

Teratogenic effects of the anticonvulsant gabapentin in mice

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ABSTRACT

Introduction: We aim to study and elucidate the safety profile of the antiepileptic doses of gabapentin during pregnancy, and to evaluate gabapentin-induced murine fetotoxicity at different dose levels.

Methods: A total of 60 pregnant mice, divided into 12 groups of five mice each, were exposed to gabapentin in four different doses of 0 (control), 113, 226, or 452 mg/kg body weight per day, at three different gestational stages including early gestation (1-6 days), mid-gestation (7-12 days), and late gestation (13-17 days). The pregnant mice were euthanised on day 18 of gestation, and foetuses were examined for teratogenic manifestations. Their brains were dissected and examined for gross changes, malformations, histological changes, and quantitative protein estimation.

Results: Foetal resorptions were observed in all treated groups with gabapentin administration at early gestation (1-6 days), and mid-gestation (7-12 days). On the other hand, growth retardation along with stunting in size of live foetuses were observed in all the mid-gestation (7-12 days), and late gestation (13-17 days) treated groups. Various gross malformations were observed with all the three doses (113, 226, and 452 mg/kg body weight per day) when gabapentin was administered at mid-gestation (7-12 days). The same trends were confirmed by gross and microscopic examination of brains along with quantitative protein estimation.

Conclusion: Gabapentin should not be prescribed during pregnancy, as no therapeutic dose of gabapentin is safe during this period as far as the foetal well-being is concerned.

Keywords: anticonvulsant drugs, birth defects, gabapentin, mice, pregnancy, teratogenicity

INTRODUCTION

The use of anticonvulsant drugs in pregnancy may have potential effects on embryogenesis, neurological development, growth and subsequent paediatric progress, which presents unique challenges to both the clinicians and their patients.⁽¹⁾ Control of maternal epilepsy including drug selection and dose adjustment, must be balanced with the foetal and neonatal risks associated with anticonvulsant drugs as well as the clinical status of the patient.⁽¹⁾ Teratogenic screening is required and also recommended as drug administration during pregnancy may result in adverse effects on the foetus. In the general population, 1% of the adults and 5% of the children suffer from epilepsy. Antiepileptics that are prescribed to epileptic pregnant women should be effective, safe and free from foetal toxicity. No antiepileptic is ideal and safe. Overall, no one drug can be specifically recommended; but monotherapy with most of the recognised first-line drugs have a satisfactory outcome. Polytherapy should be avoided, if possible, as it is associated with greater incidence of congenital malformations.⁽¹⁾ Gabapentin (GBP) and lamotrigine have the highest GP awareness rates among the newer antiepileptics.⁽²⁾ Therefore, there is a possibility that newer second-line agents, like GBP, may be safer as an add-on therapy.⁽¹⁾

In 1993, GBP was approved by the US Food and Drug Administration for use in epilepsy. The IUPAC name of gabapentin is 2-[1-(aminomethyl) cyclohexyl]acetic acid. The empirical formula is C₉H₁₇NO₂, molecular mass is 171.237 g/mol, and its chemical structure is shown in Fig. 1.

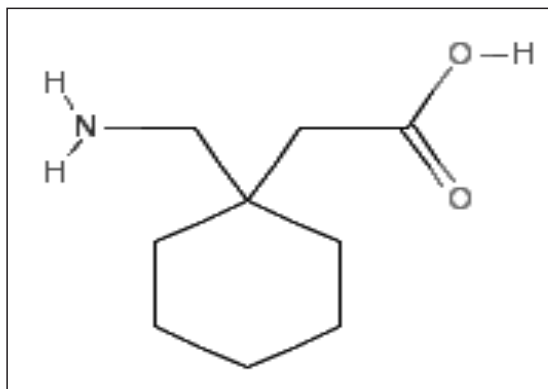


Fig. 1 Chemical structure of GBP.

