Effects of Topiramate on Pregnancy Outcome in Rats

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Background: Anti-epileptics taken during pregnancy might lead to low birth-weight and birth defects which could be associated with neonatal morbidity and mortality.

Objective: To evaluate the effects of maternal exposure to therapeutic doses of topiramate on the growth of 20-day rat fetuses.

Design: An Experimental Animal Study.

Setting: Teratology Laboratory, Anatomy Department, College of Medicine and Medical Sciences, Arabian Gulf University, Bahrain.

Method: Three groups of Sprague-Dawley pregnant rats were used in the experiment: control, Topiramate 50mg/Kg BW and Topiramate 100 mg/Kg BW. Topiramate was administered by intragastric intubation from day 6 through day 19 of gestation. Cesarean section was performed on day 20. Resorption was calculated, placental weight and umbilical cord length were measured. Fetuses were collected to assess their growth parameters: fetal weight (FW), biparietal diameter (BPD), crown-rump length (CRL) and head length (HL). Ponderal index and CRL/HL ratio were calculated to indicate the type of growth restriction.

Result: The Topiramate treated groups showed an insignificant increase in the rate of resorption, a significant decrease in umbilical cord length, placental weight and highly significant reduction in fetal growth parameters. No significant changes were noticed in fetal growth parameters between Topiramate groups. A positive correlation was found between FW and UCL, PW, CRL, HL and BPD in all examined groups. Ponderal index and CRL/HL ratio indicate symmetrical growth restriction of the fetuses in both treated Topiramate groups.

Conclusion: The doses of Topiramate, which were given to pregnant rats were equivalent to the human therapeutic range; the drug led to symmetrical fetal gross restriction with few abnormal fetuses and placentae. Topiramate attributed effects were not dose related. The drug should be taken with caution during pregnancy.

Bahrain Med Bull 2016; 38(4): 214 - 218

Intrauterine growth restriction (IUGR) is one of the causes that lead to low-birth-weight and neonatal morbidity and mortality. Drugs taken during pregnancy might induce IUGR through different mechanisms, including placental degeneration, malnutrition, and umbilical blood flow reduction or by a direct effect on the embryo/fetus or through the drug or its metabolites[1]. Topiramate is used to treat epilepsy and it crosses the placenta. FDA classified it as "Pregnancy Category-D drug". In animal experiments, high doses of more than 200 mg/kg produce different types of anomalies, growth restriction and skeletal deformities[2].

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Increased rate of IUGR with low-birth weight and congenital malformations were seen in cases of maternal epilepsy[3,4]. However, it is difficult to attribute these results to maternal epilepsy as such, drug treatment or their combinations. Few experimental studies on animals had addressed the effects of the therapeutic doses of Topiramate on growth. Singh et al found that Topiramate in doses of up to 200 mg/kg did not produce a reduction in fetal weight in rats despite its production of abnormal vertebral ossification and that its effect is dose related (40, 100 and 200 mg/kg)[5]. Topiramate is used recently for body weight loss in adults with the different hypothesis in its action[6,7]. Fadel et al. found that therapeutic doses of Topiramate (50 and 100 mg/kg BW) affect skeletal developments of ribs and vertebrae in rat fetuses[8].

The aim of this study is to evaluate the effects of prenatal exposure to Topiramate at human therapeutic doses (50 and 100 mg/kg) on growth and development of 20-day-old preterm rat fetuses.

METHOD

Sexually mature adult virgin Sprague-Dawley rats (150 to 250 grams) were kept at room temperature and received food and water ad libitum and handled only by the researcher. Two weeks later, one fertile male rat was introduced into a cage with two females and remained there overnight. Following mating, pregnancy was confirmed in the next morning by detecting sperms in vaginal smears (GD 1). Twenty-four pregnant rats were divided randomly into three equal groups (8 dams each). Topiramate was dissolved in distilled water (10 mg/ml) and was administered to healthy non-epileptic pregnant rats intragastric from day 6 through day 19 of gestation, in doses equivalent to human: group (T50) received 50mg/kg daily; group (T100) received 100mg/kg daily; the control group received corresponding volume of distilled water by intragastric route.

Pregnant rats were weighed daily and the doses of Topiramate were adjusted according to their body weight. On day 20, the pregnant rats were anesthetized by ether and their abdomens were opened. Fetuses were collected from the uterine horns. The numbers of live, dead fetuses and resorption (post-implantation loss) were recorded. The following measurements were taken: length of umbilical cord (UCL) placental weight (PW), fetal weight (FW), crown-rump length (CRL), head length (HL) and biparietal diameter (BPD). Ponderal index (FW x 100 / CRL^3) and the CRL/HL ratio were calculated to indicate the type of growth restriction if found. Each fetus was externally examined and the abnormalities were recorded.

