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The Impact of Two Selected Doses of Gabapentin on the Kidney and Brain of Adult White Male Rats: A Histological, Biochemical, and Immunohistochemical Study

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Keywords: Apoptosis, Cholinesterase, Histopathology, Brain, Kidney, Gabapentin.

Article History

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Abstract:

Gabapentin is a medication commonly used in the treatment of epilepsy and sciatic neuropathy. Histology, biochemistry, immunohistochemical changes, body, kidneys, and brain weights were investigated in this study, where 15 male albino rats were used. The animals were split into 3 sets: A, B, and C (5 rats/set). Set A was administrated in distilled water (control set). Gabapentin was administrated orally to both B and C sets at doses 21.8 and 43.6 mg/kg, respectively. The dosing period was 30 days. The findings showed glomerular atrophy and changes in renal tissue in the B and C sets. Brain sections of B and C sets showed necrotic and degenerative changes. The findings showed insignificant body and brain weight increases in B and C sets, while set B significantly decreased kidney weight. Biochemical analysis showed an insignificant increase in the urea and creatinine in both experimental sets. Both B and C sets showed insignificant increases in the cholinesterase enzyme activity in the brain. Immunohistochemical assessment of the kidney tissue showed positive and strong positive immunoreactivity of both caspases 3 and 8. Both B and C sets showed weakly positive and positive Glial Fibrillary Acidic Protein (GAFP) immunoreactivity in the brain. It was obvious that gabapentin caused the induction of apoptosis in the kidney through the overexpression of caspases 3&8. The medication also induced various lesions in the brain, especially in the axons

of the neurons. The severity of its effect depended on the given dose and duration of treatment.

Keywords: Apoptosis, Brain, Cholinesterase, Gabapentin, Histopathology, Kidney.

تأثير اثنتين من الجرعات المختارة من عقار الكابابنتين على كلية ودماغ الجرذان البيض البالغة: دراسة نسجيه، كيموحيوية، وكيميائية مناعية نسجيه

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الخلاصة:

يعد الكابابنتين عقار شائع لعلاج الصرع والتهاب العصب الوركي . في الدراسة الحالية ، استخدم ٣٠ جرذ ابيض ذكر. قسمت الحيوانات الى ثلاث مجاميع *A,B* و*C* (5جرذان/مجموعة). مجموعة *A* جرعت بالماء المقطر(مجموعة سيطرة). جرعت المجموعتين *B* و*C* فمويا بعقار الكابابنتين وعند التركيزين ٢١,٨ و٤٣,٦ ملغم /كغم من وزن الجسم .كانت مدة التجريع ٣٠ يوم. تم التحري عن التغيرات النسجيه، الكيموحيوي، الكيميائية الحيوية ،والكيميائية المناعية النسجيه ، فضلا عن التغيرات في وزن الجسم ،الكلية والدماغ .أظهرت النتائج ضمور الكبيبة وتغيرات مرضية أخرى في النسيج الكلوي للمجموعتين *B* و*C* . وأظهرت مقاطع الدماغ للمجموعتين *B* و*C* تغيرات تنكسية وتنخريه . وأظهرت النتائج زيادة غير معنوية في وزن الجسم والدماغ ، في حين كان هناك انخفاض معنوي في وزن الكلية . بينت نتائج التحليل الكيموحيوي زيادة غير معنوية في اليوريا والكيراتين في كلتا المجموعتين التجريبيتين *B* و *C*. وأظهرت المجموعتين *B* و *C* زيادة غير معنوية في فعالية أنزيم الكولين ستيريز. اظهر التحليل الكيميائي المناعي النسجي لنسيج الكلية استجابة موجبة وموجبة قوية لبروتيني *caspases 3*و٨ في كلتا المجموعتين التجريبيتن *B* و *C*. كلتا المجموعتين التجريبيتن *B* و*C* أظهرت استجابة موجبة ضعيفة وموجبة لبروتين *GAFP* في نسيج الدماغ .كان واضحاً أن عقار الكابابنتين سبب حث مسارات الموت المبرمج من خلال التعبير الجيني المفرط لبروتيني *caspases 3* و٨ . حث العقار ظهور أفات مختلفة في نسيج الدماغ وخاصة في محاور الخلايا العصبية . وكانت شدة التأثير معتمدة على الجرعة والفترة الزمنية للمعاملة.

الكلمات المفتاحية: موت الخلايا المبرمج، علم الأمراض النسيجي، الدماغ، الكلية، جابابنتين.

