Cibtech Journal of Zoology ISSN: 2319–3883 (Online)
An Open Access, Online International Journal Available at http://www.cibtech.org/cjz.htm
2015 Vol. 4 (1) January-April, pp.37-44/Nikbakht and Hejazi
Research Article

PROTECTIVE ROLE OF MELATONIN IN HISTOMORPHOMETRIC CHANGES OF FETAL MICE TESTIS BY LAMOTRIGINE INDUCED

Sajjad Nikbakht[1] and *Sajjad Hejazi[2]
[1]Department of Histological Science, Tehran Science and Research Branch, Islamic Azad University, Tehran, Iran
[2]Department of Anatomy, Tabriz branch, Islamic Azad University, Tabriz, Iran
**Author for Correspondence*

ABSTRACT
In last studies we reported the effect of LTG on some abnormalities occurrence such as cranial disorders, ventricular dilatation. In the present study, the effect of melatonin, as a well known antioxidant with free radicals removing mechanism, on fetus testis improvement of mother's under-treatment of the lamotrigine using animal model, has been done. The use of melatonin in rats protects cells from oxidative damages and in fact decreases oxidative stresses via various ways. In this study, 10 mg/kg melatonin was used as intraperitoneal injection. In order to melatonin solubility 1% ethanol was used. The animals received 75 mg/kg lamotrigine dissolved in saline peritoneally. It must be noted that lamotrigine was injected in organogenesis days of pregnancy. The testicles were in 10% formalin solution and then were stained by H&E method followed by histotechnique stages. ANOVA was used for analysis of the data; also Tukey test was used to compare the difference between groups via statistical software of SPSS 10. Lamotrigine caused a meaningful decrease in fetal testis weight compared with lamotrigine + melatonin and control groups such that the difference between the groups was meaningful. Based on Morphometric parameters of testicular tissue Lamotrigine caused a meaningful decrease in the seminiferous tubules difference of lamotrigine control , and lamotrigine treatment groups compared with the control group, also there was a difference between lamotrigine control and lamotrigine treatment groups. In the present study some vacuoles are observed in the thickness of germinal epithelium that may be created by destroyed germ cells. Lamotrigine caused no meaningful difference in asexual sertoli cells of control, lamotrigine control, and lamotrigine groups. It seems that lamotrigine causes no meaningful decrease in sertoli cells. Lamotrigine caused a meaningful decreased in leydig cell number in lamotrigine control, and lamotrigine treatment groups compared with the control group. But, the parameter was not meaningful between control and treatment groups. It seems that melatonin has a protective effect on the process of decreased leydig cells number.

Keywords: *Fetal Mice, Lamotrigine, Melatonin, Testis*

INTRODUCTION
One of the important causes of congenital disorders is the use of some medicines during pregnancy. Among medicines which have taken into consideration are anti epilepsy drugs (AEDs) due to most of their components that increase fetal abnormalities. Based on the conducted studies, 7-10% of fetuses with mothers that exposed to AED suffer from fetal abnormality (Bastaki *et al.*, 2001). Lamotrigine (LTG) is one of the novel AEDs that was administered in some epileptics since 1992 (Richens, 1994). Now, the drug is one of the common drugs in epilepsy treatment and some psychological diseases and its use is increasing (Curry *et al.,* 1998; Kusumakar *et al.*, 1997; Young *et al.*, 1997). According to the studies the drug passes the placental blood barrier and causes fetal abnormalities (Mohanty *et al.*, 2011). LTG, in pregnancy period, causes decreased total protein of fetuses that leads to decreased height and weight (Nau, 1981). In last studies the effect of LTG on some abnormalities occurrence such as cranial disorders (Encephalitis, Execephalitis), ventricular dilatation. Furthermore, genetic disorders in hydrolysis of the metabolites increase teratogenic of them. Melatonin can remove and neutralize free radicals of hydroxyl, peroxyl, and nitrate peroxy anions (Lewis-Johnes *et al.*, 1985). The use of melatonin in rats protects cells from oxidative damages and in fact decreases oxidative stresses via various ways. Melatonin can decrease

