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Effects of Gabapentin Drug on Hematological Parameters and Histological Structures in Female Mice

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ABSTRACT

Gabapentin (GPN) is an anti-seizure drug that has also been prescribed for managing nerve pain and other ailments. However, its abuse has become an increasing concern, as it might lead to a serious health hazard. This study was therefore conducted to investigate the effects of oral administration of GPN drug on hematological blood parameters, as well as examine histological changes in vital organs of laboratory mice. Twenty female mice were obtained for the study and divided into four groups (5 mice each). The control group (G1) was maintained on a standard ration and treated orally with 1 ml of distilled water. Treatment groups; G2, G3, and G4 were administered with GPN (1, 0.5, and 0.25 mg/kg body weight, respectively) for 8 weeks. At the end of the experiment, the animals were sacrificed and the blood was collected for hematological analysis and histological examination on the liver and kidney. The results revealed that in the GPN-treated G2 (1 mg/kg body weight), a significant ($p<0.05$) decrease in WBC count was observed compared with the control group (G1), but the differences among the other groups were not significant. There was a significant decrease in the RBC, MCV, and MCH values, compared to the G1. Conversely, RDW, PLT, liver enzyme function and kidney parameters in all the GPN-treated groups (G2, G3, and G4) increased significantly in relation to the G1. Furthermore, the pathohistological study revealed extensive damage to the liver tissues and hepatocyte cord in the G2; moderate damage in the G3; while no obvious change was observed in the G4. Our findings indicated that the use of GPN as an anti-arthritis drug at high doses is associated with a long-term wide range of side effects in the body, and its use is therefore recommended at lower doses.

Keyword: Anti-arthritis drug, Gabapentin, Liver enzyme, Kidney function

Introduction

Gabapentin (GPN) is an anti-seizure drug that is also prescribed for nerve pain and other ailments. It has been available in the US since 1993. GPN is chemically known as 1-(amino methyl) cyclohexane acetic acid and is one of the new anti-epileptic drugs that has been approved as adjunctive therapy in adult patients, suffering from partial seizures. It affects chemicals and nerves in the body experiencing seizures and some types of pain. Also, it is used in adults to treat neuropathic pain (nerve pain) caused by the herpes virus and after an operation,[1]. GPN comes in several doses which include 100, 300, 400, 600, and 800 mg. It is well absorbed orally and circulates mostly unbound in the plasma. The drug interacts with cortical neurons at auxiliary subunits of voltage-sensitive calcium channels and increases the synaptic concentration of a gamma-aminobutyric acid (GABA). Furthermore, it enhances GABA responses at non-synaptic sites in neuronal tissues and reduces the release of mono-amine neurotransmitters,[2].

One of the mechanisms implicated in the effect of gabapentin is the reduction of the axon excitability, measured as an amplitude change of the presynaptic fiber volley in the Ca1 area of the hippocampus. This is mediated through its binding to presynaptic NMDA receptors.[3,4] Epilepsy is one of the most common serious neurological conditions affecting approximately 1 % of the world population at any one time.[5]. GPN has effectiveness as an add-on to treatment in people with a drug-resistant seizure disorder. For effective therapy, long term therapy is advised with anti-epileptic drugs, but the abuse of the drug in recent times is of growing concern. The study aimed to examine the effect of gabapentin drug on hematological blood parameters and determine histological changes in the liver and kidney of laboratory mice.

Materials and Methods

Source of experimental animals

Twenty female mice of 12 weeks old weighing (20 –25 g) were obtained from the stock of animal house research center in the faculty of Science, Kufa University, Al-Najaf

Province, Iraq. The mice were housed in laboratory conditions in plastic cages at standard conditions, the room temperature was set at 20 - 25 oC, using air conditioners, and ventilation with the humidity was adjusted to 50 %, and natural light periods (12 hours light/dark). Food and water were provided daily, [17, 20]. The experiment was performed in compliance with the Ethical Standards for the Investigation of Experimental Animals and was approved by the Ethical Committee of the Faculty of Science, Kufa University.

Drug supplement and dose calculation

The drugs were bought from the local pharmacy in Al-Najaf City, manufactured by pioneer Company, Iraq at 300 mg dose. The drug dose used in this study was the Oral LD50 of gabapentin for mice = 5000 mg/kg.[18].

Experimental design

The experiment was conducted by dividing the 20 experimental animals into 4 groups. Each group of 5 mice was contained. The first group (G1) was taken as the control and treated with distilled water. The other three groups were treated orally by special gavage with different doses of GPN (1, 0.5 and 0.25 mg/kg body weight [b.w.], respectively). After 8 weeks, the animals were sacrificed and samples of blood collected from the heart of the experimental mice for hematological analysis. Also, vital organs which included the liver and kidney were obtained for histological examination.