1. Introduction:

Anticonvulsant medications are mostly used for managing epilepsy, although they are additionally employed to treat migraines, bipolar mood disorder, neuropathy, and inflammation of the nerves **[1].** One of the most prominent anticonvulsant drugs is gabapentin (gamma-aminobutyric acid), which is used to treat electrical brain disorders and pain resulting from damage to the nerves, especially the cranial nerve. The drug is also used to treat nerve damage caused by diabetes, which is known as diabetes mellitus neuropathy. It is also used to treat nerve pain after infection with the herpes virus. The drug also treats dysesthesia **[2].**

The drug was discovered in 1970 through a campaign conducted to discover drugs to treat neurological disorders **[3].** Following the United States FDA's confirmation, the medication was initially made available to Americans in 1993. It is functionally related to another drug, pregabalin, and the drug works to inhibit the release of irritating neurotransmitters, thus helping

in its use against pathological neurotransmission **[4].** Despite its benefits, this medication produces negative effects, such as drowsiness and loss of balance. The drug's use raises the possibility of allergy responses, breathing difficulties, and depression **[5].**

The toxic role of the drug is evident from its interaction with some other anticonvulsants **[6].** The drug falls under category C. The drug causes apoptosis at certain doses and tissue damage to the kidneys in rats **[7].** The drug is well absorbed orally and exists in plasma in an unbound form. The medication affects calcium channels that are voltage-gated in neurons in the cortex at the accessory nerve units. Thus, the concentration of GABA increases at the synapse area, and the drug reduces the monoamine transporters **[8].** The liver and kidneys are the organs concerned with getting rid of most drugs, and therefore any defect affects them and makes them unable to perform their functions properly. This causes the deposition of drugs, especially anticonvulsant. Liver and kidney dysfunction causes a prolongation of the period allocated for the elimination or excretion of the medication or from the toxic metabolic compound, leading to clinical toxicity that can impact the attaching, distribution of cellular proteins, and the metabolism process. For example, protein binding is significantly reduced for acidic, negatively charged drugs, such as phenytoin and valproate in patients with kidney failure **[9].**

Case study reports stated that gabapentin causes liver damage, but without causing symptoms. The drug caused liver cirrhosis in a 90-year-old patient when he took 900 mg/kg for two weeks **[10, 11].** When the drug is used for 5 weeks in the case of treating neuropathic pain, the drug causes neutropenic fever. Long-term use of the drug impacts the healing or recovery process of bones, especially bone fractures, through its effect on some biochemical parameters and histological composition in rats **[12].** The drug has a significant effect on the gene expression of some cancer genes **[13].** Gabapentin impacts the male and female reproductive systems in rats, as it causes a decrease in testosterone levels and the process of sperm production while in females, it inhibits the steroids of the pineal gland, stimulates gonad hormones, and increases the atretic follicles. The drug also affects the period of pregnancy, especially affecting the development of the skull **[14].** The current research aimed to identify the toxic effects of the drug gabapentin at doses 21.8 and 43.6 mg/kg on some biochemical, histopathological, and immunohistochemical variables in the liver and brain of male albino rats.

2. Material and methods:

2.1. Ethical Approve: According to The Institutional Animal Care and Use Committee (IACUC) ethical approval number UM.VET.2023.046 dated 2/10/2023, the study was carried out in an animal's housing at the Faculty of Veterinary Medicine at the University of Mosul, Iraq.

2.2. Obtaining and raising animals: 15 male rats were used in the current study. The average weight was 195±5 grams. The entire experiment was carried out in a facility of animals at the Faculty of Veterinary Medicine, University of Mosul, Mosul, Iraq after the animals were transported there from Cihan University in Erbil. The animals were kept in typical cages in normal circumstances. The animals were cared for by specialists in the animal house. The animals were fed pellets and given water daily during the experiment period for all groups.

2.3.Medication dosing and supplementation: Gabapentin was obtained from a local pharmacy in Nineveh Governorate. The drug is manufactured by JENERIS Pharmaceutica, S.A., Rua Jaoa de Deus,19. 2700-487 Amadora, Portugal. The research doses were calculated based on the oral LD50 rate, which is 5000 mg/kg **[15].** The doses chosen in the current research are 21.8 and 43.6 mg per 1 kg. Each concentration was diluted in six ml of filtered water and then the relevant dose was withdrawn depending on the weight of the animal **[16].** The medication was administered orally by gavage needle to the male rats **[17].** The selected doses were dissolved in distilled water. The medicine was given for only one month.

