Available online at www.scholarsresearchlibrary.com

Scholars Research Library

Central European Journal of Experimental Biology, 2014, 3 (1):13-24
(http://scholarsresearchlibrary.com/archive.html)

ISSN: 2278–7364

Teratogenic potential, developmental neurotoxicity and neurobehavioural impairment in ratsprenatally exposed to anticonvulsant Gabapentin

Krishna Pal Singh and Kiran Gupta

Neurobiology Lab, Department of Zoology, University of Allahabad, Allahabad, INDIA

ABSTRACT

For therapeutic management of epileptic seizures in adults,second-generation antiepileptic drugs (AEDs) are better option than classical AEDs considering their safety concerns. Among these, Gabapentin (GBP) is most prescribed drug even during pregnancy. Reports on potential teratogenicity, developmental neurotoxicity and neurobehavioral alterations induced by in utero exposure to GBPare limited and inconclusive. Therefore, present study has been undertaken to evaluate the teratogenic safety, fetal neuro-toxicity; and its long-lasting functional impairments on young-adult rat offspring, if equivalent therapeutic doses of GBP were administered to pregnant rats. The pregnant C.F. rats were exposed to equivalent therapeutic doses of GBP at 300 and 400mg/kg BW/day from gestation day 0-20. Half of the pregnant dams of both drug treated and control groups were sacrificed on GD 21, and fetuses were examined for gross congenital anomalies; then fetal brains were processed for histopathological observation, while remaining pregnant dams were allowed to deliver naturally and their pups were reared with their biological motherstill weaning,and independently up to PND 56 for neurobehavioural observations under selected mazes of anxiety and depression. There was dose-dependent significant reduction in body and brain weight of prenatally GBP treated rat fetuses. Histopathalogical observations showed substantial alterations in different neocortical layers of fetal brain.In utero GBP exposed rat offspring showed significantly increased state of anxiety like behavioural responses in open-field arena and on elevated plus-maze at PND 56. In behavioral despair maze,these rat offspring showed significant enhanced number of immobility phases (a depression sign). This study concludes that prenatal exposure toGBP during critical period of brain developmentmay induce fetal toxicity, neuroarchitectural changes and long-lasting impact on neurobehavioral alterations in young-adult offspring.

Keywords: Gabapentin, Developmental Neurotoxicity, Neurobehavioural impairments, Prenatal, Rat

---

INTRODUCTION

The clinical management of pregnant womenwith epilepsy is a challenging task tothehealth care providers, considering beneficial effects of antiepileptic drugs (AEDs) to mother and possible teratogenic risks to developing fetus [1].For prophylactic treatment of epileptic seizures, both classical (typical) and new generation (atypical) AEDs are available in the world market. The potential teratogenicity of typical AEDs has been well establishedin clinical and preclinical studies [2-3]. In utero administration to typical AEDs is the most common cause of embryo-fetal abnormalities including growth retardation and developmental delay as well as functional impairments in offspring [3-8].

Newer AEDs (Felbamate, Lamotrigine, Vigabatrin, Oxcarbazepine, Levetiracetam, Tiagabine, Topiramate, Zonisamide and Gabapentin) are known for less side effectsdue to their improved pharmacokinetic and Pharmacodynamic profiles, hence have raised the hope for a better treatment option even during pregnancy [9-11]. Among the atypical AEDs, Gabapentin (GBP) is relatively a new agent, and it was first developed to treat epilepsy and recently discovered to have some beneficial effects for neuropathic pain [12, 13]. The drug is remarkably non-toxic [16].

Animal studies indicate that some newer AEDs also have embryo-toxic potential and some degree of developmental neurotoxicity [15-17]. The current literature survey revealed that a few reports are available on reproductive toxicity and teratogenicity of newer AEDs but these observations are mostly limited to external / internal gross anomalies. Among newer AEDs like GBP has not been shown to be teratogenic in animals [18, 19], whereas Prakashet al [20] demonstrated that GBP is teratogenic in mice.There are insufficient data to make a definite conclusion about the teratogenic safety of NAEDs in general and GBP in particular [21,22].

There are few reports available on prenatal exposure to GBPon developmental neurotoxicity (restructure of neuroanatomical organization) in fetal brain [20]and their long-lasting behavioural alterations in rodent offspring [23, 24]**.**Some reports are available in clinical trials but these are preliminary and inconclusive [22, 25].

Therefore, keeping above views in consideration present study has been undertaken to elucidate the effect of in utero exposure to Gabapentin during critical period of brain development, onteratogenic potential, developmental neurotoxicity(neurohistopathological and cytoarchitectural changes in neocortex of fetal brain)and its long-lasting impact on neurobehavioural impairments (anxiety like changes)in young/adult ratoffspring.

MATERIAL AND METHODS

Animals

Inbred Charles-Foster rats (150±10g) were acclimatized before experimentation. These rats were maintained under standard laboratory conditions (24±2ºC room temperature, 60±10 relative humidity and 12h light (06.00 to 18.00h)/12h dark cycle (18.00 to 06.00h).All animals were housed in transparent polypropylene cages (39×24×15cm) and dry rice bran was used as bedding material. Beddings were changed twice a week to avoid any unhygienic condition. The food pallets and fresh tap water were provided *ad libitum* throughout the experiment. The adult male (10-12 weeks old) and nulliparous female rats (9-10 weeks old) were caged together (2:1 ratio) overnight for mating. On next day (at 08.00h), mating was checked in female rats and inferred by the presence of sperms in the vaginal swab. Sperm positive dams were housed individually in the same sized cages and at similar laboratory conditions. The sperms positive dams were designated as gestation day zero (GD 0). All the experiments were executed as per standard guidelines for care and use of laboratory animals and their required numbers were approved by the Institutional Animal Ethics Committee (IAEC).

Experimental design, drug exposure andrationale for dose selection

All positive female rats (n=16) were randomly separated into three groups: Group A (n=8, vehicle exposed/ control), Group B (n=4, 300mg/kgGBP exposed) and Group C (n=4, 400mg/kg GBP exposed). Freshly prepared selected doses of the drug (GBP) were given to experimental pregnant rats through gavage with the help of cannula at 09.00 hrs from GD0-20. The control dams were treated with equal dose of vehicle (water) through same route and time.The rationale for selection of two doses of GBP was in accordance with maximum humanrecommended doses (MHRD, i.e. 3600mg/kg/day); and considering the higher metabolic rate of rats, 4-6 times faster than humans [26]. Therefore, 300mg/kg (5x MHRD), 400 mg/kg (7x MHRD) doses of GBP were selected on the basis of mg/kg body weight per day to mimic with therapeutic dose range [27].