toxicity and side effects of medicines (Lewis-Johnes *et al.,* 1985). Melatonin, as the most important of epiphysis secretion, is a very effective antioxidant and free radicals counteractive (Redins *et al.,* 2002). Melatonin has the antioxidant property since it can induce the activity or occurrence of antioxidant enzymes such as: super oxide dismutase, Glutathione reductase, Glutathione peroxidase (Redins *et al.,* 2002). The anti apoptotic effects of melatonin on different tissues have been shown in various experiments (Schrader *et al.,* 2001). Since that the teratogenic effects of LTG on the fetus and newborn have been established (Mark *et al.,* 2004; Mohanty *et al.,* 2011; Rahmani *et al.,* 2006) and the use of the drugs is increasing, it has been tried to examine, in the present study, the effect of melatonin, as a well known antioxidant with free radicals removing mechanism, on fetus testis improvement of mothers under-treatment of the lamotrigine using animal model.

MATERIALS AND METHODS

Methods

The study was an interventional one in which 30 female and 10 male mice fetus weighing about 30±5 g were used (they were bought from Pastor ins.). The cages were kept at 22±2℃ with humidity of 38%±2, and 300 LUX light in the room center with 12 hours of successive periods of darkness and light. Water and concentrated food were available efficiently. In order to mate, three female mice were kept with one male mouse in a cage from evening to the next day morning, and then they were examined in terms of vaginal plug formation. The female mice with plugs were isolated from others. The date of plug observation was considered a zero day of pregnancy. In this study, 10 mg/kg melatonin was used as intraperitoneal injection. In order to melatonin solubility 1% ethanol was used according to the study of Ata Shahin (Ateşşahin *et al.,* 2006). The animals received 75 mg/kg lamotrigine dissolved in saline peritoneally (Bastaki *et al.,* 2001).

Attention, Administration of doses higher than 75 mg/kg lamotrigine causes mouse death (Bastaki *et al.,* 2001). It must be noted that lamotrigine was injected in organogenesis days of pregnancy (Mark *et al.,* 2004).

In the present study, the groups were as 1 control and 2 experimental groups.

- Group 1: involved of mice at 9, 10, and 11 days of pregnancy, which received 75 mg/kg saline solution (Bastaki et al., 2001) intraperitoneally (n=10).
- Group 2: (lamotrigine control group) involved of mice at 9, 10, and 11 days of pregnancy, which received 75 mg/kg lamotrigine dissolved in saline solution intraperitoneally (n=10).
- Group 3: (lamotrigine+melatonin control group) involved of mice at 9, 10, and 11 days of pregnancy, which received 75 mg/kg lamotrigine dissolved in saline solution and 10 mg/kg melatonin orally (by gavage) (n=10).

At the end of the pregnancy the numbers of newborns in different groups were recorded. Then the CRL of fetus was measured using a caliper with an accuracy of 0.1 mm. Their weights were measured using a digital weigh with an accuracy of 0.10. Then the newborns were placed in 10% formalin fixative solution to perform the stages of histological films preparation. The testicles were in 10% formalin solution and then were stained by H&E method followed by histotechnique stages.

Statistical analysis: The mean and SD of fetal length and weight, testis weight, spermatogonia, Sertoli, and Leydig cell number, seminiferous tubule diameter, and the thickness of the testicular capsule were expressed as Maen±SEM. ANOVA was used for analysis of the data, also Tukey test was used to compare the difference between groups via statistical software of SPSS 10.

RESULTS AND DISCUSSION

Results

The observations of testicular tissue of the lamotrigine group (medical control group) revealed spermatogonia cell damage and their disorganization, as well as the presence of large vacuoles in the seminiferous tubule. It seemed that the vacuoles were the places of cells, which dislodged from their original place because of the cell degeneration process. Expansion of the interstitial spaces between

seminiferous tubules and the lower number of the seminiferous tubules were of other changes of this group. The samples of the group which received lamotrigine and melatonin simultaneously, the seminiferous tubules compression and decrease of interstitial spaces were observed. The seminiferous tubule epithelium was in a normal condition such that there were no large vacuoles. Spermatogonia cells were in a normal condition with a large spherical nuclear, euchromatin, and several nucleoli. In interstitial spaces of the medical intervention group the presence of Leydig cells with a high density compared with the medical control group was seen.