2.4. Study design: A, B, and C were the three sets of fifteen mature male rats. Every set has five animals in it. A set was considered a control group. B&C sets were given gabapentin orally (21.8 and 43.3 mg/kg) body weight [b.w.] by a special dosing needle for 30 consecutive days. Weekly weigh-ins were conducted on the animals.

2.5. Histopathological preparation: The kidney and brain were obtained after dissection and placed in a 10% neutral buffered formalin to fix the tissues for 3 consecutive days. The weight of the previous organs was recorded. The tissue samples were washed with running water for 1 hour to remove traces of the fixative **[18].** The histological slides were prepared routinely. The paraffin blocks were sectioned by typical microtome. The histological sections were between 4-5 micrometers in thickness. Harri's Hematoxylin and Eosin were used to stain histological sections **[19].** DPX was used for mounting the slides. It was cleaned, prepared, and mounted with D.P.X. as a suitable medium **[20].** The microscopic assessment and photography were done using a compound microscope attached to a digital single-lens reflex camera (type MDCE-6A, Japan) **[21].**

2.6. Detection of the kidney Urea, and creatinine: After a full month of dosing with gabapentin, the rats were euthanized, and blood samples (1.5-2 ml) were collected from the eye's corner using capillary tubes. The taken blood was stored in small containers with anticoagulants, and samples of it went through a centrifuge (3000 rpm) for over 10 minutes to produce serum for biochemical evaluation. Before use, the serum had been kept in a freezer at -20 C. **[20].** Biosystems S.A.UREA/ BUN color, Costa Brava30,08030, Barcelona, Spain kit

was used.

2.7. Detection of cholinesterase enzyme in the brain tissue: The brain was ground and homogenized using a homogenizer Electroporation for 30 seconds at a speed of 400 r/min to the brain and it was a tube. The homogenizer was immersed in ice during the homogenization process as the brain was triturated in a Phosphate buffer solution with a pH of 8.1 after adding three ml/100 mg of tissue weight **[21].** The samples were stored in test tubes immersed in crushed ice until cholinesterase tests were performed immediately after the previous process was done.

2.8. Immunohistochemical assessment: The avidin-biotin immunoperoxidase method was employed to conduct immunohistochemistry. CASP1, CASP3, and CASP8 (Elabscience, USA, catalog No. E-AB-70300, Catalog No. E-AB-70388, Catalog No. E-AB-19664, respectively were investigated in the kidney. GFAP (Elabscience, USA, catalog No. E-AB- 650669) was investigated in the brains. A quantitative cell scoring method was used to detect the immunoreactive bodies in cells. A four-point score based on the IHC staining intensity of the cells was employed for negative (-), weak positive (+), positive (++), and strong positivity (+++).

2.9. Data analysis: The criteria chosen to be measured in the current research were statistically analyzed, including variations in body weight and specific body parts, criteria for measuring kidney and brain function, and changes in the cholinesterase enzyme using Graph Pad Prism 5.0 (San Diego, USA) software. All previous parameters were described as an average ± standard deviation (SD). The P-value was estimated as; significant ($P<0.05$), and very significant ($P<0.01$), respectively.

3. Results:

The light microscopic assessment of the kidney belongs to A set (control set) showed the normal histological structure of the kidneys **Figure 1**.

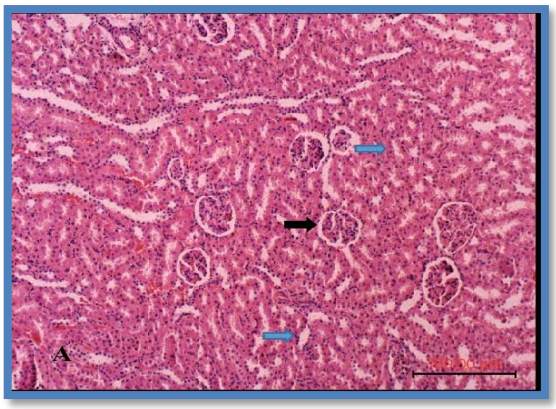

Figure 1: The histological section of the cortical part of the rat kidney of set A (control group) shows the normal histological structure of the glomeruli (black arrow) and surrounding tubules (blue arrow). (200µm H&E).

It is represented by Malpighian corpuscles (glomeruli) and renal tubules. Sections of set B (21.8 mg/kg) demonstrated the presence of significant and evident glomerular atrophy, oedema, Bowman's gap expansion, and alterations, including partial cell death, in the urine tubules **Figure 2**.