Fetal body and brain weight, and Histopathological procedure

Half of the pregnant dams of all the groups were sacrificed after anesthetization with pentobarbitone on GD 21 at 0900 h, and their near term fetuses were collected by uterectomy and weighed. Fetal brains were dissected out by carefully peeling the cartilage then washed and weighed. Prior to weighing, brains were rolled over a piece of dry absorbent paper to remove excess water. To record the neurohistopathological observations, brains were then fixed by immersion in 10 % neutral formalin, further processed for paraffin microtomy; and 7 µm thin serial sections were cut, processed as per standard protocol and stained with H & E stain. These stained sections were identified with the

help of stereotaxic atlas of developing rat brain [28].For photomicrography of histological slides Eclipse CCD camera of Nikon 831 was used.

Measurements of Cortical Thickness

The cortical thickness was measured in the medial frontal cortex of the fetal brain sections with the help of Image J software (1.46r). Firstly, scalewas set in the software according to bar of the photographs, then line tool was selected for measurement oftotal cortical thickness, from outer surface of the cortex (molecular layer) to inner surface of the neuroepithelium. Likewise, measurements of different layers (I to V) werealso taken separately. In each experiment, sixsections of medial frontal cortex of fetal brains of each group were selected for cortical thickness measurements.

Postnatal rearing for neurobehavioural tests

Further, remaining 50% vehicle and drug exposed rats were allowed to deliver naturally. At birth i.e. postnatal day 1(PND 1),all the litters of each groups were weighed individually, examined for external anomalies, if any. Further,newborn pups were culled (n=6 per group) and reared with their biological mothers up to postnatal day 21(PND 21). After weaning period, rat offspring were segregated into different cages (n=4 per cage) for social interaction up to PND 70.The offspring used in this study were weighed at birth, then once a week till 8 weeks of age.Theseyoung-adult rats were subjected to selected neurobehavioural testslike open- field arena and elevated-plus maze, to test anxiety-like behavioral responses in a new environment and forced swimming test for depressive sign. All behavioral tests were performed between 09.00 to 11.00 hrs.

Behavioural tests and procedures

Open-field exploratory test

An open-field apparatus made of plywood measuring 60.96×60.96×60.96 cm was used to record the locomotor activity, and to test the open-field exploratory behavior of rats [29,30]. The floor of the apparatus was divided into 16 evenly spaced squares surrounded by opaque high walls of 60.96 cm. The entire apparatus was painted black except the 6 mm wide white lines that divided the floor into 16 squares. The open-field apparatus was illuminated by a 100W bulb focusing onto the field from a height of about 100 cm from the floor. The entire room except the open-field was kept dark during the experiment. In the novel test situation, each animal was centrally placed in the test apparatus for a maximum test period of 5 min. to observe the following behavior: (1) Ambulation- the number of squares crossed by the rat; (2) Rearing- the number of times rat stood on its hind limbs (supported and unsupported); (3) Self-grooming –the number of responses of grooming, scratching, licking and washing made by individual rat; (4) Faecal boli –the number of faecal boli exuded by individual rat. Before each trial, floor and walls were cleaned with cotton soaked in 70% alcohol.

Elevated plus-maze test

This model was used to test the anxiety pattern in rodents [31,32]. The detailed description of the design may be obtained in our earlier reports [11, 35]. In brief, the plus-maze consists of two opposite arms 50×10 cm, connected with a central square (10×10 cm), giving the apparatus a shape of plus (+) sign. One arm, painted white, was kept open, whereas the other arm was enclosed with a 40 cm high wall and painted black together with the surrounding walls. Thus, each maze consists of two open and two enclosed arms with a central square. The maze was kept in a dimly lit room and was elevated 50 cm above the floor. The experimental animals were placed individually in the center of the maze facing towards the enclosed arm. The number of entries and the time spent on open and enclosed arms were recorded during the next 5 min for each rat. An arm entry was recorded when all four paws of the rat entered into the arm. The floor and walls of the open and enclosed arms were cleaned with 70% alcohol before each trial.

Behavioral despair test

To evaluate the depression status in rodents, this maze was used [34, 35].The individual rat was placed in a circular glass chamber, 45 cm. in diameter containing 25 cm depth of water, so that rat could not touch the bottom of the cylinder with its hind limbs or climb over the edge of the chamber. Two swim sessions were conducted, an initial 15 min pretest, followed by a 5 min test, 24hrs later. The period of immobility (remain floating in water without struggling) andfrequency during 5 min test period was noted and evaluated.

The question of reliability, validity and sensitivity of mazes are of prime importance in establishing the paradigms used in this study. These mazes have been subjected to thorough critical appraisal and validated as animal models of

anxiety and depression [29, 35]. The paradigms established in our laboratory, their validity and reliability have earlier been tested and documented [9, 33].

Statistical Analysis

All data were represented as mean and standard error (Mean ±S.E). The variables were analyzed using one way analysis of variance (ANOVA) followed by post hoc Tukey's multiple comparison test to determine differences amongst groups for neocortical thickness and behavioral tests. For all statistical values, alpha level was $p<0.05$. All calculations were done with the help of Microsoft Excel and Statistica-10 software.

RESULTS

Effect of prenatal exposure toGBP on fetal body and brain weight

One way ANOVA followed by Tukey'spost hoc test showed highly significant dose- dependent reductionin fetal body weight ($F_{(2,44)}=162.8$, $p<0.001$) and brain weight ($F_{(2,27)}= 958.06$, $p<0.001$) respectivelyin prenatally GBP (300 and 400 mg/kg) exposed groupsas compared to vehicle treated group. The percentage of reduction in fetal body weight (20.43% and 28.43%)and brain weight (45.00 % and 51.66 %) were also founddose-dependent at 300 mg and 400 mgdoses of the drug respectively. The effect of the drug was more intense (almost double) in fetal brain weight than body weight (Fig-1, Fig-2).