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GBP, the amino acid antiepileptic drug (AED), is indicated for adjunctive use in individuals older than 12 years for the treatment of partial seizures, with or without becoming secondarily generalised.⁽³⁾ Biochemical effects enhancing the ratio of gamma-aminobutyric acid (GABA) to glutamate, ion-channel actions (direct or indirect), and/or enhancement of nonsynaptic GABA release are the different possible mechanisms of action.⁽³⁾ The anticonvulsant effect produced is related to the concentration of GBP in neurons, by the L-system amino acid transporter that has been involved in its absorption from the gut.⁽³⁾ Oka et al studied the effect of gabapentin on Ca²⁺ channels which involved the activation of nitric oxide synthase (NOS) in the primary neuronal culture of cerebral cortex in mice.⁽⁴⁾ Results suggested that GBP inhibits depolarisation-induced NOS activation in murine cortical neuronal culture. Blockade of both P/Q-type and L-type Ca²⁺ channels was associated with NOS activation.⁽⁴⁾

Rose and Kam suggested that GBP has a unique effect on the voltage-dependent calcium ion channels at the postsynaptic dorsal horns.⁽⁵⁾ This may therefore interrupt the series of events leading to the experience of a neuropathic pain sensation. GBP is considered an important drug in the neuropathic pain syndrome management.⁽⁵⁾ GBP is usually considered effective in doses of 900–1,800 mg daily, in three divided doses, although dosage may be increased to 3,600 mg in some patients to achieve reasonable seizure control.⁽⁶⁾ One-third of women with epilepsy have an increase in seizures during the gestational period.⁽⁷⁾ Ohman et al studied the pharmacokinetics of GBP during delivery, lactation, and in the neonatal period, and reported an active transplacental transport of GBP, with accumulation in the foetus as an important consequence.⁽⁸⁾ Crawford opined that the two newer anticonvulsants (GBP and lamotrigine) appear to be less harmful to the foetus as compared to the rest.⁽⁷⁾ GBP has been labelled category C on the basis of effects produced in rodent foetuses.⁽³⁾ Drugs that have been found to be teratogenic in Man have produced similar effects in experimental animals.⁽⁹⁾ The second generation antiepileptic drugs are reported to have produced teratogenic effects in various experimental animals; however, such data with reference to Man is still inconclusive.⁽⁶⁾

The developmental toxicity of the anticonvulsant agent GBP was studied and evaluated by Petre and Anderson in mice treated by gavage throughout organogenesis.⁽¹⁰⁾ Mice received different doses of 500, 1,000, or 3,000 mg/kg on gestation days (GD) 6–15. In mice, both body weight and food consumption were recorded on GD 0, 6, 12, 15, and 18. Each near term (mouse, GD 18) female was euthanised, necropsies were performed, and litter and

foetal data was collected. They reported that no adverse maternal or foetal effects were produced in mice treated with GBP in doses up to 3,000 mg/kg.⁽¹⁰⁾ Montouris reported human pregnancy registry data of 51 foetuses, including three twin gestations.⁽¹¹⁾ 39 women with epilepsy and other disorders were exposed to GBP during pregnancy. Montouris claimed that the rates of different teratogenic manifestations, such as miscarriage, low birth weight, and malformation, were reduced or similar to those seen in the general population, or among women with epilepsy. He concluded that GBP exposure during pregnancy is not associated to an increased risk for adverse maternal and foetal events.⁽¹¹⁾

The objectives and the scopes of the present study were to: (1) study and elucidate the teratogenic profile of GBP in pregnant mice; (2) evaluate GBP-induced murine fetotoxicity at different dose levels; (3) assess the impact of GBP-induced teratogenicity; (4) discern if GBP teratogenicity is predictable and/or observed as pronounced specific organ defects; (5) describe the microscopical changes that occurs after GBP administration; and (6) assess changes induced by GBP in the brains of foetuses by protein estimation.