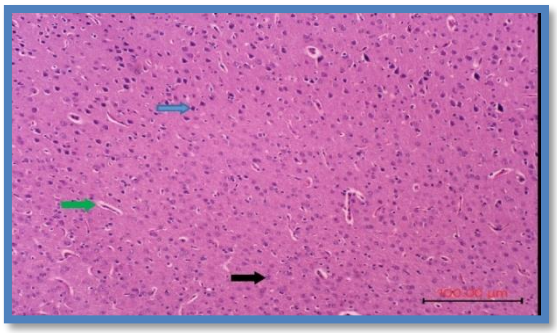

Figure 2: The histological section of the cortical part of the rat kidney of set B shows the presence of atrophy in the glomeruli, effects of oedema, widening of Bowman's space (black arrows), and changes in the urinary tubules, including partial destruction of cells (blue arrows) and congestion in the blood vessels (green arrow) (100µm H&E).

Sections from set C (43.6 mg/kg) showed the presence of severe atrophy of the Malpighian corpuscles, effects of oedema, damage to the tiny, filtering blood vessels, widening of Bowman's space, and destruction of most of the urinary tubules, including the destruction of cells **Figure 3**.

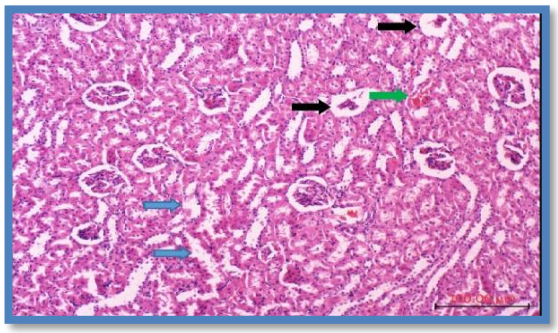

Figure 3: The histological section of the cortical part of the rat kidney of set C shows severe atrophy of the glomerulus, expansion of Bowman's space (black arrows), and destruction of the renal tubules (blue arrows) (100µm, H&E).

The light microscopic assessment of the brain belongs to the A set (control set) and shows normal histology of the brain that is represented by neurons, glial cells, and normal blood vessels that exist in the cortical part of the brain **Figure 4**.

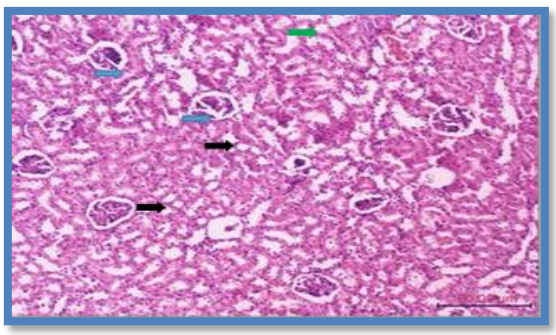

Figure 4: The histological section of the brain belongs to the A set (control set) showing normal neurons (black arrow), glial cells (blue arrow), and blood vessels (green arrow) in the brain cortex (100µm H&E).

Brain sections of set B revealed neuronal necrosis, degenerative changes, and blood vessel congestion **Figure 5**.

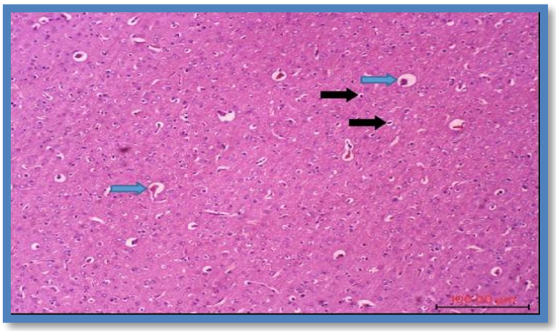

Figure 5: The histological section of the brain belonging to the B set shows cell death (necrosis) of nervous cells (black arrow), vacuolation of the neuronal cytoplasm (green arrow), and blood vessel congestion (blue arrow). (100µm, H&E)

while sections of set C revealed severe necrotic and degenerative changes in nervous tissue as well as congestion **Figure 6**.