Based on Wilson method, approximately half of the fetuses were randomly collected from each mother to be used for skeletal staining (parallel study) and the other half were fixed in Bouin's solution for two weeks before their reexamination to confirm any external abnormalities, see table 1[9].

Data was entered and analyzed using SPSS version 20. Statistical analysis of the recorded data was performed using chi-square test X^2 and One Way Analysis of Variance (ANOVA) test.

Table 1: Number of Animals Used in the Different Groups in the Study

Groups (Number of Mothers)	Control (8)	T50 (8)	T100 (8)
Live Fetuses	94	104	105
Number of Fetuses for Skeletal Staining	45	49	50
Number of Fetuses for Bouin's Fixation	49	55	55
TOTAL	**188**	**208**	**210**

RESULT

The changes in maternal weight gain during the period of gestation are summarized in table 2. Analysis of variance showed that there is no significant difference in maternal weight gain between the study groups (P=0.696). One fetus was dead in the control group while all fetuses were seen alive in the treated groups. Resorption was found in two litters out of eight (25%) in the control group while in Topiramate groups they were found in four litters out of eight (50%) in each treated group. Chi-Square test for resorption indicated that there is an increase in the rate of resorption in the Topiramate-treated groups compared to the control, but the increase is not significant (P=0.689).

Table 2: Influence of Topiramate on Maternal Weight Gain and Embryolethality

Groups (Number of Mothers)	Control (8)	T50 (8)	T100 (8)
Maternal Weight Gain (g) / Mean ± SD	80.62 ± 19.27	74.37 ± 15.93	79.37 ± 9.34
Total Implantations	100	111	114
Resorption (%)	5 (5.0%)	7 (6.3%)	9 (7.9%)
Dead Fetuses (%)	1 (1.0%)	0	0
Live Fetuses (%)	94 (94.0%)	104 (93.%7)	105 (92.1%)

Placental weight (PW) and umbilical cord length (UCL) showed a significant decrease in both treated Topiramate groups compared to the control, see table 3. No significant differences were noticed between treated groups.

Table 3: Influence of Topiramate on Placental Weight and Umbilical Cord Length

Groups (Number of Fetuses)	Control (94) Mean ± SD	T50 (104) Mean ± SD	T100 (105) Mean ± SD
Placental Weight (g)	0.558 ± 0.111	0.521 ± 0.736*	0.522 ± 0.740*
Umbilical Cord Length (cm)	2.22 ± 0.30	1.99 ± 0.27*	2.08 ± 0.35*

ANOVA test: * P < 0.01 compared to control group

In this study, fetal weight (FW), crown-rump length (CRL), head length (HL) and biparietal diameter (BPD) growth parameters were evaluated, see table 4. Growth parameters of both Topiramate-treated groups were significantly reduced compared to the control group, see figure 1 (A and B) and table 4. No significant differences regarding growth parameters values were found between Topiramate-treated groups.

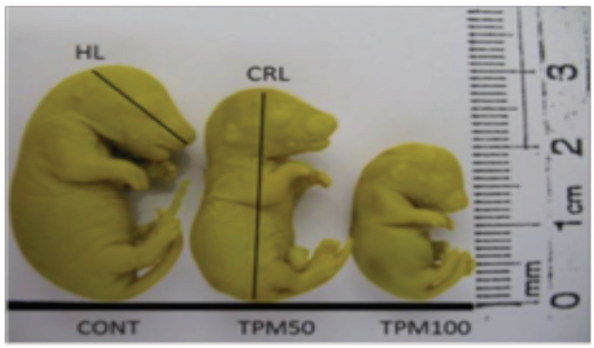


Figure 1 (A)

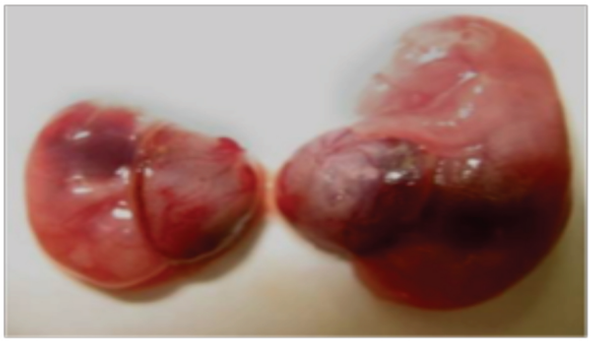


Figure 1 (B)