METHODS

Sexually mature adult Swiss white (ICR) female mice with average age of 10–12 weeks were mated with males of the same stock for observing foetal teratogenic effects. Optimum and appropriate humane care was provided to the animals bred and kept in the department animal house. A total of 60 pregnant mice, divided into 12 groups of five mice each, was used in the study. GBP was administered in four different doses of 0, 113, 226, or 452 mg/kg body weight per day, at three different gestational stages including early gestation (1–6 days [length 6 days]), mid gestation (7–12 days [length 6 days]), late gestation (13–17 days [length 6 days]). The day on which sperms were seen in the vaginal smear was taken as day 0 of pregnancy. The pregnant mice were euthanised with an overdose of ether anaesthesia on day 18 of gestation, and through abdominal incision, uterine horns were exteriorised and inspected for foetal resorption. Live foetuses were collected for further examination. 0.5 ml per 20 g body weight normal saline was used as vehicle and was injected intraperitoneally with drugs. GBP, with trade name gabapin, (Intas Pharmaceuticals, Ahmedabad, Gujarat, India) was employed for the study. GBP, in doses of 0, 113, 226, or 452 mg/kg body weight equivalent to 0, 900, 1,800, or 3,600 mg, respectively, of the adult human dose (dosages for mice were calculated as described by Ghosh),⁽¹²⁾ in 0.5 ml saline per 20 g body weight, was divided into three equal doses per day; these were administered at

intervals of eight hours to different treatment groups of mice. Groups receiving 0 mg/kg body weight of GBP (vehicle alone) were treated as the controls.

Foetuses were examined for external changes and gross malformations. The brain was dissected and examined for gross changes and malformations as well. Bouin's solution was used as a fixative for histological study of the brains. They were dehydrated by application of ascending grades of alcohol and were embedded in paraffin wax. Random histological sections were selected and stained by haematoxylin and eosin. Brains were serially cut at 10 µm thickness in the coronal and transverse plane, and were stained with haematoxylin and eosin. A total of 120 foetuses (ten per group and two per litter) were selected randomly and one section per brain (total 10 × 2 = 120 slides) was examined under microscope. Slides were studied at different magnifications for observing histological changes. Both gross photography and photomicrography were performed. Further study for protein estimation was performed in four groups treated with GBP during mid-gestation; positive teratogenic findings manifested. Two foetuses from each of 20 litters (divided into four groups) were selected randomly and used for the assay mentioned below.

Quantitative estimation of proteins: protein contents in different samples of cell lysates, prepared by repeated freeze thaw were determined by standard Folin's method. 200 µL of reagent (alkaline copper solution: 25 ml of reagent A [2% Na₂CO₃ in 0.1 M NaOH] + 0.5 ml of reagent

B (0.5% of CuSO₄.5H₂O 1 M 1% sodium potassium tartarate]) was mixed with 40 µL of cell lysate followed by incubation at room temperature for 10 min. 20 µL of Folin-Ciocalteu's phenol reagent (freshly diluted with water in 1:1 ratio) was added to the above reaction mixture and allowed to stand at room temperature for 30 min. Absorbance was measured on an Elisa plate reader (Lab System, Finland) at 620 nm, with water taken as blank. Different forms of data were tested for statistical significance by using one-way analysis of variance (ANOVA) test with the help of statistical software programmes (GraphPad Prism 5.0, GraphPad Software, San Diego, CA, USA). Any value of $p < 0.05$ was regarded as significant.

RESULTS

GBP exposure resulted in foetal resorption in different study groups and were statistically significant in treated groups with drug administration on early and mid gestations, as compared with the corresponding controls. The incidence increased with an increase in dosage from 113 to 452 mg/kg body weight (Table I). Incidence of foetal resorption was highest (52.5%), when 452 mg/kg body weight GBP was administered during early gestation. GBP administration resulted in growth retardation along with stunting in size (Fig. 2, Table II). Crown-rump length of live foetuses were decreased significantly in treated groups with drug administration during mid and late gestations, as compared with the corresponding controls, and the incidence

Table I. Incidence of resorptions and live foetuses in different study groups.