Hematological and biochemical analysis

The blood was collected from the mice through heart puncture and put 2 ml in EDETA tube for hematological analysis (WBC, RBC, MCV, MCH, RDW and platelets) by fully automatic hematology analyzer (ROBY, Germany). In addition, 3 ml of the blood removed by using gel tube centrifuged at 3000 r. p. m for 20 minutes for serum test of liver enzyme analysis (GOT, GPT, and ALP), and kidney functions (urea and creatinine). Determination performance by enzymatic colorimetric method by using kits provided by (Spinreact S.A. company). [19]

Histological preparation and examination

Histological examination was carried out on livers and kidneys obtained from experimental animals according to the procedure outlined in Bancroft.[7]. The preparations were dehydrated by increasing concentrations of ethyl alcohol sequentially (70, 80, 90 and 100 %), 2 hr in each concentration. The samples were cleared with two successive rounds of xylene for 2 hours in each step. In the final step of infiltration, the samples were dipped into a warm paraffin at 56 ºC. The cassettes were opened in the following day for embedding the tissues in paraffin blocking. A rotary microtome was used for sectioning the paraffin blocking into slices (4-5 µm in thickness), fixed on clean slides which were brushed with egg albumin. At the end of the experimental procedure, histopathological staining was done with Hematoxylin and Eosin. The sections were examined under a light microscope and photomicrographs taken.

Statistical data analysis

Statistical analysis of data was performed using Statistical Package for Social Sciences (SPSS, Version 24, Chicago, USA). All data were presented as mean ± standard error (SE). Multiple comparisons and determination of significant differences between treatment groups were done with one-way ANOVA, while post hoc test was achieved by least significant difference (LSD). A difference was considered significant when the probability value was $p<0.05$.

Results and Discussion

Hematological and biochemical parameters

Gabapentin (GPN), a gamma-aminobutyric acid (GABA) is effective in treating neuropathy or arthritis pain. Recent studies using animal and human models showed that the selective inhibition of the alpha 2 delta subunit of voltage-gated calcium channels is one possible mechanism of action.[8] In the present study, the effects of different doses (1, 0.5 and 0.25 mg/kg body weight) of GPN on hematological parameters of female mice is presented in Table 1. The results revealed that the oral use of GPN caused a significant ($p<0.05$) decrease in WBC, RBC, MCV and MCH in the G2 (1 mg/kg b.w. treatment) compared to the control group (G1). There were significant differences (LSD=1.968, 1.218, 2.170, 10.278; $p=0.001$) among the G1, G2, G3 and G4 groups, respectively. A significant ($p<0.05$) increase was observed in the RDW and PLT (LSD= 4.345 and 1499.49, respectively; $p<0.001$) in relation to the control group.

These results indicated that prolonged exposure of mice to therapeutic dose of GPN resulted in a decrease in RBC, MCV and MCH. Our observations are in agreement with the study of Almowalad,[9] who found the effects of high levels of pregabalin on RBCs and RBCs in albino rat. In a similar study,[10] it was reported that the hematological tests carried out indicated that anemia hemoglobin was 9.6 g/dL, renal impairment (urea 89.1 mg/dL, creatinine 1.56 mg/dL, eGFR 34 mL/min/1.73 m^2), and abnormal liver function tests (ALP 368 u/L, gamma-glutamyl transferase 142 u/L). A recent study also showed that a high dose of pregabalin (150 mg/kg/day and 300 mg/kg/day) interrupted the extracellular signal-regulated kinase (ERK) / the c-JUN N-terminal kinase (JNK)/ p38- mitogen-activated protein kinases (MAPKs) signaling pathway, reversed the bax / bcl2 ratio and triggered oxidative stress. It also reduced the production of dopamine, glutamate, and norepinephrine, and raised the number of degenerated neurons[11].

In the present study, GPN caused significant elevations in the blood urea, creatine of all the treatment groups, in relation to the control group. The increased levels of urea in GPN-treated groups indicated renal malfunction and might be due to impaired renal glomerular filtration rate (GFR), which was caused by GPN. We opined that the impairment or effect of GPN on GFR might be due to the renal overload which might be caused by unchanged secreted GPN, an observation in line with the finding of Al-Uboody.[12]

