.*,2014;). Such prenatal interferences could be associated with redundant pre and postnatal outcomes of pregnancy like

intrauterine growth restrictions, small for gestational age and mental retardations among others. Despite the known effects of antiepileptic drugs phenytoin being one of them in pregnancy, use is still preferred because convulsive disorders

can equally pause great danger to both the mother and the developing embryo or fetus (Kakkar*et al.*, 2013; Waltman*et al.,*2003.). Many studies that have been done through animal models or clinical trials link the antiepileptic drugs such as phenytoin to various pregnancy outcomes (Bromley *et al.*, 2017; Lander *et al.*, 2008; Saetre*et al.*, 2009; Shar*et al.*, 2010). However, there is no clear data on the dose and time of prenatal phenytoin exposure in relation to pregnancy outcome. Phenytoin (PTH) otherwise called Dilantin is one of the oldest wildly used antiepileptic drugs with low sedative effects (Nulman*et al.*, 1997). It is also available and affordable and therefore findings of the appropriate dose adjustments during its prenatal use will save both the lives of mothers suffering from convulsive disorders as well as the children of these mothers.

1.2 Study Objectives

1. To establish the pregnancy outcomes following *in-utero* exposure to varied doses of phenytoin at different gestation periods.

2. To establish whether the pregnancy outcomes following in-utero exposure to varied doses of phenytoin are dose dependent.

3. To establish whether the pregnancy outcomes following in-utero exposure to varied doses of phenytoin are time dependent.

1.3 Hypothesis (H_0)

Prenatal exposures to phenytoindo not affect the pregnancy outcomes.

1.4 The study assumptions

In carrying out this study it was assumed that the albino rat (*RattusNorvegicus)* model would replicate the actual teratogenic induction scenario that would occur in humans due to the known close association of this kind of rat species with human biological and functional outcomes when exposed.

2.0 Materials and Methods

2.1 Study site/Location

All experiments that included breeding, handling, weighing, phenytoin administration and measurements of fetal parameters was done at the Small Animal Facility for Research and Innovation (SAFARI) situated in Jomo Kenyatta University of Agriculture and Technology (JKUAT).

2.2 Study Design

A static group laboratory based experimental study design was adopted

2.3 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).

2.4. 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 and water *adlibitum*. They were kept in spacious polycarbonate plastic cages in the animal house as determined by (Allen *et al.,* 2016).

2.5 Sample size calculation

In calculation of the sample size, resource equation was applied to get 30 albino ratsas 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=Total number of Animals-Total number of groups. E is therefore is 30-10 which is 20

2.6 Grouping of animals

A total of 30 nulliparous albino rat dams of the species Rattusnorvegicus weighing between 150-250g were derived from a pure colony were used as the animal experimental model in this study. The 30 dams were calculated using the resource equation method (E=TA-TG), (Charan& Biswas, 2013) since the standard deviation from previous studies was not available as well as the effect size.

2.7 Determination of phenytoin doses

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 mg / kg = Rat dose mg / kg Animal K /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 mg / kg = Animal dose mg / kg 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.Phenytoin administration was done using an oral gavage needle gauge 16.

2.8 Administration of phenytoin

All rats in first trimester (TM_1) group in Low, Medium and High dose categories received phenytoin doses from gestation day GD_1-GD_{20}while the rats in second trimester (TM_2) group in Low, Medium and High dose categories received phenytoin from gestation day GD_7-GD_{20}. Rats in third trimester (TM_3) group in Low, Medium and High dose categories received phenytoin from gestation day GD_{14}-GD_{20}

2.9 Determination of fetal growth parameters

Fetal growth parameters that included fetal and organ weights, crow-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 and the Vernier caliper.

2.10 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 REF: JKU/2/4/896A**).**

3.0 Results

This study evaluated maternal pregnancy outcome following prenatal exposure of phenytoin varying doses administered during different gestational periods and manifested through intra and intergroup comparison of these parameters; Litter size, placental weights, resorbed endometrial glands and devoured fetuses.