Groups*	Dose of GBP (mg/kg BW route i.p.)	GBP exposure on gestational period	No. implants	No. (%) live foetuses	Average no. live foetuses per mice	No. (%) resorptions
1 (C)	0	Early	38	37 (97.37)	7.4	1 (2.63) ^b
2 (C)	0	Mid	39	39 (100)	7.8	0 (0)
3 (C)	0	Late	40	40 (100)	8.0	0 (0)
4 (T)	113	Early	37	28 (75.68)	5.6	9 (24.32) ^a
5 (T)	113	Mid	40	33 (82.50)	6.6	7 (17.50) ^a
6 (T)	113	Late	36	35 (97.22)	7.0	1 (2.78) ^b
7 (T)	226	Early	38	25 (65.79)	5.0	13 (34.21) ^a
8 (T)	226	Mid	37	28 (75.68)	5.6	9 (24.32) ^a
9 (T)	226	Late	38	37 (97.37)	7.4	1 (2.70) ^b
10 (T)	452	Early	40	19 (47.50)	3.8	21 (52.50) ^a
11 (T)	452	Mid	39	26 (66.67)	5.2	13 (33.33) ^a
12 (T)	452	Late	36	34 (94.44)	6.8	2 (5.56) ^b
Total			458	381 (83.19)	6.35	77 (16.81)

* 5 mice per group; total number of mice = 60

^a p-value < 0.05 as compared to the corresponding control

^b p-value > 0.05 as compared to the corresponding control

GBP: gabapentin; BW: body weight; C: control group; T: treated group; early gestation: 1–6 days; mid-gestation: 7–12 days; late gestation: 13–17 days.

Table II. Incidence of growth retardation and stunting in size in different study groups.

Groups*	Dose of GBP (mg/kg BW route i.p.)	GBP exposure on gestational period	Crown-rump length of live foetuses (mm) [mean \pm SD]	Body weight of live foetuses (g) [mean \pm SD]	Weight of foetal brains (mg) [mean \pm SD]
1 (C)	0	Early	27.2 \pm 2.05	1.57 \pm 0.09	32.23 \pm 2.13
2 (C)	0	Mid	25.9 \pm 1.92	1.51 \pm 0.12	31.47 \pm 2.08
3 (C)	0	Late	26.5 \pm 1.86	1.52 \pm 0.14	30.89 \pm 1.97
4 (T)	113	Early	25.3 \pm 1.80 ^b	1.48 \pm 0.10 ^b	30.12 \pm 2.06 ^b
5 (T)	113	Mid	20.9 \pm 1.77 ^a	1.06 \pm 0.07 ^a	19.12 \pm 0.87 ^a
6 (T)	113	Late	21.4 \pm 1.81 ^a	1.20 \pm 0.08 ^a	20.72 \pm 1.31 ^a
7 (T)	226	Early	25.8 \pm 1.94 ^b	1.49 \pm 0.11 ^b	29.67 \pm 1.87 ^b
8 (T)	226	Mid	19.2 \pm 1.72 ^a	0.88 \pm 0.06 ^a	16.86 \pm 0.88 ^a
9 (T)	226	Late	21.2 \pm 1.69 ^a	0.95 \pm 0.07 ^a	18.23 \pm 0.94 ^a
10 (T)	452	Early	25.2 \pm 1.67 ^b	1.46 \pm 0.09 ^b	29.72 \pm 1.78 ^b
11 (T)	452	Mid	16.8 \pm 1.59 ^a	0.61 \pm 0.05 ^a	14.86 \pm 0.99 ^a
12 (T)	452	Late	18.1 \pm 1.66 ^a	0.70 \pm 0.05 ^a	16.02 \pm 0.90 ^a

* 5 mice per group; total number of mice = 60

^a p-value < 0.05 as compared to the corresponding control

^b p-value > 0.05 as compared to the corresponding control

GBP: gabapentin; BW: body weight; C: control group; T: treated group; early gestation: 1–6 days; mid-gestation: 7–12 days; late gestation: 13–17 days.

increased with an increase in dosage from 113 to 452 mg/kg body weight (Figs. 2 & 3, Table II). Maximum reduction in crown-rump length (16.8 \pm 1.59 mm) when compared to the corresponding control (25.9 \pm 1.92 mm), was observed when GBP was injected in doses of 452 mg/kg body weight during late gestation.