Morphometric Results of Growth Parameters

The one day old newborns were prepared to growth parameter evaluations. The newborns at first were weighted by a digital weigh with an accuracy of 0.01 mg, also their length was measured by caliper. Finally, the samples were placed in 10% formalin fixative solution in order to conduct the histotechnique stages.

The Effect on Weight

In medical control group (lamotrigine) the mean weight of newborns was 1.2±0.3 g. In medical Interventional group (lamotrigine + melatonin), it was 1.4±0.3 that there was a meaningful difference between the two groups ($p<0.05$). Also, there was a difference between the medical control group and control group, but there was no meaningful difference between medical Interventional group and control group (table 1).

The Effect on CRL

In medical control group, the mean CRL of newborns was 22.5±0.37mm. In a medical Interventional group it was 26 ±0.8 that there was a meaningful difference between the two groups ($p<0.05$). Also, there was a difference between the medical control group and control group, but there was no meaningful difference between medical Interventional group and control group (table 1).

The Effect on Testis

Its weight in medical control group and medical Interventional groups was 0.7±0.09 and 0.78±0.5mg, respectively, which had no difference compared with each other (table 1).

Table 1: The mean of fetal growth parameters followed by the administration of lamotrigine in medical control group and lamotrigine + melatonin medical interventional group at organogenesis period of pregnancy. Each parameter was expressed as mean±SEM (n=20)

Groups Variables	Medical control group (lamotrigine)	Interventional group (lamotrigine+melatonin)	Control group	Number	P value
Neonate weight (gr)	1.2±0.3	1.4±0.3	1.49±0.6	20	p<0.05
Neonate Length (mm)	22.5±0.7	26.5±0.8	27.3±0.5	20	p<0.05
Testis weight(gr)	0.7±0.09	0.78±0.5	0.81±0.3	20	p<0.05

**The different letters show a meaningful difference among groups (P<0.05*)

The Effect on Morphometric Parameters of Testicular Tissue

Seminiferous tubule: in order to examine and calculate the diameter of seminiferous tubule, 20 tubules of both groups were measured cross-sectional, then the mean was determined. The mean of seminiferous tubules in control, lamotrigine control, and lamotrigine treatment groups were 38.8±0.5, 29.9±0.4, and 34.9±0.8 µm, respectively. There was a meaningful difference among three groups ($P<0.05$) (table 2).

Spermatogonia Cells

In order to determine the cell number, 20 seminiferous tubules of each mouse were evaluated cross-sectionally. The mean of Spermatogonia cells number in control, lamotrigine control, and lamotrigine

treatment groups were 49.9±0.3, 36.8±0.5, and 46.2±0.1 mm^2, respectively. There was a meaningful difference among three groups (P<0.05) (table 2).

Sertoli Cells

The mean of Sertoli cell number in control, lamotrigine control, and lamotrigine treatment groups were 22.4±0.5, 19.34±0.9, and 19.6±0.3mm^2, respectively. There was no meaningful difference among three groups (P>0.05) (table 2).

Leydig Cells

The mean of Leydig cell number in control, lamotrigine control, and lamotrigine treatment groups were 343.3±0.5, 302.2±0.7, and 310.5±0.7 mm^2, respectively. There was no meaningful difference between lamotrigine control and lamotrigine treatment groups, but a meaningful difference was seen between the two groups and control group (P<0.05) (table 2).

Table 2: Parameters of testicular tissue Morphometrics followed by lamotrigine administration in medical control group and lamotrigine+melatonin in medical intervention group at organogenesis days of pregnant mice. Each parameter has been presented as mean±SEM (n=20).