Figure 1 (A and B): Fetal Growth Restriction

Table 4: Influence of Topiramate on Fetal Growth

Groups (Number of Fetuses)	Control (94)	T50 (104)	T100 (105)
Fetal Weight (g) / Mean ± SD	2.53±0.28	2.06 ±0.20*	2.11 ±0.20*
Crown-Rump Length (CRL) / Mean ± SD	2.73 ± 0.19	2.61 ± 0.18*	2.54 ± 0.29*
Head Length(cm) (HL) / Mean ± SD	1.32 ± 0.08	1.26 ± 0.08*	1.23 ± 0.09*
Biparietal Diameter / Mean ± SD	0.76 ± 0.07	0.69 ± 0.05*	0.68 ±0.07*
Ponderal Index	12.4	11.7	12.8
CRL/HL	2.06	2.07	2.06

ANOVA test: * P < 0.01 compared to control group

Ponderal Index was not decreased in T50 and T100 groups compared to control group. The ratio CRL/HL was almost equal in T50 and T100 groups compared to control group which indicates a symmetrical intrauterine growth restriction, see table 4.

The decrease in FW in both topiramate groups is accompanied by a corresponding reduction in placental weight, umbilical cord length and all growth parameters (CRL, HL and BPD). A significant positive correlation was found between the fetal weight and these measurements as shown in table 5.

Table 5: Pearson Correlation Coefficient (R) Between Fetal Weight and Other Developmental Growth Parameters in Control and Topiramate-Treated Groups

	UCL	PW	CRL	HL	BPD
Fetal Weight (C)	0.297	0.400**	0.385**	0.289**	0.425**
Fetal Weight (T50)	0.409**	0.421**	0.650**	0.448**	0.199*
Fetal Weight (T100)	0.236*	0.053	0.304**	0.428**	0.261**

* Correlation is significant at (0.05) (2 tailed)
** Correlation is significant at (0.01) (2 tailed)

Very few external abnormalities are seen. One fetus (severe kyphosis with subcutaneous hematoma) in Topiramate 100 group and five abnormal placentae in both Topiramate-treated groups together were detected, see figure 1 (C to F). Bilobed placenta, placenta succenturiata, twin placenta, malposition of the placenta and gangrenous placenta were seen.

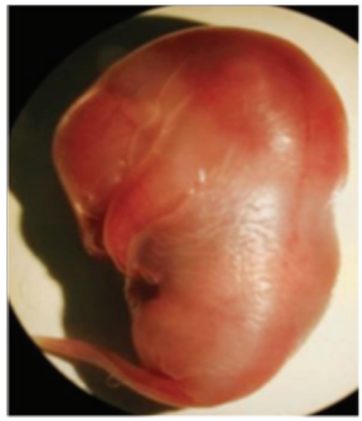


Figure 1 (C): Abnormal Fetus with Kyphosis

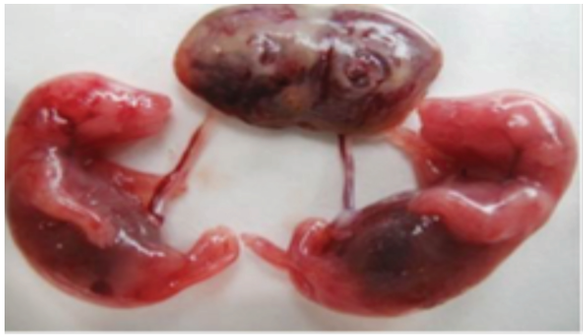
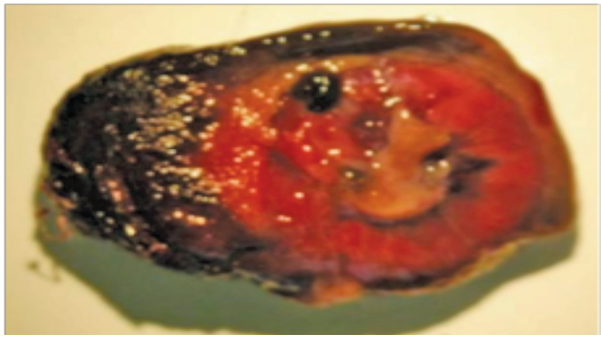


Figure 1 (D)



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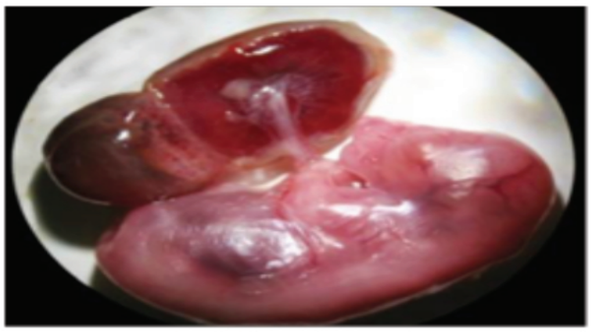

Figure 1 (F)

Figure 1 (D to F): Abnormal Placentae

DISCUSSION

Pregnant women suffering from psychological illness usually take different anxiolytic and antiepileptic drugs and possibly other medications. This multi-drug therapy makes it difficult to assess the risk of malformations associated with Topiramate use[10].