Body weight of live foetuses was reduced in all the treated groups; although they were statistically significant only with drug administration in mid and late gestations, as compared with the corresponding controls in all three doses given (Figs. 2 & 3, Table II). Maximum reduction in body weight (0.61 \pm 0.05 g) when compared to the corresponding control (1.51 \pm 0.12 g), was observed, when GBP was injected in doses of 452 mg/kg body weight in mid-gestation. Gross malformations, including brachygnathia and pointed snout, open eyes and cataracts, thick short necks, rudimentary limbs, and malrotated limbs were observed with all the three doses (113, 226, and 452 mg/kg body weight). They were statistically significant when GBP was administered in mid-gestation, as compared with the corresponding controls; whereas the incidence of gross malformations increased with an increase in dose from 113 to 452 mg/kg body weight (Figs. 2 & 3 and Table III). Brachygnathia and pointed snout were the most common malformations (38.46%) observed when GBP dose of 452 mg/kg body weight was administered during mid-gestation.

GBP exposure resulted in gross changes, such as reduction in size and distortion in shape in the brains

collected from the foetuses (Fig. 4). Weight of foetal brains decreased significantly in treated groups with drug administration during mid and late gestations, as compared with the corresponding controls. The incidence increased with an increase in dose from 113 to 452 mg/kg body weight (Fig. 4, Table II). Maximum reduction in brain weight (14.86 \pm 0.99 mg) as compared to corresponding controls (31.47 \pm 2.08 mg), was observed when GBP was injected in doses of 452 mg/kg body weight in mid-gestation. Important microscopic changes observed in histological sections from the frontal cortex were vacuolisation and cavity formation surrounded with irregular arrangement of brain cells (Figs. 5c & d), when GBP was injected in doses of 452 mg/kg body weight during mid-gestation. With regard to the effect GBP administration on protein contents of the foetal brain (Fig. 6), a dose-dependent inhibition in the protein content of the brain tissue was observed following GBP exposure during mid-gestation of pregnancy, as compared to brains of foetuses obtained from the control mice ($p < 0.05$). The protein contents were found to be as low as 50% in the 452 mg/kg body weight GBP-treated group.

DISCUSSION

Tomson and Battino reported the lack of availability of systematic information on the pharmacokinetics of other newer AEDs (e.g., GBP, pregabalin, tiagabine, topiramate or zonisamide) during pregnancy.⁽¹³⁾ Johannessen and Tomson suggested that the pharmacokinetic variability



Fig. 2 Photographs of fetuses from four groups treated with GBP during mid-gestation and collected on day 18 of gestation. 1: Control group with GBP 0 mg/kg body weight administration; 2: Control group with GBP 0 mg/kg body weight administration; 3: Treated group with GBP 113 mg/kg body weight administration: growth retardation and stunting in size, malrotated left hind limb (arrow); 4: Treated group with GBP 226 mg/kg body weight administration: growth retardation and stunting in size, malrotated right fore limb (arrow) and malrotated left hind limb (arrow); 5: Treated group with GBP 452 mg/kg body weight administration: growth retardation and stunting in size, malrotated right hind limb (arrow) and malrotated left hind limb (arrow); 6: Treated group with GBP 452 mg/kg body weight administration: growth retardation and stunting in size, open right eye (arrow) and malrotated left hind limb (arrow).

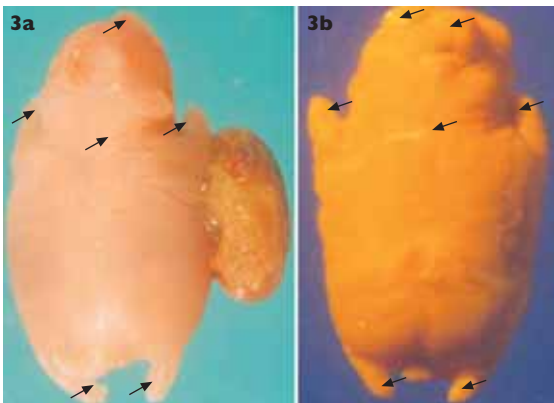


Fig. 3 (a) Photograph shows treated group with GBP 452 mg/kg body weight administration: growth retardation and stunting in size, pointed snout (arrow), thick short neck (arrow), rudimentary right and left fore and hind limbs (arrows). (b) Photograph shows treated group with GBP 452 mg/kg body weight administration: growth retardation and stunting in size, open right eye with cataract (arrow), brachygnathia and pointed snout (arrow), thick short neck (arrow), rudimentary right and left fore and hind limbs (arrows).