Intragroup comparisons of fetal weights showed statistical significant difference between the control and LPG, MPG and HPG. P<0.05. On the other hand, intragroup comparisons of crown rump length and fetal heart weights showed statistical significant difference between the control group and LPG, MPG. P<0.05. However, the intergroup comparison showed no statistical significance difference between the control group and LPG in trimester three.

Table 1: Mean of the litter size, Resorbed endometrial glands, placental weights and dead fetuses of the LPG, MPG, and HPG against the control at Trimester 1

Parameter	Control	Low dose Phenytoin administered at trimester I (LPG_{T1})	Medium dose phenytoin administered at trimester I (MPG_{T1})	High dose phenytoin administered at trimester I (HPG_{T1})	F	P-value
Litter size	13±0.577a	9.33±0.333b	7±0.577b	4±0.577c	52	0.000*
resorbed endometrial glands	0.33±0.333a	1.67±0.333b	2.33±0.333b	5±0.333c	23.1	0.000*
placental wts	0.43±0.009a	0.30±0003b	0.29±0.006b	0.27±0.003c	161	0.000*
dead fetuses	0.33±0.333a	1±0.577a	1.33±0.333a	1.67±0.333a	1.94	0.201

*The means, followed by the same letter in a row are not statistically different at (P□ 0.05) using one way ANOVA with Tukey test on post-hoc t-tests. * indicates significance (p<0.05)*

From table 1 above;

Litter size mean of the control (13±0.577) was found to be significantly higher than that in LPG (9.33±0.333), MPG (7±0.577) and HPG (4±0.577), p = < 0.0001. However, there was no statistical significant difference between LPG and MPG but compared to the HPG there was statistical difference

Resorbed endometrial glands mean among the control group (0.33±0.333) was found to be significantly different from all the experimental groups; LPG (1.67±0.333), MPG (2.33±0.333) and HPG (5±0.333) p<0.0001 which was less than 0.05 significance level.

Placental weights mean in trimester 1 between phenytoin treated groups and the control showed significance difference. However, there was no significance difference between mean placental weights of LPG and MPG. Control group (0.43±0.009), LPG (0.30±0003), MPG (0.29±0.006) and HPG (0.27±0.003) P<0.05

The mean of the **dead fetuses** were different between phenytoin treated groups and the control however it was not significant p value=0.201.

Table 2: Mean of the litter size, Resorbed endometrial glands, placental weights and dead fetuses of the LPG, MPG, and HPG against the control at Trimester 1I

Parameter	Control	Low dose Phenytoin administered at trimester II (LPG_{T2})	Medium dose phenytoin administered at trimester II (MPG_{T2})	High dose phenytoin administered at trimester II (HPG_{T2})	F	P-value
Litter size	13±0.577a	11.3±0.67a	8±0.58b	6±0.58b	27.8	0.000*
resorbed endometrial glands	0.33±0.333a	0.67±0.33ab	1.33±0.333b	3±0.577b	8.44	0.007
placental wts	0.43±0.009a	0.35±0.003b	0.32±0.003c	0.287±0.003d	144.6	0.000*
dead fetuses	0.33±0.333a	0.67±0.333ab	1.33±0.333b	2.67±0.333b	9.6	0.005*

*The means, followed by the same letter in a row are not statistically different at (P□ 0.05) using one way ANOVA with Tukey test on post-hoc t-tests. * indicates significance (p<0.05)*

From the results in table 2;

Litter size mean of the control (13±0.577) was found to be significantly different from MPG (8±0.58) and HPG (6±0.58). However, there was no significant difference between the litter size of the control and the LPG (11.3±0.67) when phenytoin was administered during the second trimester. There was also no significance difference between the litter size of the MPG and HPG when phenytoin was given during the second trimester.