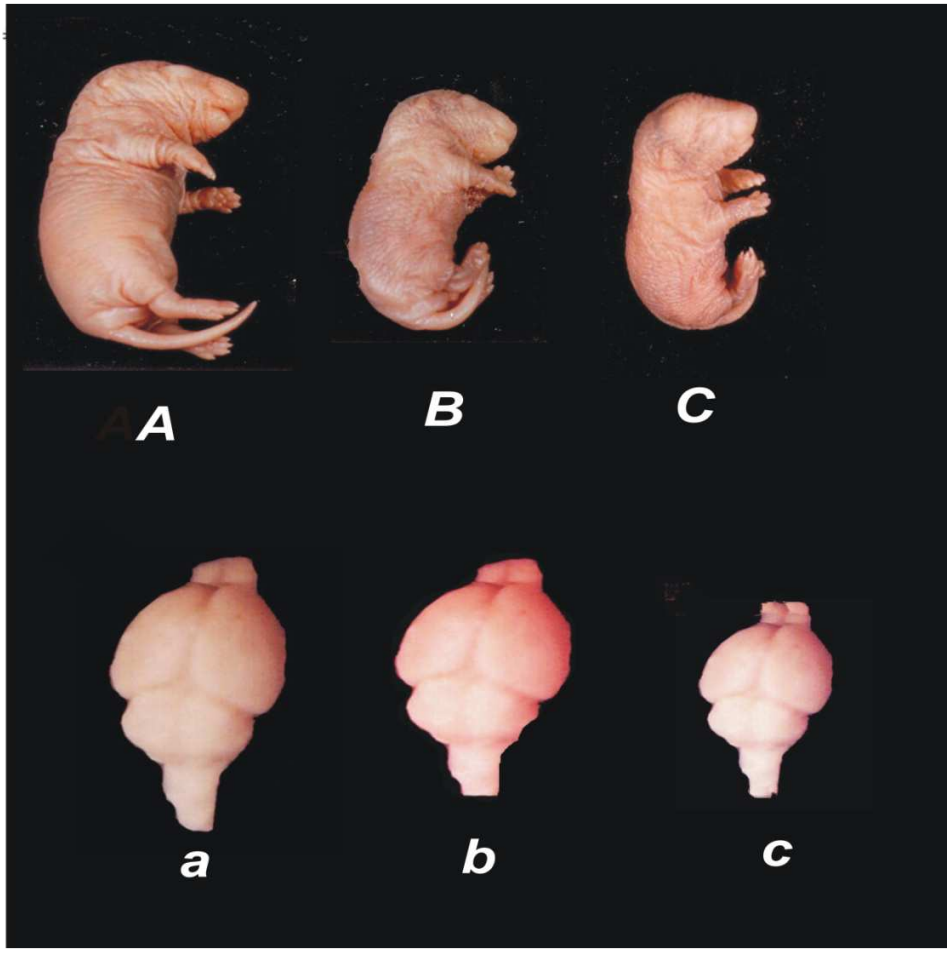

Fig-1 showing effect of prenatal exposure to GBP on fetal body size. (A) Control, (B) 300mg GBP and (C) 400mg GBP; as well as on fetal brain size (a) Control (b) 300mg GBP and (c) 400mg GBP.

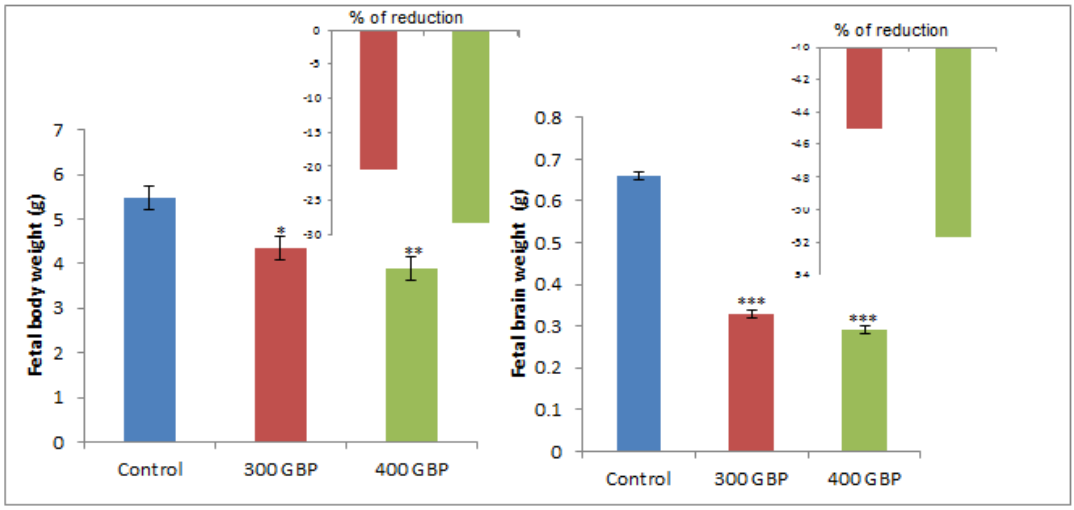

Fig. 2 Showing effect of prenatal exposure to GBP on fetal body and brain weight.

*All data represent Mean ± SEM value. *, **,*** indicate level of significance at p<0.05, p<0.01 and p<0.001 respectively between control and GBP exposed groups for one way ANOVA followed by post hoc Tukey's multiple comparison test. The percentage of reduction in body and brain weight in comparison to control was represented as inverted histograms.*