Variables Groups	Seminiferous diameter	Spermatogonia cells number	Sertoli cells number	Leydig cells number
Control	38.8 ± 0.5	49.9± 0.3	22.4± 0.5	343.3 ± 0.5
Medical control group (lamotrigine)	29.9± 0.4	36.8± 0.5	19.34± 0.9	302.2± 0.7
Interventional group (lamotrigine+melatonin)	34.9± 0.8	46.2± 0.1	19.6± 0.3	310.5± 0.7

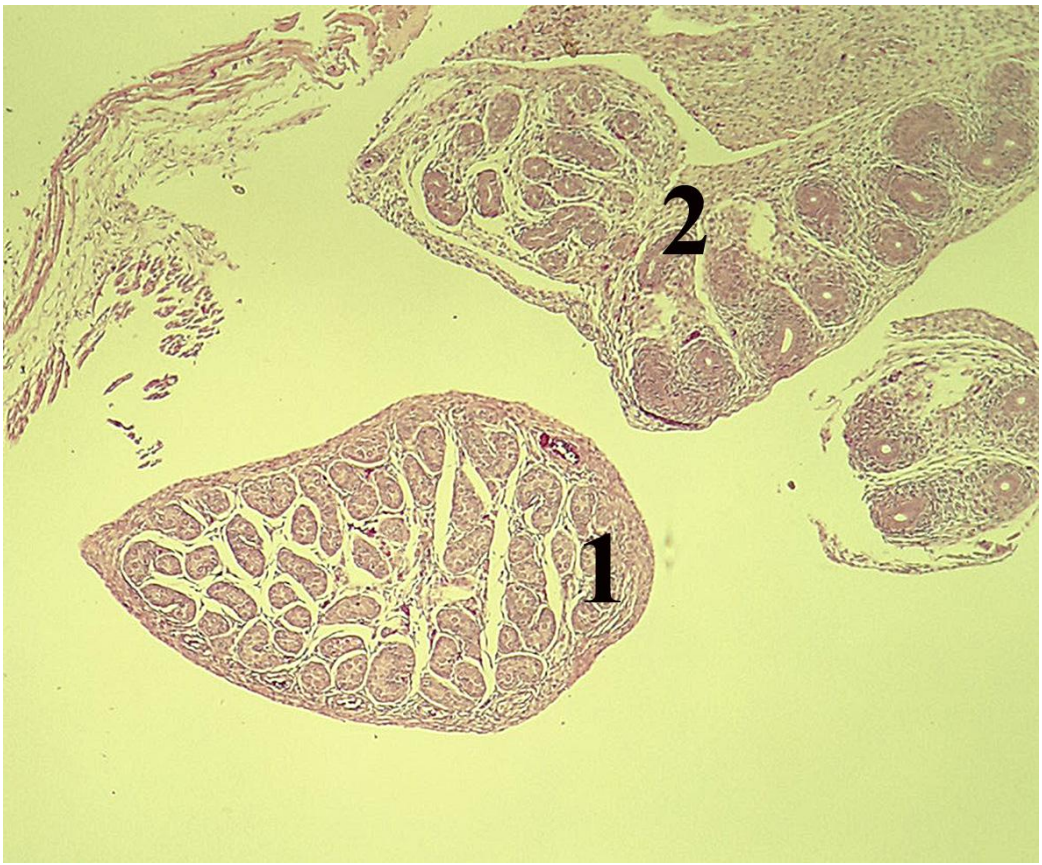

Figure 1: Whole section of one-day old fetal testis tissue of lamotrigine – melatonin group (Nikon microscope, ×10, H&E staining) 1. Testis 2. Mesonephric Remains