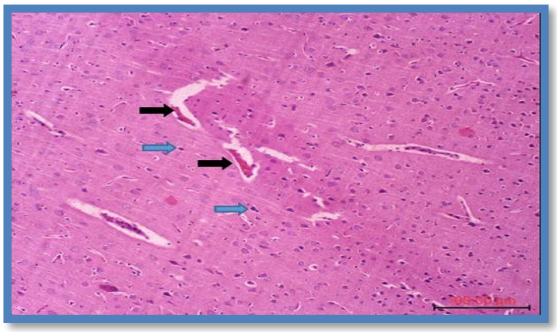

Figure 6: The histological section of the brain belonging to the C set shows severe necrotic (black arrows) and degenerative changes (blue arrows). (100µm, H&E).

3.1. Effect of gabapentin on body, kidney, and brain weights: The findings revealed an insignificant increase in the body weight for both experimental groups (21.8, 43.6 mg /kg) compared to set A **Figure 7.**

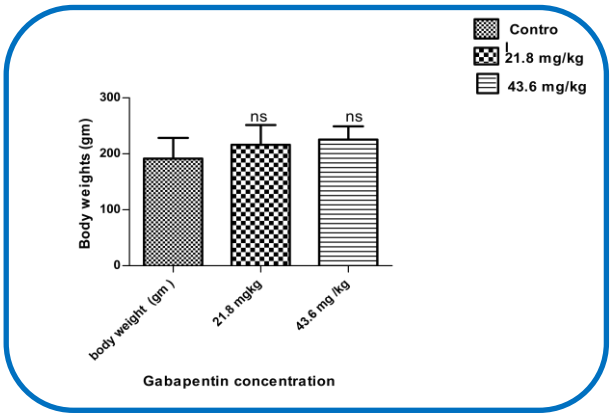

Figure 7: The effect of gabapentin at the doses 21.8 and 43.6 mg/kg (sets B and C) on the body weight. The medication was given for 30 consecutive days (1 month). P< 0.01 is considered highly significant, and n = not significant in comparison to set A. By comparing the average of each concentration in the two test sets to the means of set A, Dunnett's test was utilized to demonstrate the discrepancies.**

Brain weight showed an insignificant increase for both previous sets **Figure 8**.

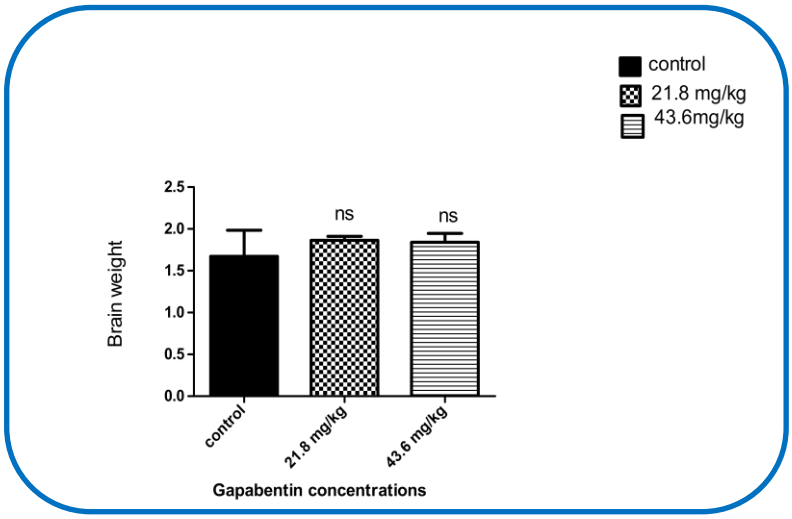

Figure 8: The effect of gabapentin at the doses 21.8 mg /kg (sets B and C) on the brain weight. The medication was given for 30 constitutive days (1 month), n = not significant in comparison to set A. By comparing the average of each concentration in the two test sets to the means of set A, Dunnett's test was utilized to demonstrate the discrepancies.

In addition, kidney weight revealed a highly meaningful (P > 0.01) abate at the concentration of 21.8 mg/kg and an insignificant decrease in weight at the dose of 43.6 mg/kg in comparison with the set A **Figure 9**. When comparing the results of the two experimental sets, we note that the kidneys of the third set (43.6 mg /kg) showed a non-significant increase compared to the second set (21.8 mg /kg).