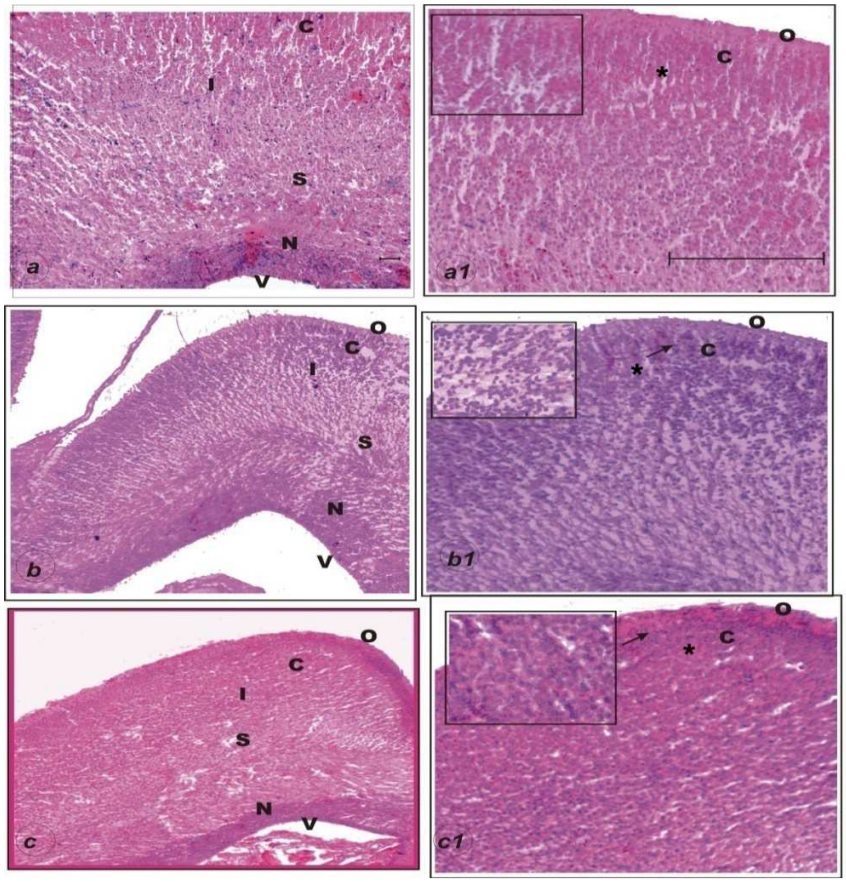

Fig-3 Showing effect of prenatal exposure to GBP on neocortex of fetal brain.

(a) Control (b) 300mg GBP exposed and (c) 400mg GBP exposed at 8X (Scale bar at 50µm); (a1), (b1) and (c1) represent cortical plate at 32X magnification.* indicates area of marked cellular degeneration and reduced neuronal deficit in GBP exposed groups (Scale bar at 100 µm). Super imposed area of selected region of cortical plate depicts neuronal deficit at 72X.

Abbreviation: V= Lateral ventricle, N=Neuroepithelium, S= Subventricular plate, I= Intermediate zone, C=Cortical plate, O=Molecular layer.

Effect of *in utero* exposure toGBP on fetal gross anomalies

In this study, no major gross external anomalies were found except kinking of tail and hemorrhages on tail and neck region in GBPtreated rat fetuses at 400 mg/kg.

Effect of prenatal exposure to GBP on neuroarchitectural pattern of frontal cortex

One way ANOVA displayed substantially altered ($F_{(2,19)}$= 7089, p< 0.001) total neocortical thickness. Post hocTukey's multiple comparison test displayed significant (p< 0.001) decrease in GBP exposed groups in comparison to vehicle treated (control) group. On examination of neuroarchitectural pattern of different neuronal layers of neocortex in GBP administered fetal brains,thickness of molecular and cortical layers (layer I & II), intermediate layers and subventricular zone (III & IV)were foundto be substantially (p<0.05) decreased, while neuroepitheliam layer (layer V) in exposed groups was found to be significantly (p<0.05) increased than control brain sections.Histological observations of neocortex area in GBP exposed brain sections showed loose arrangement of neuronal cells, and neuronal deficit was also found. (Fig-3, Fig-4)

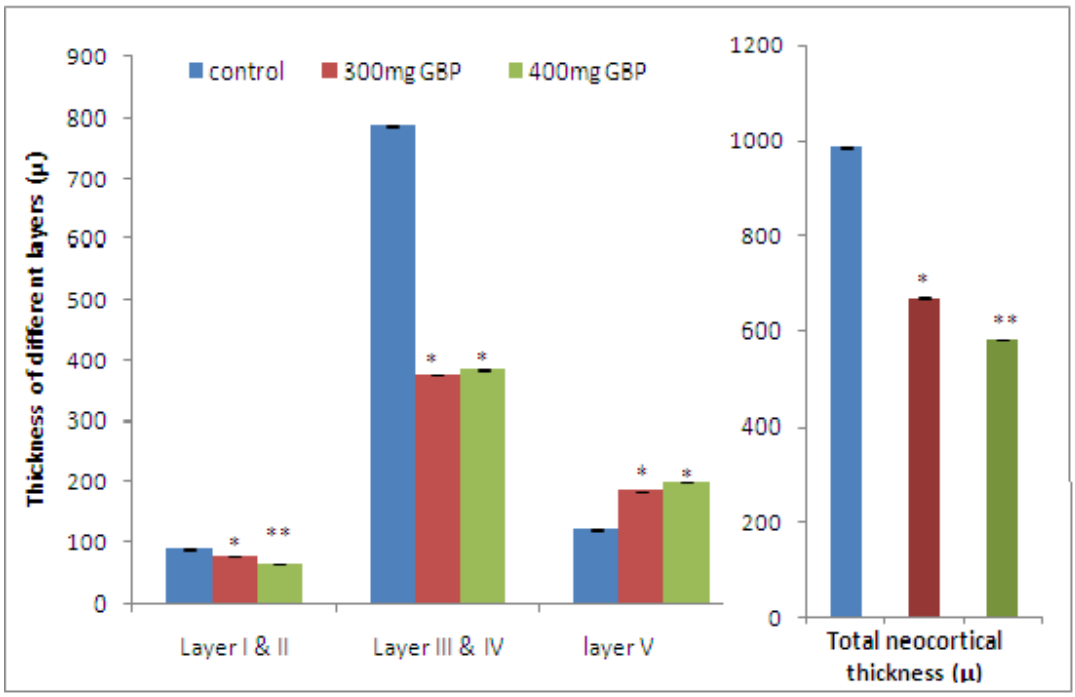

Fig.4Showing effect of prenatal exposure to GBP on thickness of neocortical layers and total neocortical layer.

*All data represent Mean ± S.E. value. *, **,*** indicate level of significance at p<0.05, p<0.01 and p<0.001 respectively between control and GBP exposed groups for one way ANOVA followed by post hoc Tukey's multiple comparison test.*

Effect of prenatal exposure to GBP on choroid plexus in the lateral ventricles of fetal brain

The intense folding of plexus with complex convoluted structure including tubules, as depicted in control, was changed severely into thread like structure due to excessive shrinkage of choroid plexus at 300 mg/ kg, whereas there wassubstantial reduction of plexus volume, loss of lobular and tubular structure, condensation of plexus at one site of lateral ventricle and fragmentation at several places at 400 mg/kg dose. Thus, there was a loss of volume and typical architectural pattern of multiple convolutions of lobular plexus with tubules at both the doses of GBP(Fig-5).