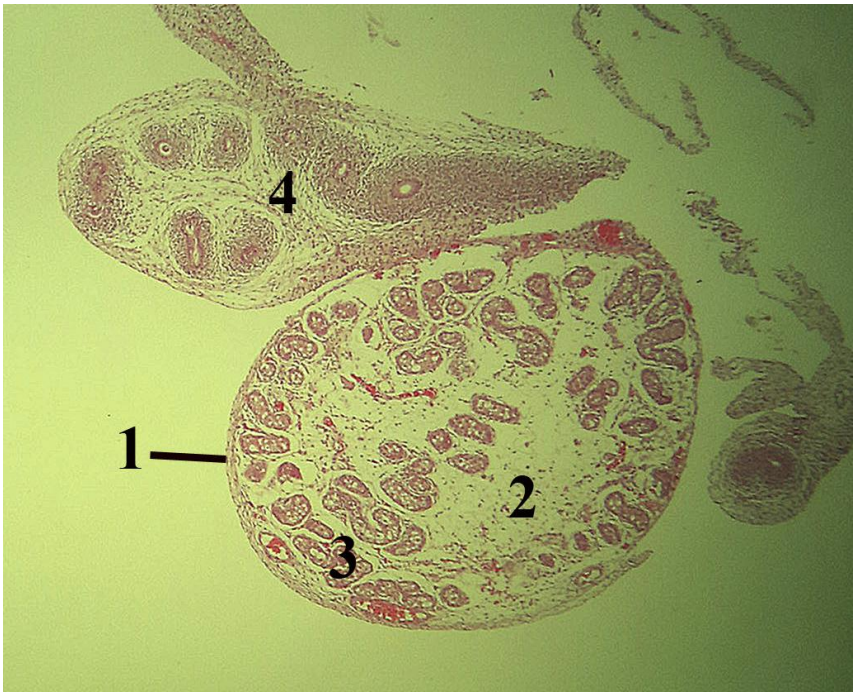

Figure 2: Whole section of one-day old fetal testis tissue of the lamotrigine control group (Nikon microscope, ×10, H&E staining) 1. Testis 2.Increased interstitial space 3.Decreased seminiferous tubule density 4. Mesonephric remains

Discussion

Antiepileptic drugs are of teratogenic chemical compounds that most studies have been conducted about them (Berkowitz *et al.*, 2004). Epilepsy is a chronic neurological disease that millions of people across the world suffered from and they have to use at least one of the antiepileptic drugs. Medical treatment of epilepsy during pregnancy always has been associated with several complications. Consequently, fetuses of epileptic mothers are more prone to abnormalities (Bastaki *et al.*, 2001).

The main reason of congenital abnormality followed by using antiepileptic drugs is attributed to the formation of free radicals by the drugs. Furthermore, the presence of genetic disorders in metabolite hydrolysis increases teratogenic. Despite of several studies, teratogenic events by antiepileptic drugs in different days and special fetal days is considered as a problem in medicine, Obstetrics and Gynecology (Bastaki *et al.*, 2001).

Melatonin is one the secretions of epiphysis gland and effective in some physiologic events. It has neuronal-hormonal function as well as the productivity, immunity and temperature regulator. Also, it affects cell Proliferation and differentiation (Sanchez-Hidalgo *et al.*, 2009). Melatonin can remove and neutralize free radicals such as hydroxyl, peroxyl radicals and nitrate peroxy anions (Padmanabhan *et al.*, 2003). It also protects cells of laboratory large mice against oxidative damages; in fact decreases oxidative stresses via different ways (Sönmez *et al.*, 2007). High doses of melatonin, 100 mg/kg, at the presence of X ray protect large laboratory mice from acute testicular damage (Guneli *et al.*, 2008). Melatonin also can decrease drugs toxicity (Reiter *et al*., 2001). Being as the most important secretion of epiphysis, melatonin is the most effective and neutralizing antioxidant of free radicals (Sanchez-Hidalgo *et al.*, 2009). It passes easily from the cell membrane and spreads in whole-cell because of small size and lipophilic property. The melatonin concentration in cell nuclear is too high and protects DNA against degenerative factors (Sanchez-Hidalgo *et al.*, 2009). The strong antioxidant property of melatonin can induce activity or occurrence antioxidant enzyme genes such as super oxide dismutase, glutathione reductase, glutathione peroxidase (Sanchez-Hidalgo *et al.*, 2009). Based on the microscopic observations there were signs of spermatogonia degeneration and disorganization, and presence of vacuoles in