Liver enzyme function and kidney parameters as presented in Table 2 showed a significantly increased levels of the GPT, GOT and ALP (LSD= 10.096, 10.830 and 11.683, respectively; $p<0.001$), compared to the G1 control group. Furthermore, creatinine and urea levels were observed to be significantly high in all the GPN-treated groups of G2, G3 and G4 in comparison with the G1. Our study revealed that an increase in liver parameters due to GPN caused elevated liver enzyme functions (GOT, GPT and ALP), an observation which correlates with the finding of Meshkibaf and coworkers.[13] They showed that serum levels of ALP, AST, ALT, LDH, total bilirubin and direct bilirubin were enhanced significantly with higher dose of GPN. This might be explained by the inducing effect of GPN on liver enzymes, where it causes increased hepatic nitric oxide and induces

hepatotoxicity by increasing free radical liberation.[14,15]

Histopathological study

The results (Figure 1) of the histopathological study indicated that marked histological changes occurred with varying concentrations of GPN. In the control group, the features of liver displayed normal hepatocyte, normal central vein and hepatocyte cord (Fig. 1A). Meanwhile, no change was observed in the 0.25 mg/kg b.w. treatment group (Fig. 1B), while these changes were moderate in the 0.5 mg/kg b.w. treatment group (Fig. 1C), but severe changes, including extensive damage in the hepatocyte, vacuolation, nuclear fragmentation and apoptotic body formation were seen after treatment with 1 mg/kg b.w. of GPN (Fig. 1D).

Figure 2 shows the histological changes in the kidney of the experimental mice. Normal glomeruli, proximal and distal convoluted tubule were observed in the control group, which was treated with distilled water only (Fig. 2A), while no histological changes were observed in the 0.25 mg/kg b.w. treatment group (Fig. 2B). Moderate histological changes were seen in the kidney after treatment with the 0.5 mg/kg b.w. of the test drug and damage to the glomeruli, vacuolation in cell cytoplasm were detected (Fig. 2C). Severe damage and extension of the urinary tubules were observed in the treatment group with 1 mg/kg b.w. (Fig. 2D).

The extensive damage in the liver and kidney occurred as a result of prolong exposure to GPN treatment which have 5-6 hours half-life of absorption. This was as a result of a linear relationship that exists between dose and plasma concentrations over the therapeutically effective dose range. In other words, the effect of GPN is cumulative and this agrees with the study of Mohammed et al.[16] A moderate damage was observed in the group treated with 0.5 mg/kg b.w. of GPN because the concentration of the drug in the blood was less than the fatal dose. This finding is in agreement with the work of Meshkibaf and coworkers,[13] where they reported no death or fatal liver damage when GPN was used at low dosage. A recent study with low dose of GPN-antidepressant combination with opioids was effective in managing neuropathic cancer pain without severe adverse effects, while the low dose (0.25 mg/kg b.w.) was effective in the histological feature of liver and kidney.

Conclusion

The results from this study indicated that high dose of GPN had significant adverse effects on the hematological and biochemical parameters of the experimental animals. Consequently, the high dose of the drug led to a severe side effect on histological structures of the liver and kidney, compared with the moderate effect at a lower dose. It is therefore recommended that a precautionary measure be taken in its use.

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Conflicts of Interest

The authors declare no conflict of interest.

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Tables

Table 1. Effects of gabapentin on hematological parameters in female mice

Mean±SE						
Group	**WBC (×10^9 /L)**	**RBC (×10^{12} /L)**	**MCV (fL)**	**MCH (pg)**	**RDW (%)**	**PLT (×10^9 /L)**
G1	5.07±0.03^a	4.37±0.07^a	83.00±1.16^a	29.00±1.53^a	12.00±0.29^a	191.67±6.01^a
G2	2.63±0.03^{abc}	2.73±1.33^{ab}	45.00±0.001^{ab}	13.53±0.27^a	20.23±0.53^{ab}	2231±646.0^a
G3	4.37±0.87 ^{b}	3.39±0.29 ^{a}	39.00±0.001^{abc}	13.67±0.33^a	19.83±1.17^a	2039.67±216.33 ^{a}
G4	5.80±1.60 ^{c}	3.85±0.22^b	55.33±0.67^{abc}	17.90±6.10^a	16.57±2.33^{ab}	2043.67±617.67 ^{a}
LSD	1.968	1.218	2.170	10.278	4.345	1499.49
p-value	0.001	0.001	0.001	0.001	0.001	0.0001

G1: Control (Distilled water); G2: 1 mg/kg b.w.; G3: 0.5 mg/kg b.w.; G4: 0.25 mg/kg b.w.; a: significant difference at p<0.05 comparison between treated and control groups; bc: significant difference at p<0.05 comparison within groups.