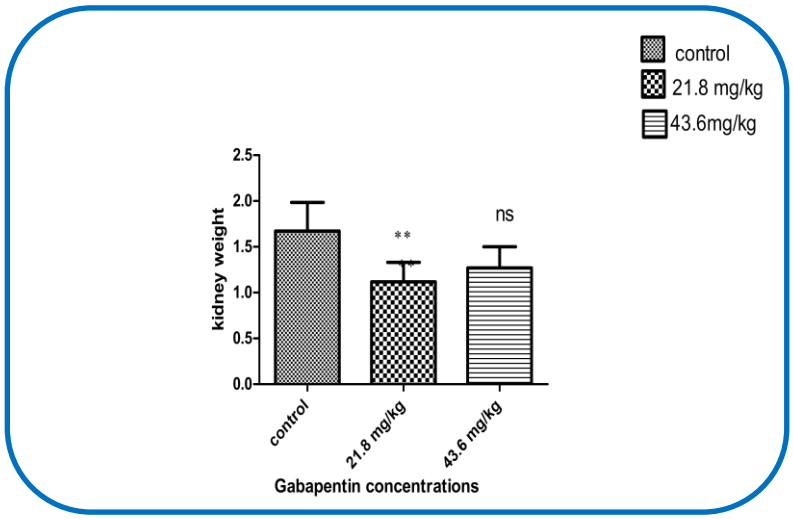

Figure 9: The effect of gabapentin at the doses 21.8 and 43.6 mg/kg (Sets B and C) on the kidney's weight. The medication was given for 30 consecutive days (1 month). P>0.01 is considered highly significant, and n = not significant in comparison to set A. By comparing the average of each concentration in the two test sets to the means of set A, Dunnett's test was utilized to demonstrate the discrepancies.**

3.2. Effect of Gabapentin on the level of Urea and creatinine: The findings showed a significant (P> 0.05) and insignificant increase in the urea level of both sets B and C, respectively, in comparison to set A **Figure 10**. Creatinine levels showed an insignificant increase at the two selected doses **Figure 11**.

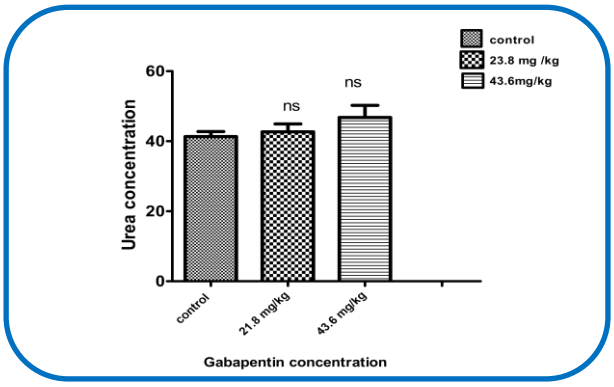

Figure 10: The effect of Gabapentin medication on the Urea level at the concentration 21.8 (set B) and 43.6 (set C) mg/kg. The medication was given for 30 consecutive days (1 month). * Considered as significant at P<0.05, and ns = not meaningful in comparison to set A. By comparing the average of each concentration in the two test sets to the means of set A, Dunnett's test was utilized to demonstrate the discrepancies

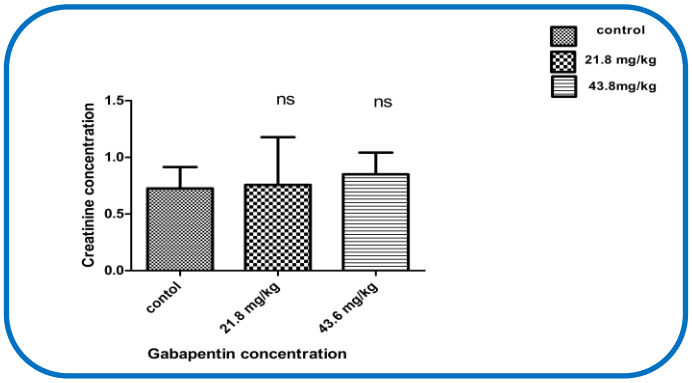

Figure 11: The effect of Gabapentin medication on the Creatinine level at the doses 21.8 (set B) and 43.6 (set C) mg/kg. The medication was given for 30 consecutive days (1 month). ns = not significant in comparison to set A. By comparing the average of each concentration in the two test sets to the means of set A, Dunnett's test was utilized to demonstrate the discrepancies

3.3. Effect of Gabapentin on the cholinesterase level in the brain: The findings revealed that there is an insignificant change in the brain cholinesterase activity in set B and insignificant increase in its activity in the set C in comparison with set A **Figure 12**.