is less pronounced and more predictable, for drugs that are eliminated completely unchanged, renally (GBP, pregabalin and vigabatrin).⁽¹⁴⁾ On the other hand, the pharmacokinetic variability of GBP is related to its dose-dependent absorption. Adab concluded that higher doses of GBP and newer antiepileptic drugs may increase its teratogenic potential, but it has yet to be ascertained if the long-term adverse effects are related to intrauterine exposure in the second half of pregnancy.⁽¹⁵⁾



Fig. 4 Gross and microscopic photographs of brain of fetuses from four groups treated with GBP during mid-gestation and collected on day 18 of gestation. 1, 2, & 3: Control group with gabapentin 0 mg/kg body weight administration; 4: Treated group with GBP 113 mg/kg body weight administration: growth retardation and stunting in size; 5: Treated group with GBP 113 mg/kg body weight administration: growth retardation and stunting in size; 6: Treated group with GBP 113 mg/kg body weight administration: growth retardation and stunting in size along with distortion in shape.

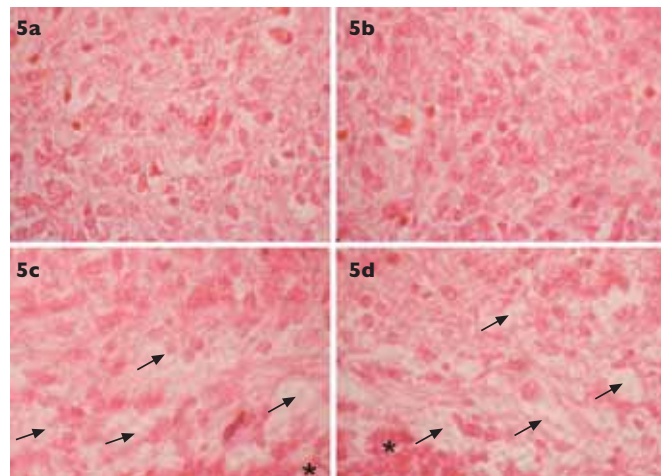


Fig. 5 (a & b) Photomicrographs (coronal plane from frontal region of brain) shows uniform distribution of brain cells (Haematoxylin & eosin, x 672). (c & d) Photomicrographs (coronal plane from frontal region of brain) shows vacuolisation and cavity formation (arrows) along with irregular aggregation of brain cells (asterix) (Haematoxylin & eosin, x 672).

Petere and Anderson studied the teratogenic effects of GBP in mice with different doses of 500, 1,000, or 3,000 mg/kg on gestational days 6–15.⁽¹⁰⁾ They observed no adverse maternal or foetal effects in mice when GBP was administered in doses up to 3,000 mg/kg. In contrast, in our study, GBP in daily doses of 113, 226, or 452 mg/kg body weight (equivalent to 0, 90, 1,800, or 3,600 mg, respectively, of adult human dose) on three separate gestational periods resulted in different teratogenic manifestations. Foetal resorption was observed in all treated groups with drug administration in early and mid gestations (i.e., gestational days 1–6 and 7–12). On the other hand, growth retardation, along with stunting in size of live

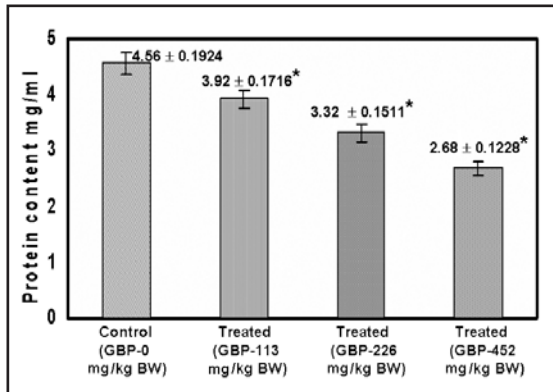


Fig. 6 Bar chart shows the effect of *in vivo* administration of GBP to pregnant mice on total protein content of the foetal brain.