seminiferous tubules of epithelium. It seemed that vacuoles were the place of cells that dislodged from their original place due to degeneration. It has been reported in a study that in the cell degeneration process membrane phospholipids lead to release arachidonic acid and its decomposition by associating enzymes. The production of metabolites induces free radicals production such as oxygen free radicals that may play an important role in edema. The aggregation of oxygen free radicals causes osmotic oxidative that in turn has some effects of chemical reactions and induces cell necrosis and apoptosis (Nulman *et al.*, 1999). On the other hand, the conducted studies propound other theories about abnormalities followed by antiepileptic drug administration. They believe that the produced free radicals by antiepileptic drugs have toxic effects in cells of consumer as well as the fetus. According to the obtained results from Morphometric parameters of fetus growth it seems that lamotrigine has been a meaningful decreasing effect on the newborns which have been under the effect of antiepileptic drugs. The result is consistent to the results obtained by (Padham *et al.*, 2010).According to the obtained results from Morphometric parameters of fetus growth it seems that melatonin has a protective effect as an antioxidant against free radicals due to the administration of lamotrigine, such that there was a meaningful difference with medical control group. The obtained results are consistent with the results presented by (Nulman *et al.*, 1999). Anti apoptotic characteristics of melatonin inhibit spermatogenesis degeneration via controlling the germ cell apoptosis (Pizarro *et al.*, 2008). Melatonin inhibits spermatogenesis degeneration via controlling the germ cell apoptosis (Ateşşahin *et al.*, 2006). In the present study, anti apoptotic property of melatonin caused the absence of spermatogonia degeneration process in the melatonin + lamotrigine group compared with the lamotrigine control group. Based on the obtained results form morphometric parameters of fetal growth, lamotrigine caused a meaningful decrease in the newborns' weight compared with lamotrigine + melatonin and control groups, whereas it increased weight of newborns treated with lamotrigine. Lamotrigine caused a meaningful decrease in fetal testis weight compared with lamotrigine + melatonin and control groups such that the difference between the groups was meaningful. It seems that melatonin increases the weight of fetal treated with lamotrigine. Lamotrigine caused a meaningful decrease in fetal length compared with lamotrigine + melatonin and control groups, but there was no difference between lamotrigine + melatonin and control groups. It seems that melatonin increases the length of newborns treated with lamotrigine.Based on Morphometric parameters of testicular tissue Lamotrigine caused a meaningful decrease in the seminiferous tubules difference of lamotrigine control, and lamotrigine treatment groups compared with the control group, also there was a difference between lamotrigine control and lamotrigine treatment groups. It seems that melatonin increases the seminiferous tubules diameter meaningfully but the difference was not so much that was not consistent to control group. Lamotrigine caused a meaningful decrease in the spermatogonia cell number difference of lamotrigine control, and lamotrigine treatment groups compared with the control group. The effect of melatonin is, to the extent that created an increased difference compared with lamotrigine control group, but the difference was not so much that consistent to control group. Although a large number of spermatogonia cells had been destroyed, there were some spermatogonia in to the tubules and on the base membrane. May be the remaining spermatogonia suggest that the cells can create germ cells in seminiferous tubule by self-division. In the present study some vacuoles are observed in the thickness of germinal epithelium that may be created by destroyed germ cells. In fact, these vacuoles can represent the loss of cell adhesion or decreased adhesive molecules such as cadherins, as well as one of the signs of apoptosis that (Newton *et al.*, 1993) has been reported such status in his study (Newton *et al.*, 1993). Administration of melatonin in laboratory large mice protects cells against oxidative damage and in fact decreases oxidative stresses via different ways (Mark *et al.*, 2004). Melatonin has anti apoptotic property via inhibition of germ cell apoptosis in testicular tissue (Bastaki *et al.*, 2001).

Conclusion

Lamotrigine caused no meaningful difference in asexual sertoli cells of control, lamotrigine control, and lamotrigine groups. It seems that lamotrigine causes no meaningful decrease in sertoli cells. Lamotrigine caused a meaningful decreased in leydig cell number in lamotrigine control, and lamotrigine treatment groups compared with the control group. But, the parameter was not meaningful between control and

treatment groups. It seems that melatonin has a protective effect on the process of decreased leydig cells number.

REFERENCES

Ateşşahin A, Sahna E, Türk G, Ceribaşi AO, Yilmaz S and Yüce A (2006). Chemoprotective effect of melatonin against cisplatin-induced testicular toxicity in rats. *Journal of Pineal Research* **41**(1) 21-7.

Bastaki SAM, Padmanabhan R, Abdulrazzaq YM, Chandranth SI and Shafiull M (2001). Studies on the teratogeniceffect of lamotrigine in mouse fetuse. *Frontier Infetal Health* **3**(11-12) 295.

Berkowitz B and Katzung B (2004). *Basic and Clinical Pharmacology* (New York; McGraw-Hill) 123.

Curry WJ and Kulllling DL (1998). Newer Anticonvulsant drugs; gabapentin, lamotrigine, febamate, tiagbin, fosphentyoin. *American Family Physician* **57** 513-520.