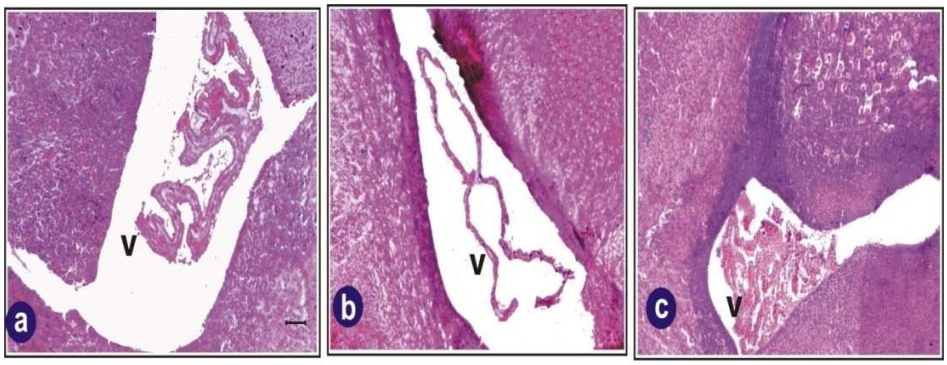

Fig-5 Showing effect of prenatal exposure to GBP on architecture of choroid plexus of lateral ventricle of fetal brain at 8X magnification.(a) Control, (b) 300mg GBP with reduced convoluted pattern and thread like structure, and (c) 400 mg GBP with condensed and fragmented structure (Scale bar at 50µm).

Effect of prenatal exposure to GBP on exploratory behavior in open-field arena

One way ANOVA followed by Tukey's multiple comparison test demonstratedsignificantly decreased ambulation ($F_{(2, 47)}$ = 400.82, p<0.001), rearing ($F_{(2, 47)}$= 88.31, p<0.001) and self-grooming ($F_{(2, 47)}$ =112.56, p<0.001) scores in open-field arena by in utero GBP exposed rat offspring in comparison to control rats while defecation scores were increased substantially ($F_{(2,47)}$ = 18.02, p<0.05) in GBP exposed offspring than control (Fig-6).

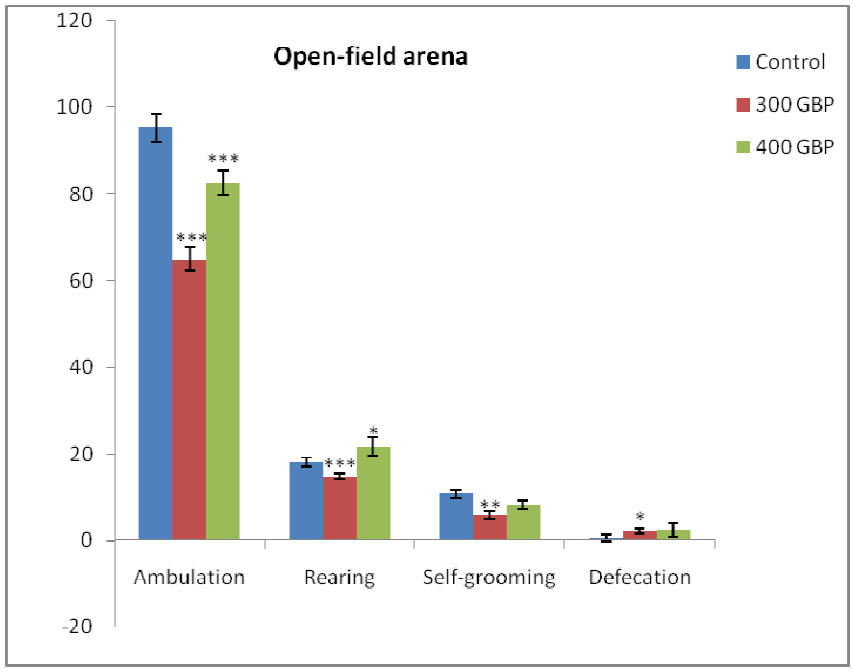

Fig.6 Showing effect of prenatal exposure to GBP on exploratory behaviour in open-field arena.

*All data represent Mean ±S.E. value. *, **,*** indicate level of significance at p<0.05, p<0.01 and p<0.001 respectively between control and GBP exposed groups for one way ANOVA followed by post hoc Tukey's multiple comparison test.*

Effect of prenatal exposure to GBP on exploratory behavior on elevated plus-maze

One way ANOVA revealed that prenatally drug exposed rat offspring spent significantly less time [$F_{(2,47)}$= 126.80, p<0.001] on open arms, whereas these offspring spent substantially more time [$F_{(2,47)}$= 20.22, p<0.001] on enclosed arms. Post hoc Tukey's multiple comparison test showed that young-adult offspring of both 300 and 400 mg/kg drug

treated groups spent less time ($p<0.001$) on open-arms than offspring of control group. In contrast to this, these offspring spent more time ($p<0.001$) on enclosed arms in comparison to vehicle treated rats (Fig-7).

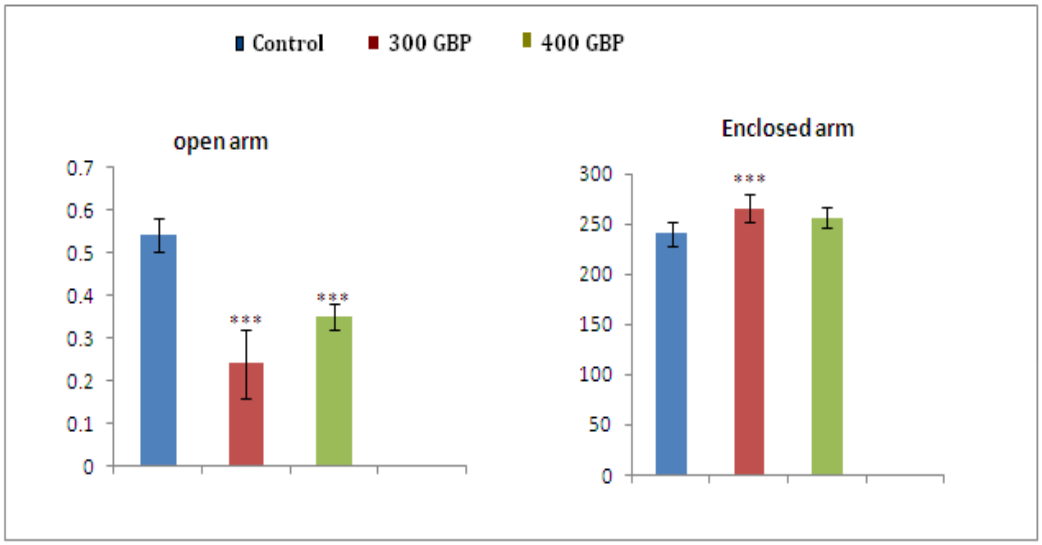

Fig.7 Showing effect of prenatal exposure to GBP on exploratory behaviour on elevated plus-maze.
*All data represent Mean ± S.E.value. *, **,*** indicate level of significance at p<0.05, p<0.01 and p<0.001 respectively between control and GBP exposed groups for one way ANOVA followed by post hoc Tukey's multiple comparison test.*