Resorbed endometrial glands mean among the control group (0.33±0.333) was found to be significantly different from, MPG (1.33±0.333) and HPG (3±0.577) but there was no significant different between the control group and the LPG (0.67±0.33) likewise to the mean of resorbed endometrial glands of the MPG and HPG when phenytoin was administered in trimester 2 $p< 0.05$ significance level.

Placental weights mean in trimester 1I between all phenytoin treated groups and the control showed statistical significance difference. Control group (0.43±0.009), LPG (0.35±003), MPG (0.32±0.003) and HPG (0.287±0.003) $P<0.05$

The mean of the **dead fetuses** among the control group (0.33±0.333) was found to be significantly different from, MPG (1.33±0.333) and HPG (2.67±0.333) but there was no significant different between the control group and the LPG (0.67±0.333) likewise to the mean of dead fetuses of the MPG and HPG when phenytoin was administered in trimester 2 $p< 0.05$ significance level.

Table 3: Mean of the litter size, Resorbed endometrial glands, placental weights and dead fetuses of the LPG, MPG, and HPG against the control at Trimester III

Parameter	Control	Low dose Phenytoin administered at trimester III (LPG_{T3})	Medium dose phenytoin administered at trimester III (MPG_{T3})	High dose phenytoin administered at trimester II (HPG_{T2})	F	P-value
Litter size	13±0.577a	11.7±0.333a	11±0.577a	8±0.577b	16.1	0.001*
resorbed endometrial glands	0.33±0.333a	0.67±0.333a	1±0.577a	1.33±0.333a	1.111	0.400
placental wts	0.43±0.009a	0.387±0.003b	0.363±0.003b	0.333±0.003c	64.1	0.000*
dead fetuses	0.33±0.333a	1.33±0.333ab	2.33±0.333bc	3.67±0.333c	18.3	0.001*

*The means, followed by the same letter in a row are not statistically different at (P□ 0.05) using one way ANOVA with Tukey test on post-hoc t-tests. * indicates significance (p<0.05).*

From the results in table 3 above;

Litter size mean of the control (13±0.577) was found to be significantly different from HPG (6±0.577). However, there was no significant difference between the litter size of the control and the LPG (11.7±0.333) and MPG (11±0.577) when phenytoin was administered during the third trimester. There was also no significance difference between the litter size of the LPG and MPG when phenytoin was given during the third trimester p<0.05.

Resorbed endometrial glands mean between phenytoin treated groups; LPG (0.67±0.333), MPG (1±0.577), HPG (1.33±0.333) and the control (0.33±0.333) did not have significance difference when phenytoin was administered in trimester III.

Placental weights mean when phenytoin was administered in trimester III had a significant difference between phenytoin treated groups and the control (0.43±0.009). There was no significance difference between the mean placental weights of LPG (0.387±0.003) and MPG (0.363±0.003) but the difference between HPG (0.333±0.003) and the later was significant. in trimester 1I between all phenytoin treated groups and the control showed statistical significance difference. Control group (0.43±0.009), LPG (0.35±003), MPG (0.32±0.003) and HPG (0.287±0.003) P<0.05.

The mean of the **dead fetuses** when phenytoin is administered in the third trimester was statistically significantly different between MPG (2.33±0.333), HPG (3.67±0.333) and the control (0.33±0.333) but there was no statistical significant difference between LPG (1.33±0.333) and the control p< 0.05 significance level.

4.0 Discussion

From this experimental, prenatal use of phenytoin influence on the pregnancy outcome was time and dose dependent. The findings on inter and intragroup comparison of the the litter size, resorbed endometrial glands, placental weights and dead fetuses were statistically significant. The pregnancy outcome result of the experimental group(LPG,MPG,HPG) that received phenytoin were significantly different (lower) compared to the control group (higher) through the gestational period but marked in trimester 1 and II .In a study that was conducted by Danielson and the group on phenytoin teratogenic effects due to hypoxia and vascular disruption strongly support this study findings (Danielsson, 2005).Nutrients that include oxygen are important and lay a key role in the normal physiology of pregnancy and also proper development of embryonic structures and therefore hypoxia interfere with the normal physiology of pregnancy hence resulting in undesirable pregnancy outcome like reduced litter size, increased resorption, reduced placental weights and even dead of the fetuses.