Guneli E, Tugyan K, Ozturk H, Gumustekin M, Cilaker S and Uysal N (2008). Effect of melatonin on testicular damage in streptozotocin-induced diabetes rats. *European Surgery Research* **40**(4) 354-60.

Kusumakar V and Yathman Ln (1997). Lamotrigine Treatment ofrapid cycling bipolar disorder. *American Journal of Psychiatery* **154** 1171-2.

Lewis-Johnes DI and Kerrigan DD (1985). A modified johnsen's count for evaluation of spermatogenesis in the rat. *IRCS Medical Science* **13** 510-11.

Mark S, Yerby MS, Kaplan P and Tran T (2004). Risks and management of pregnancy in women with epilepsy. *Cleveland Clinic Journal of Medicine* **71**(2) 25-37.

Mohanty C, Shah N, Dhungel SH and Das BK (2011). Effect of lamotrigine on fetal rat brain. *People Journal of Scientific Research* **4**(2) 5-7.

Nau H (1981). Pharmacokinetices of valproic acid and its metabolites in pregnant patient. In: *Epilepsy, Pregnancy and the Child*, edited by Janz D (Raven press) New York 131-44.

Newton SC, Blaschuk OW and Millette CF (1993). N-cadherin mediates Sertoli cell-spermatogenic cell adhesion. *Developmental Dynamics* **197**(1) 1-13.

Nulman I, Laslo D and Koren G (1999). Treatment of epilepsy in pregnancy. *Drugs* **57** 535-544.

Padmanabhan R, Abdulrazzaq YM, Bastaki SM, Nurulain M and Shafiullah M (2010). Vigabatrin administered during late gestation lowers maternal folate concentration and causes pregnancy loss, fetal growth restriction and skeletal hypoplasia in the mouse. *Reproductive Toxicology* **29**(3) 366-77.

Padmanabhan R, Abdulrazzaq YM, Bastaki SM, Shafiulla M and Chandra SI (2003). Experimental studies on reproductive toxicologic effects of lamotrigine in mice. Birth Defects Research Part B. *Developmental and Reproductive Toxicology* **68**(5) 428-38.

Pizarro JG, Yeste-Velasco M, Esparza JL, Verdaguer E, Pallàs M and Camins A (2008). The antiproliferative activity of melatonin in B65 rat dopaminergic neuroblastoma cells is related to the downregulation of cell cycle-related genes. *Journal of Pineal Research* **45**(1) 8-16.

Richens A (1994). Safety of Lamotrigine. *Epilepsia* **35**(5) 37-40.

Reiter RJ, Tan DX, Manchester LC and Qi W(2001). Biochemicalreactivity of melatonin with reactive oxygen and nitrogen species: a review of the evidence. *Cell Biochemistry and Biophysics* **34** 237-56.

Rahmani F, Delaram M and Forouzandeh N (2006). The teratogenic effects of Lamotrigine on mouse fetus. *Journal of Reproduction and Infertility* **7**(1) 45-52.

Redins CA, Redins GM and Novaes JC (2002). The effects of treatment with melatonin on the ultrastructure of mouse Leydig cells: a quantitative study. *Brazilian Journal of Biology* **62**(3) 517-23.

Schrader M, Müller M, Straub B and Miller K (2001). The impact of chemotherapy on male fertility: a survey of the biologic basis and clinical aspects. *Reproductive Toxicology* **15**(6) 611-7.

Sanchez-Hidalgo M, de la Lastra CA, Carrascosa-Salmoral MP, Naranjo MC, Gomez-Corvera A and Caballero B (2009). Age-related changes in melatonin synthesis in rat extrapineal tissues. *Experimental Gerontology* **44**(5) 328-34.

Sönmez M, Yüce A and Türk G (2007). The protective effects of melatonin and Vitamin E on antioxidant enzyme activities and epididymal sperm characteristics of homocysteine treated male rats. *Reproductive Toxicology* **23**(2) 226-31.

Young LT, Robb JC and Patelis-Siotis I (1997). Acutetreatment of bipolar depression with gabapentine. *Biology of Psychiatery* **42** 851-53.