Values are mean ± SD of independent experiments done in triplicate. * $p < 0.05$ vs. values for corresponding control.

foetuses, was observed in all the treated groups with drug administration in mid and late gestations. Gross malformations, including brachygnathia, pointed snouts, open eyes, cataracts, thick short necks, rudimentary limbs and malrotated limbs, were observed with all three doses (113, 226, and 452 mg/kg body weight), when GBP was administered during mid-gestation. All the aforementioned teratogenic manifestations were highest in incidence when GBP was administered in daily doses of 452 mg/kg body weight. The effects of GBP exposure, during pregnancy in experimental animals, on the foetal brain, including histological observations along with quantitative protein estimation, have not been previously

reported by any study group.

There an ongoing debate regarding the safety of newer AEDs, such as lamotrigine, GBP, tiagabine or levetiracetam. This is compounded by insufficient data concerning use of specific AED combinations and their resultant teratogenicity; there is therefore necessity for ongoing definitive commentary on this issue.⁽¹⁶⁾ AED-specific national and international birth registries have reported different dose-related concerns of valproic acid, carbamazepine and lamotrigine.⁽¹⁶⁾ Our study on experimental animals (mice) report a wide spectrum of teratogenic manifestations induced by GBP. Human pregnancy registry studies are observational studies, thus limiting their relevance. On the other hand, the results related to animal studies, including our present study, have the difficulties of extrapolating the results to human disease. Hence, before recommending GBP administration during pregnancy in humans, reports of both animal experimental studies and human pregnancy registry studies should be considered. Maternal and foetal risks, along with the personal priorities of patients should also be factors for consideration. In general, multiple drug therapy is considered more dangerous for the foetus than monodrug therapy.^(16,17) GBP, is indicated for adjunctive use in the treatment of partial seizures, with or without secondary generalisation.^(3,6) Hence, keeping in mind the aforementioned context, GBP should not be prescribed, as no therapeutic dose of GBP is safe during pregnancy as far as the foetal risk is concerned.

Table III. Incidence of gross malformations in foetuses following gabapentin exposure *in utero*.

Groups*	Dose of GBP (mg/kg BW route i.p.)	GBP exposure on gestational period	Total no. foetuses studied	Gross malformations				
				Brachygnathia and pointed snout (%)	Open eyes and cataract (%)	Rudimentary limbs (%)	Malrotated limbs (%)	Thick short neck (%)
1 (C)	0	Early	37	0	0	0	0	0
2 (C)	0	Mid	39	2.56 ^b	0	0	0	0
3 (C)	0	Late	40	0	0	0	0	0
4 (T)	113	Early	28	0	0	0	0	0
5 (T)	113	Mid	33	15.15 ^a	12.12 ^a	3.03 ^b	12.12 ^a	15.15 ^a
6 (T)	113	Late	25	0	0	0	0	0
7 (T)	226	Early	35	0	0	0	0	0
8 (T)	226	Mid	28	17.86 ^a	17.86 ^a	7.14 ^a	10.71 ^a	14.29 ^a
9 (T)	226	Late	37	0	0	0	0	0
10 (T)	452	Early	19	0	0	0	0	0
11 (T)	452	Mid	26	38.46 ^a	26.93 ^a	19.23 ^a	26.93 ^a	30.77 ^a
12 (T)	452	Late	34	0	0	0	2.94	0

* 5 mice per group; total number of mice = 60

^a p -value < 0.05 as compared to the corresponding control

^b p -value > 0.05 as compared to the corresponding control

GBP: gabapentin; BW: body weight; C: control group; T: treated group; early gestation: 1–6 days; mid-gestation: 7–12 days; late gestation: 13–17 days.

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