Table 1, 2 and 3 results showed reduced litter size and placental weights (lowest) in the HPG and higher litter size and placental weights in the control group when phenytoin was administered during trimester I, II and III. Watkinson also did a study on phenytoin that demonstrated its possibility to deppress maternal cardiovascular functions causing uterine ischemia hence reducing placental oxygen delivery hence interfering with the pregnancy state (22).However,it was not reported interms of dose and time, this explains statistical significant difference in litter size, resorbed endometrial glands, placental weights and dead fetuses from this study findings between the experimental group that was dose and time dependent.

From this study findings, intra group comparison of the resorbed endometrial glands mean were significantly different between HPG (high) and LPG (low) and the inter group comparison between trimester I groupas (high) and trimester III (low) was also statistically significantly different. This is supported by early studies done by Danielson and Walkinson when they reported about phenytoin effects due hypooxia and reduced placental oxygen delivery (Daielsson, 1992).In addition, Zhu did a study demonstrating effects of supplimenting folic acid to patients using phenytoin prenatally and the results was significantly different between the group that used folic acid suplimentsin terms of fetal weight and length (higher) from the group that did not use folic acid suppliments (low) in terms of fetal weight and length (Zhu,1989). When the absorbtion of nutrient such as folic acid is reduced during pregnancy which have been attributed to prenatal phenytoin use, it has an impact to the pregnancy outcome because it is one of the essential requirements for proper development of the embryonic structures

This study also found out that the number of dead fetuses were higher when phenytoin was administered during trimester III as compared to trimester I as well as the high in HPG compared to LPG. This is supported by a study that was done by Cart when phenytoin was compared to class III antiarrhythmic drugs that both block the sodium channels causing low heart rate and cardiac arrest (Azarbayajani, 2006).In trimester III, embryologically the cardiovascular system of the developing embryo has started to function and therefore phenytoin use during this period of gestation cause may cause bradycardia hence reduction of blood supply to embryonic tissues leading to growth retardation. Furthermore, it may cause cardiac arrest which helps explains the fetal death.

5.0. Conclusion

In conclusion of the study has established phenytoin use during pregnancy affects the pregnancy outcome particularly when administered during the first and second trimester regardless of the dosage. When administered in trimester three the effects are not significant except when administered on high doses. The most vulnerable window period for phenytoin teratogenicity in addition established to be the first trimester while the most critical dose was 124mg/kg/bw.

6.0 Recommendations

The study recommends that;
1. The use phenytoin during pregnancy should be avoided as it has been shown to affect the pregnancy outcome particularly in trimester one (TM_1) and trimester two (TM_2) by seeking appropriate alternatives that are safer to the fetus.

2. Should expectant mothers be on prolong use of phenytoin 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 pregnancy.

3. Due to time and dose dependent teratogenic effects of phenytoin, health care workers including clinicians, nurses, midwives and others, need to be educated on how they will need to be educating women of reproductive age and are on chronic usage of phenytoin of its teratogenicity during pregnancy, on the need for early planning of their pregnancies for effective introduction of alternative medicines, to enable them avoid use phenytoin during pregnancy.

4. Further studies be carried out in non-human primates that have close phylogenetic relations to humans, to ascertain its effects to the pregnancy outcome in relation to doses.

Acknowledgements

Author is grateful to Dr. Joseph.KKweri, Chairman of Department in Human Anatomy, Jomo Kenyatta University of Agriculture & Technology for his dedication and support to success of this study.