Effect of prenatal exposure to GBP on forced swimming behaviour in despair-maze

One way ANOVA followed by Tukey's multiple comparison test demonstrated that prenatally GBP exposed rat offspring displayed significant enhanced number of immobility phases ($F_{(2,47)}=476.75$, $p<0.001$), and spent more time in un-struggled (immobile) phases ($F_{(2,47)}=22.39$, $p<0.05$).Thus, GBP exposed offspring expressed more depressive signs in despair maze test (Fig-8).

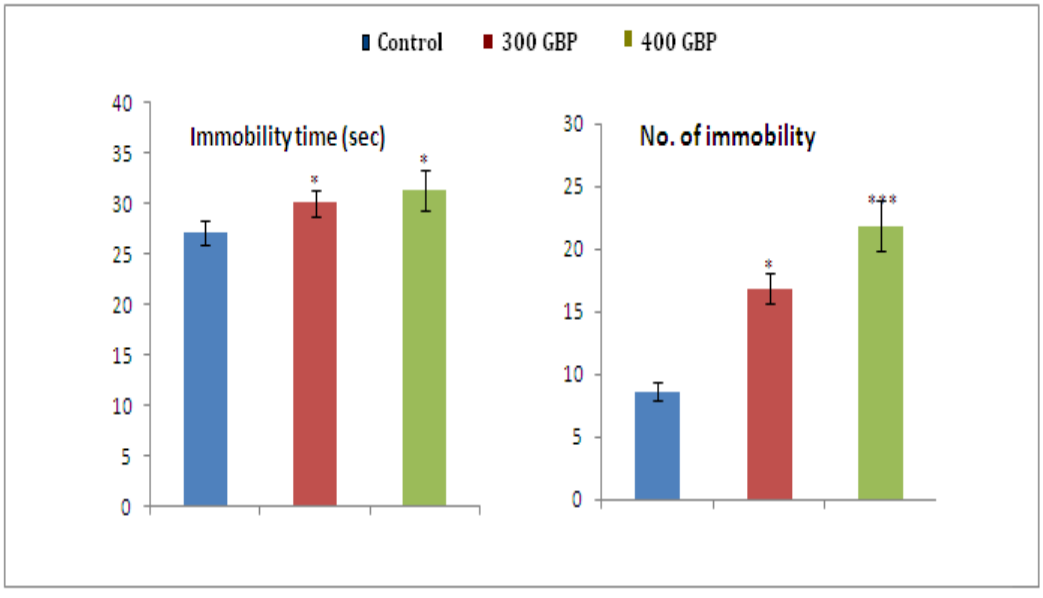

Fig.8 Showing effect of prenatal exposure to GBP on forced swimming behaviour in despair-maze.
*All data represent Mean ± S.E. value. *, **,*** indicate level of significance at p<0.05, p<0.01 and p<0.001 respectively between control and GBP exposed groups for one way ANOVA followed by post hoc Tukey's multiple comparison test.*

DISCUSSION

The present study revealed that prenatal administration to equivalent therapeutic doses of GBP induced fetal toxicity as substantial reduction of fetal body and brain weight, impaired neuronal organization of frontal cortex of fetal brains as decreased thickness of cortical layers, cellular structure and choroid plexus as well as neurobehavioural changes in young-adult rat offspring as increased state of anxiety like responses. In this study, hemorrhages were found on body and tail at 400 mg GBP exposed fetuses as external fetal malformations.

The percentage of fetal body weight reduction was more severe at 400 mg (28.43%) than 300 mg (20.43%) GBP exposed group. Our results corroborate well with those investigators who have reported fetal toxicity as significant fetal growth retardation when GBP was administered to pregnant dams at equivalent therapeutic doses (MHRD) during selected gestation period [20,36,37]. In the recent past, Prakesh et al [20] elucidated the feto-toxicity as reduced body weight (growth retardation) and stunting in size of live fetuses when they were maternally exposed to different doses of GBP (113, 226 and 452 mg/kg) at different gestation period (early, mid and late). The product monograph of the Neurontin package, Park Davis [37] 1998 also revealed that GBP was found to be fetotoxic in rodents when pregnant dams were received oral dose, about 1-5 times the maximum human doses (3600mg/day) during organogenesis on the mg/m^2 basis. In another study, GBP was found fetotoxic at higher doses (1800-4800mg/day) in rodents [36]. Still, experimental studies are limited to draw a definite conclusion on fetotoxicity of atypical AEDs in general and GBP in particular. Clinical literature on maternal exposure to GBP and its risks on lower fetal birth weight and/or intra uterine growth retardation (IUGR) islimited [38].GBP pregnancy registry of Boston, USA elucidated that GBP exposure during pregnancy did not lead to an increased risk for adverse fetal events [3,39]. In our study, although GBP was administered to pregnant rats throughout the gestation period but could not produce major anomalies; and were limited to hemorrhages on certain body parts and kinking of tail in a few fetuses. Our results on teratogenic potential of GBP were in agreement with those workers who have reported no evidence of teratogenicity of GBP at doses up to 1500mg/kg from GD 6-15 in rats [3, 18]. In contrast to this, Prakash et al [20] reported the gross malformations including brachygnathia, pointed snouts, open eyes, cataracts, rudimentary limbs and malrotated limbs at higher dose, 452 mg/kg GBP.

A few clinical studies also indicate that GBP exposure during pregnancy (I^{st}, II^{nd} and/or III^{rd} trimester) did not lead to an increased risk for adverse fetal events/congenital abnormalities [39, 40].