Table 2. Effects of gabapentin on biochemical parameters

Mean±SE					
Group	**GOT (U/L)**	**GPT (U/L)**	**ALP (U/L**	**Creatinine (mg/dl)**	**Urea (mg/dl)**
G1	383.67± 3.93^a	86.00±0.58^a	112.33±0.88^a	0.74±0.00^a	37.44±0.73^a
G2	648.33±1.67^{ab}	300.00±6.43^{ab}	249.67±1.20^{ab}	0.82±0.01^{ab}	51.85±1.08^{ab}
G3	421.00± 1.53^{bc}	303.00±1.00^{ac}	256.67±1.67^{ac}	0.80 ±0.01^a	40.68±0.34^{abc}
G4	410.33±5.55^{abc}	120.67±1.20^{abc}	210.00±5.77^{abc}	0.75±0.03^b	35.50±1.33^{bc}
LSD	10.096	10.830	11.683	0.056	3.088
p-value	0.001	0.001	0.001	0.001	0.001

G1: Control (Distilled water); G2: 1 mg/kg b.w.; G3: 0.5 mg/kg b.w.; G4: 0.25 mg/kg b.w.; a: significant difference at p<0.05 comparison between treated and control groups; bc: significant difference at p<0.05 comparison within groups.

Figures

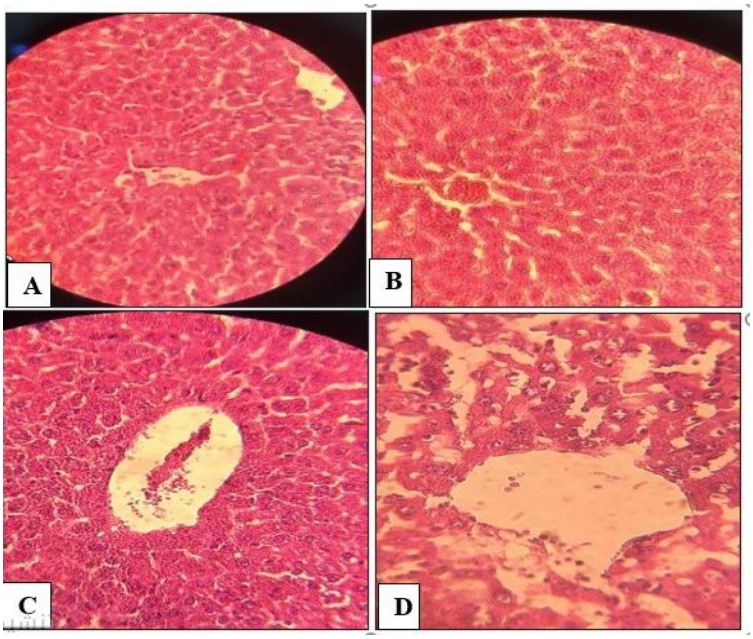

Figure 1. Cross section of liver tissues of female mice treated with varying concentrations of gabapentin. A: Control group (no treatment); B: Treatment with 0.25 mg/kg b.w.; C: Treatment with 0.5 mg/kg b.w.; D: 1 mg/kg b.w.

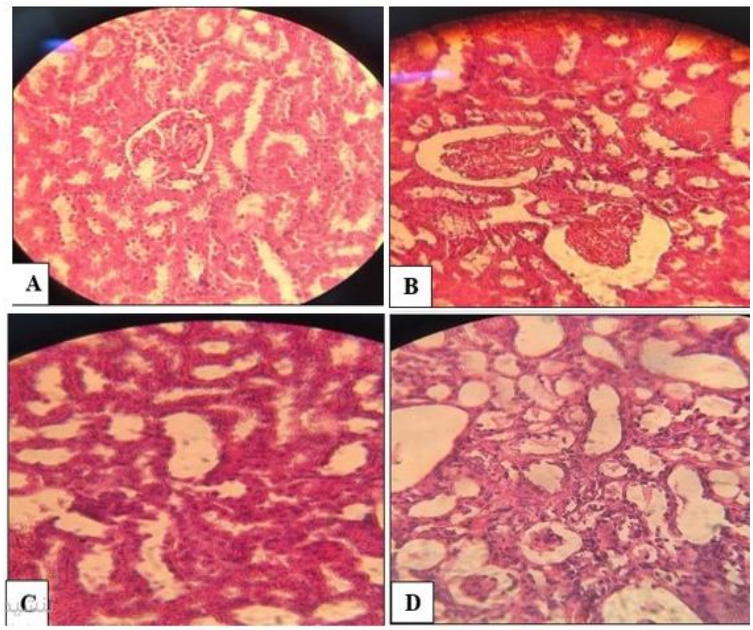

Figure 2. Cross section of kidney tissues of female mice treated with varying concentrations of gabapentin. A: Control group (no treatment); B: Treatment with 0.25 mg/kg b.w.; C: Treatment with 0.5 mg/kg b.w.; D: 1 mg/kg b.w.