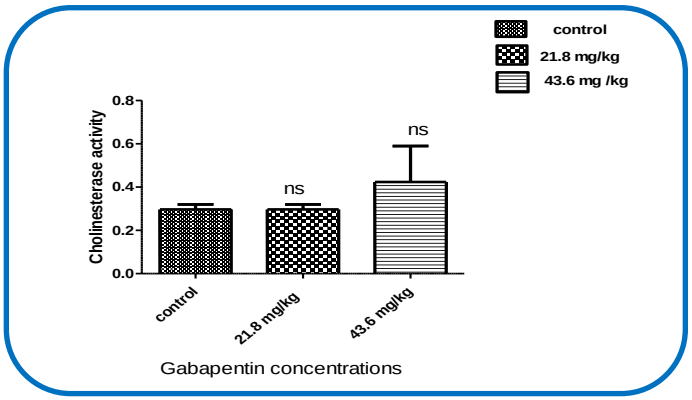

Figure 12: The effect of Gabapentin medication on the cholinesterase activity at the doses 21.8 and 46.6 mg/kg . The medication was given for 30 constitutive days (1 month). ns = not meaningful in comparison to control. By comparing the average of each concentration in the two test sets to the means of set A, Dunnett's test was utilized to demonstrate the discrepancies

3.4. Effect of Gabapentin on the expression of CASP3 and CASP8 in the kidney: To determine the impact of 21.8 and 43.6 mg/kg of gabapentin in the enhancement programmed cell death in the kidney of a male rat, the positivity of caspase-3 and caspase-8 was examined.

The microscopical assessment of renal tissue of set A showed negative immunoreactivity of CASP3 (-) **Figure 13**. A positive and a strong positive (+++) of CASP3 expression in the renal epithelial cytoplasm were detected in sets B **Figure 14** .

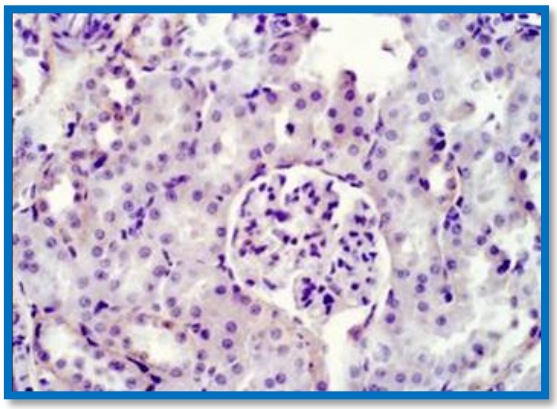

Figure 13: A photomicrograph of the kidney cortex of set A showing revealed negative (-) caspase-3 immunoreactivity in the renal tubule's epithelia. Immunohistochemical stain, 400 x

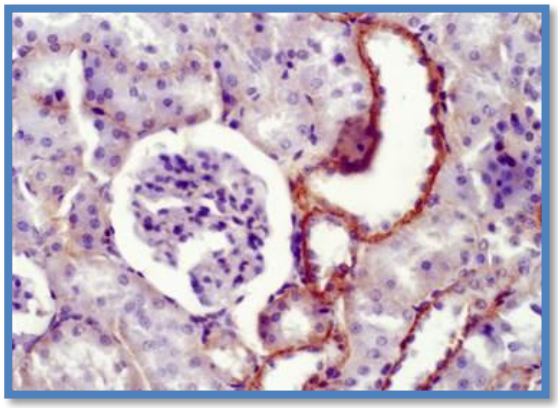

Figure 14: A photomicrograph of the kidney cortex of set B shows a positive (++) immunoreactivity (black arrow) of caspase-3 in set B in the renal tubule epithelia. Immunohistochemical stain, 400 x

In set C there was a similar response to set B compared to set A **Figure 15**.

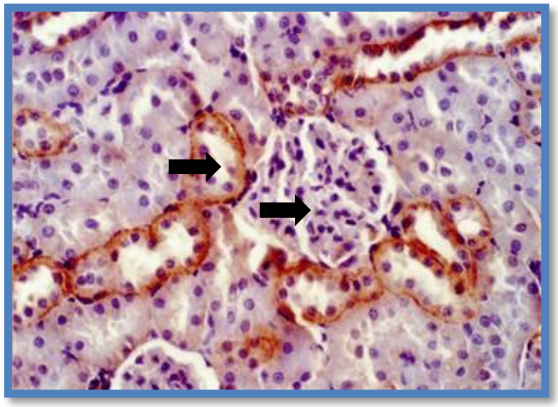

Figure 15: A photomicrograph of the kidney cortex showing a strong positive (+++) caspase-3 immunoreactivity (black arrow) in set C in the renal tubule's epithelia. Immunohistochemical stain, 400 x

As for investigating the immunoreactivity of caspase- 8 in the renal tissue, the microscopical examination revealed a weak positive reaction (+) which appears as golden-brown granules in epithelial cells lining renal tubules in set A **Figure 16** .