References

1. 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.
2. Allen M., Ahrens K.A, Bosco J.L. (2016). Use of antiepileptic medications in pregnancy in relation to risks of birth defects. *Annals of epidemiology* 2(1), 842–50.
3. Azarbayjani, F., Borg, L. A. H., Danielsson, B. R. (2006). Increased Susceptibility to Phenytoin Teratogenicity : Excessive Generation of Reactive Oxygen Species or Impaired Antioxidant Defense. *Basic clinical pharmacology & toxicology*. *99*(4), 305–311.
4. Bailey, J., Thew, M., Balls, M. (2014). An analysis of the use of animal models in predicting human toxicology and drug safety. *Alternative Laboratory Animals*. *42*(3), 181-199.
5. Bromley RL, Baker GA. Fetal antiepileptic drug exposure and cognitive outcomes. *Seizure Eur J Epilepsy* [Internet]. 2017;44:225–31. Available from: http://dx.doi.org/10.1016/j.seizure.2016.10.006
6. Charan, J., Biswas, T., How to calculate sample size for different study designs in medical research (2013). *Indian Journal of Psychological Medicine*. *35*(2), 121-126.

7. Danielsson, B. R., Johansson, A., Danielsson, C., Azarbayjani, F., Blomgren, B., Sko, A. (2005). *Phenytoin Teratogenicity : Hypoxia Marker and Effects on Embryonic Heart Rhythm Suggest an hERG-Related Mechanism.Birth Defects Clinical and Molecular Teratology. 73*(3), 146–153.
8. Gelder, M., Rooij, IA., Miller, RK., Zielhuis, GA., Jong, LT., Roeleveld, N. (2010) Teratogenic mechanisms of medical drugs. *Human Reproductive Update. 16*(4), 378-394.
9. Kakkar, A., Chilkoti, G., Arora, M. (2013). Phenytoin induced sinoatrial bradyarrhythmia in the perioperative period. *Indian Journal of Anaesthesia. 57*(6), 628-630.
10. Katsiki, N., Mikhailidis, D. P., Nair, D. R. (2014). The effects of antiepileptic drugs on vascular risk factors : A narrative review. *Seizure: European Journal of Epilepsy*, *23*(9), 677–684.
11. Lander, C. M. (2008). Antiepileptic drugs in pregnancy and lactation. *Neurology. 31*(3), 70–72.
12. 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.
13. Nulman, I., Scolnik, D., Chitayat, D., Farkas, LD., Koren, G. (1997) Findings in children exposed in utero to phenytoin and carbamazepine monotherapy: independent effects of epilepsy and medications.*American journal of medical genetics . 68*(1), 18-24.
14. Ornoy A. Valproic acid in pregnancy : How much are we endangering the embryo and fetus ? Valproic acid in pregnancy : How much are we endangering the embryo and fetus ? 2017;(August 2009).
15. Saetre E, Abdelnoor M, Amlie P, Tossebro M, Perucca E, Taubøll E, et al. Cardiac function and antiepileptic drug treatment in the elderly : A comparison between lamotrigine and sustained-release carbamazepine. 2009;50(8):1841–9.
16. Shah, R. R. (2010). Cardiac Effects of Antiepileptic Drugs. *British Journal of Pharmacology. 159*(1), 58-69
17. Waltman, P. A. (2003). Epilepsy and Pregnancy. *Journal of Biomedical and Pharmaceutical Research. 23*(2), 93–99.
18. Webster, W. S., Danielsson, B. R., Azarbayjani, F. (1997). Pharmacologically Induced Embryonic Bradycardia and Arrhythmia Resulting in Hypoxia and Possible Free Radical Damage at Reoxygenation. *Teratology 63*(3), 152–160.
19. Zhu, M., Zhou, S. (1989). Reduction of the Teratogenic Effects of Phenytoin by Folic Acid and a Mixture of Folic Acid , Vitamins , and Amino Acids . *Journal of the International Leaque Against Epilepsy. 25(2), 205-216*