Several investigators have postulated different mechanisms of action for AEDs induced fetal/ neonatal weight loss [1, 41]. Among these workers Goldenberg et al [41] reported in a clinical study that fetal birth weight reduction may be due to more preterm deliveries, or to a reduced growth potential, or to intrauterine growth restriction, or to multiple births, or to infant gender, or to specific drug induced effect. In the present study, no substantial difference was observed in offspring of control and exposed groups for preterm deliveries, infant gender and growth potential as well as litter size [8]. Hence, reduced fetal weight (IUGR) could be associated to direct drug induced effect. The IUGR may be associated to maternal aphagia followed by severe under nutrition [42, 43]. In the present study, since no significant difference of food consumption was found between control and GBP exposed dams, hence, this could not be an inducing factor [8]. Thus, GBP may induce somatic development and growth, directly or indirectly in rat feuses. The mechanism underlying the association between low fetal weightand AAEDs including GBP, during pregnancy is still unclear. However, involvement of other possible inducing mechanisms may not be ruled out; and are needed to be explored.

Prenatal antiepileptic drug exposure during critical period of brain development induces neurochemical and structural alterations in various fetal brain areas. The basic scaffolding of neurons, glia and nerve connections are assembled early, begins prenatally in humans. In rodents and non-human primates, brain growth spurts starts prenatally (mid gestation) and ends postnatally (PND 30). During this period, cell proliferation, migration, differentiation, synaptogenesis, gliogenesis and apoptosis begins from GD7 and lasts at different postnatal days accordingly [44]. Hence, prenatal exposure to any xenobiotic agent including GBP during critical period of CNS development may induce default programming of neurogenesis, followed by gross structural anomalies in the fetal brain including gross stunting of brain (brain weight deficit), and related neuroarchitectural (reduced neuronal thickness), neurohistopathological changes in different areas of the brain (like frontal cortex) as depicted in the present study.

Since several neurotransmittersare thought to have trophic effects during morphogenesis prior to their function as transmission or nerve conduction. There is increasing evidence that a number of neurotransmitters, including Dopamine and Serotonin, and possibly GABA can play a trophic role in regulating brain growth and development. A number of studies indicate that early exposure to compounds that block dopaminergic and gabargic transmission may block cellular proliferation or growth [22, 45]. Recent studies on CNS development showed that these neurotransmitters may serve as molecules that regulate specific aspects of cell proliferation, survival, migration, circuit formation and establishment of topography [17, 45]. It has been presumed that early disturbances in neurotransmitters level may be coupled with changes in cellular energy metabolism which ultimately leads to functional disturbances in neurotransmitters [46]. Therefore, neurotransmitters might be one reason to alter the process of neurogenesis in fetal brain.

Experimental evidences also indicate that in immature rodents, AEDs can cause profound apoptotic neuronal death in many areas of the developing brain. Interestingly, during the developmental period which corresponds to the last trimester of pregnancy in humans, robust cell death can be induced by AEDs in the hypothalamus and the basal ganglia [47]. Thus, in the present study it may be speculated that AEDs may also induce apoptotic neurodegeneration within the cortical region. This laboratory has also reported recently ROS induced increased apoptotic neurodegeneration, enhanced level of proapoptotic protein, Bax and decreased level of antiapoptotic protein, Bcl-2 in neocortex of prenatally Venlafaxine (a novel antidepressant drug) exposed fetal brain as compared to control brains [48].

Another possible inducing mechanism could be associated with low molecular weight of the drug (<600g/mol) which may cross placental and blood-brain barrier (BBB) easily, and may influence neurotransmitter receptors, ion channels, choroid plexus in brain ventricles, including Cerebro- spinal fluid. These drugs have the potential to alter neuronal development through both acute and chronic effects on cellular behavior and gene expression. The molecular weight of GBP is 383.51g/mol therefore, it crosses easily the placental and BBB through diffusion, and interfere the neuronal development.

In utero GBP exposed rat offspring displayed significantly altered exploratory and locomotory responses (anxiogenic) in the open –field arena and on elevated- plus maze. In such mazes developed for specific paradigms, abnormally increased or decreased locomotion, rearing, defecation and urination etc. are considered to be indices of increased emotionality and fearfulness (anxiety), as they represent more primitive responses [49]. In this study, GBP exposed rat offspring when subjected to behavioral despair test, they exhibited increased number of immobility phase and spent more time as immobile period; thus indicate depressive sign in *in utero* exposed rats. The present findings suggest that GBP treated young rat offspring when subjected to stressful or new environments, they found difficult to cope with the new environment resulting in slower habituation.

The second and third weeks (day 7-21) of pregnancy in rats appears to be most vulnerable to the action of CNS drugs, because this is the critical period for synaptogenesis, formation of specific neural circuits, rapid cell proliferation and functional maturation of dopaminergic and other neurotransmitter systems. Neurobehavioural dysfunctions due to in utero exposure to psychotropic drugs indicate that last week of gestation and / or lactation is the most sensitive period for inducing long-lasting effects in mammals [50, 51]. Reports on brain biochemistry revealed that early neurochemical alterations in the CNS may lead to functional deficits, resulting in abnormal behavioral pattern in F_1 progeny. It is generally accepted that central dopamine facilitates hyperactivity, locomotion and aggression whereas acetycholine (ACh) is responsible for cognitive performances. Locomotor hyperactivity has been reported in rats by depleting brain Dopamine level [52]. The role of other neurotransmitters during early stage of neuronal maturation, synthesis and release in pre or post synaptic receptors may also be considered. The present results are in agreement with those workers who have reported in utero exposure of AEDs and its long-lasting effects on behavioural alterations in rodent offspring [53]. The exact cause of neurobehavioural disturbances of prenatally GBP exposed rat offspring has not been well established so far. Thus, it may be revealed that prenatal exposure to GBP may induce long-lasting impact of the drug as overt neurobehavioral (anxiety like) impairments in young-adult rat offspring.

The present findings speculate that multifactorial mechanisms might be involved to dys-regulate the neural developmental processes when GBP is administered during critical and vulnerable period of brain development and growth. Therefore, doses of therapeutic range may adversely induce neuroanatomical alterations in fetal rat brain.

Therefore, the drug, GBP should be manifested critically to the potential women with epilepsy considering the possible neuro-organizational and neurobehavioural impairments in animal models but extrapolation of animal data to clinical setting are always warranted after weighing the risks to developing fetuses.

CONCLUSION

In summary, this is the first report of its kind elucidating the potential neurobehavioural teratogenicity of the atypical AEDs like GBP expressing long-lasting behavioural aberrations in F_1 rats exposed to GBP throughout gestation period; and possible involvement of developing neurotransmitters (maturation, synthesis and release as well as default conduction of impulses) may not be ruled out. This riddle may be solved by involving the well controlled animal studies especially designed for 'CNS-neurotransmitters-behavioral axes.