Topiramate was reported to increase the rate of malformation in infants and to be associated with fetal growth restriction[11]. Most of the previous animal studies used a moderate and high dose of Topiramate[2,5].

In this study, a double increase in the rate of resorption (post-implantation loss) was noticed in the litters of Topiramate-treated groups (50 % of litters) compared to the control (25 % of litters). These results were similar to previous studies, but they used higher toxic doses or long-term exposure of female rats before fertilization[5,7,12]. However, the insignificant increase in the total number of resorption in our study could be explained by the fact that we used Topiramate only during pregnancy (day 6 to 19 of gestation) and not prenatal; therefore, the preimplantation loss was not included.

The growth of the fetus is a complex process which may be affected by some factors including maternal nutrition, hormones, gene regulation, placental changes and others environmental factors[13-16]. Many anthropometric measurements have been used as diagnostic tests to distinguish malnourished fetuses from fetal growth restriction due to other causes. Birth weight is a relatively crude measure of fetal growth[17]. Birth length is not considered a good indicator of fetal growth because it is more difficult to measure and it shows relatively small variations[18]. Ponderal index is more reliable; it is the closest equivalent of body mass index (BMI) in adults and it measures the thinness at birth[19]. The placental weight is also considered a good indicator for fetal growth[13]. In the present study, the growth parameters of the fetuses (FW, CRL, HL, BPD) from the Topiramate-treated groups were reduced, indicating intrauterine growth restriction (IUGR), which is similar to other studies[5,11,20]. The placental weight was also reduced. No dose relation was noticed between the two therapeutic doses.

Previous anthropometric indices studies comparing the lengths of the different regions in the body classify growth of the fetus into proportionate or disproportionate[21,22]. The fetuses of both Topiramate groups in the present study were symmetrically small as the ponderal index was equal in all groups, and the rate of reduction of CRL/HL ratio was relatively uniform denoting proportionate growth restriction.

The nutrients and oxygen are the most relevant factors of the intrauterine environment in determining the limits of fetal growth[23]. Growth restriction of the fetuses discovered in the Topiramate groups was probably not attributed to maternal malnutrition as there was no reduction in maternal weight gain which found to be almost uniform in all groups[24]. However, the reduction in placental weight and the shortage of the length of umbilical cord may suggest dysfunction of the placentae which probably led to a decrease in uteroplacental and or fetoplacental circulation. Histopathological degenerative changes of the Topiramate on placentae were described in the previous study and reported to increase in severity if high dose is used[25,26].

Multiple studies reported statistically significant associations between Topiramate exposure during pregnancy and fetal anomalies as hypospadias and brain maldevelopment, jaw or oral cleft[27-29]. Other researchers found that the recurrence risks for malformations in pregnancies exposed to Topiramate are higher compared with carbamazepine and lamotrigine, also for pregnancies exposed to polytherapy regimens, but in our study none of these types of anomalies were found[30]. Ornoy et al revealed that Topiramate reduces birth weight but does not increase the risk for structural defects, which is similar with our results[7].

CONCLUSION

The study reveals that Topiramate, at doses equivalent to the human therapeutic doses, induces symmetrical intrauterine growth restriction and a remarkable increase in the rate of resorption and few gross abnormalities in rat fetuses and placentae. The effects of these therapeutic doses are not dose related and Topiramate should be taken with caution during pregnancy as the drug is frequently used by women in childbearing period.

Author Contribution: All authors share equal effort contribution towards (1) substantial contribution to conception and design, acquisition, analysis and interpretation of data; (2) drafting the article and revising it critically for important intellectual content; and (3) final approval of manuscript version to be published. Yes.

Potential Conflict of Interest: None.

Sponsorship: Arabian Gulf University, Bahrain.

Acceptance Date: 5 October 2016.

Ethical approval: Approved by the Research and Ethics Committee, College of Medicine and Medical Sciences, Arabian Gulf University, Bahrain.

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