Acknowledgement
Authors are thankful to Head, Department of Zoology, University of Allahabad, Allahabad, India for providing laboratory facilities. Financial support from U.G.C., New Delhi to KG is thankfully acknowledged.

REFERENCES

[1] L Etemad; M Moshiri; S AMoallem; *J Res Med Sci*. **2012**, 17(9), 876.
[2] S H Lee; JW Kang; T Lin; J E Lee;Dong Il J.**2013**, Article ID 726478.
[3] DS Hill; B J Wlodarczyk; R H Finnell; *Expert R Neurother*. **2010**, 10, (6), 943.
[4] KJ Meador; GA Baker; RH Finnell; LA Kalayjian; JD Liporace; DW Loring; *Neurology*.**2006**,67: 407.
[5] K.Gupta; K.P.Singh; *NASL*.2007,49.
[6] KP Singh; M Singh; *Indian J. Exp. Biol*. **2001**, 39, 223.
[7] KP Singh; M Singh; *Neurotoxicology and Teratology*,**2002**, 24, 497.
[8] K Gupta; D Phil. Thesis,University of Allahabad, Allahabad, U.P. India, **2010**.
[9]M Ghaffarpour; H Pakdaman; B Siroos; *Iran J.Neurol*.**2013**,12(4):157.
[10] G Motamedi; K Meador; *Epilepsy Behav*.**2003**,4:S25.
[11] CH Meischenguiser; SM Giano; *Epilepsy Behav*.**2004**,5,163.
[12] O KVasant; T ADundappa; S Rajkumar; *Arc of Applied Sci Research*, **2010**, 2 (4): 85.
[13]NGharamaleki;NFaraz; S Saeid;*Annals of Biological Research*, **2011**, 2 (4) :301.
[14] Y Wise; Gabapentin: Neuropathic Pain and Body Weight Gain.carecure.org.
[15] H Claude;W Michael; O Iyabo;*BMC Pharmacol Toxicol*. **2013**, 14, 51.
[16] LG Costa;LSteardo; Cuomo V;*Pharmacol Rev*.**2004**, 56,103.
[17] P Bittigau; M Sifringer; K Genz; E Reith; D Pospischil; S Govindarajalu; M Dzietko; SPesditschek; I Mai; K Dikranian; JW Olney; C Ikonomidou; PNAS. USA,**2002**,99,15089.
[18] JA Petrere; JA Anderson; *Fundam Appl Toxicol*.**1994**,23, 585.
[19] C Palmieri; R Canger; *CNS Drugs*, **2002**, 16,755.
[20] PLV Prakash; V Rai; MM Pai;*Singapur Med J*,**2008**, 49, 47.
[21] E Gedzelman; J Kimford; MD Meador; *Ther Adv in Drug Safe.* **2012**,3(2), 71.
[22] C Ikonomidou; L Turski; *Epilepsy Res*.**2010**, 1.
[23] T Levav; *Int. J. Devl. Neuroscience*, **2004**, 22,137.
[24]MR Cilio; AR Bolanos; Z Liu; R Schmid; Y Yang; C E Stafstrom; *Neuropharmacology*.**2001**, 40,139.
[25] KJ Meador; G Baker; MJCohen; E Gaily; M Westerveld;*Epilepsy Behav*.**2007**, 11(3), 292.
[26] S Kapur; SC Vanderspek; BA Brownlee; JN Nobrega;*JPET*.**2003**, 305, 625.
[27] S Reagan-Shaw; M Nihal; N Ahmad;*The FASEB*.**2007**, 22, 659.
[28] J Altman; SA Bayer; Atlas of Prenatal Rat Brain Development,CRC,Press,**1995**.
[29] FA Malek; KU Möritz; J Fanghänel; *Indian J Med Res*.**2003**, 118, 90.
[30] L Prut; C Belzung;*Eur J Pharmacol*.**2003**, 463, 3.
[31] FOhl; Clinical Neuroscience Research. **2003**, 3, 233.
[32] AAWalf; CA Frye; *Nature Protocols*.**2007**, 2, 322.
[33] KP Singh; AK Jaiswal; M Singh; SK Battacharya; *Indian J.Exp.Biol*. **1998**,36,1102.
[34] E Poleszak; P WlaŸ; B Szewczyk; E Kêdzierska; E Wyska; TLibrowski; J Szymura-Oleksiak; *Pharmacol Rep.* **2006**,58(5),746.
[35] K M Wicke; A Rex; A Jongen-Relo; I Groth; G Gross; *Psychopharmacology* (Berl). **2007**, 195, 95.
[36] MJ McLean; *Epilepsia*. **1995**, 36(2):S73.

[37] Neurontin package, Park Davis, **1998**.
[38]BJ Wlodarczyk; A M. Palacios; T M George; R H Finnell; *Am J Med Genet Part A.* **2012**,158A (8), 2071.
[39] G Montouris; *Epilepsy &Behaviour*. **2003**, 4, 310.
[40] LV Wilton; S Shakir; *Epilepsia*.**2002**, 43,983.
[41] RL Goldenberg; SP Cliver; Y Neggers;*Acta Obstet Gynecol Scand*. **1997**, 165,8.
[42] M E Gilbert; R Macphail; J Baldwin; V C Moser; N Chernoff*; Neurotoxicol.Teratol.* **2010**, 32, 362.
[43]AS Fraser; *Epidemiol Rev*. **2010**,32, 5.
[44]D Rice; S Barone ; *Environ. Health Perspect.***2000**,208, 511.
[45] B Kolb; R Gibb; *J Can Acad Child Adolesc Psychiatry*. **2011**, 20(4), 265.
[46] NS Marchi; R Azoubel; WA Tognola; *Arq.Neuropsiquiatr*. **2001**,59,362.
[47] J Dobbing; J Sands;*Early Hum. Dev*. **1979**, 3,79.
[48] M Singh; D Phil. Thesis, University of Allahabad, Allahabad, U.P. India,**2013**.
[49] J C BGacona; AMeloy*; J. Nervous & Mental Disease*.**1991**, 178,546.
[50] FM Scalzo; SF Ali; RR Holson; *Biochem.Behav*. **1989**,34,727.
[51] J Zhang; L Wang; D Pitts;*Neurotoxicol.Teratol*. **1996**, 18, 49.
[52] FE Miller; TG Heffner; C Kotake; LS Seiden; *Brain Res.* **1981**,229,123.
[53] J Nicolai; SH Johan; PA Albert; *Neurological Sciences*, **2008**, 271, 1.