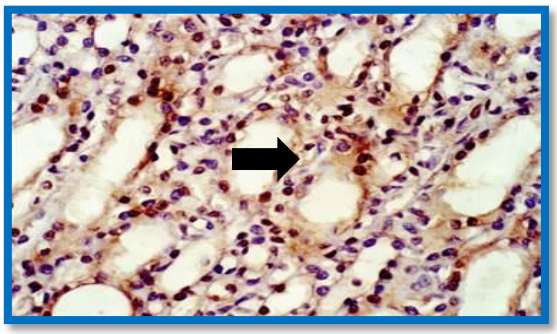

Figure 16: A photomicrograph of the kidney cortex shows weak caspase-8 immunoreactivity (-) in the renal epithelial cells: immunohistochemical stain, 400 x.

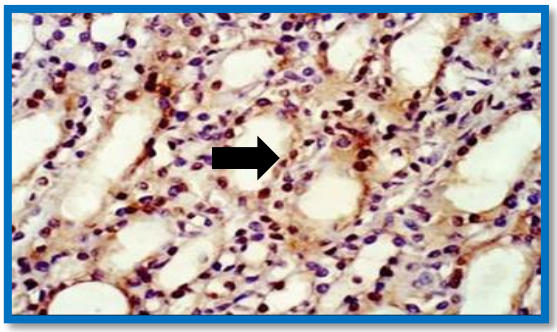

Figure 17: A photomicrograph of the kidney cortex of set B shows positive (++) immunoreactivity) in the renal epithelial cells. Immunohistochemical stain, 400 x.

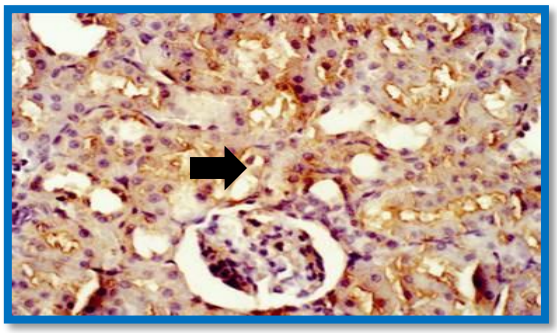

Figure 18: A photomicrograph of the kidney cortex of C shows revealed a highly (strong) positive (+++) immunreactivity (black arrow). Immunohistochemical stain, 400 x.

3.5. Effect of Gabapentin on the expression of GFAP in the brain: The positivity of the intermediate filament-III protein (GFAP) was investigated in the brain tissue of adult male rats at doses 21.8 and 42.6 mg/kg. The microscopical assessment of the brain tissue sections of set A revealed negative (-) immunoreactivity of GFAP expression as golden-brown fibers neurons **Figure 19**.

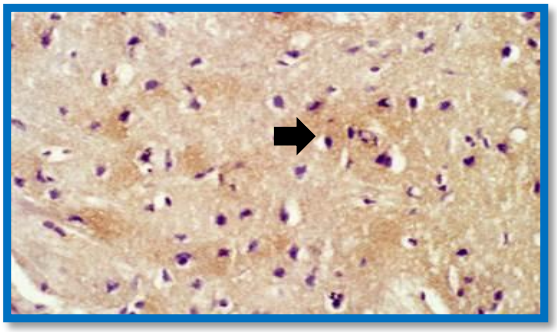

Figure 19: A photomicrograph of GFAP expression in the brain of set A revealed a negative (-) response (black arrow). Immunohistochemical stain, 400 x.

A weak positive (+) response was detected in set B **Figure 20**

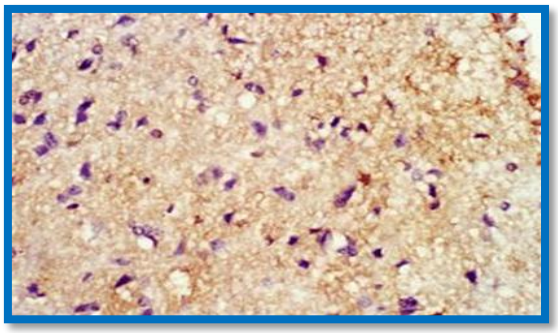

Figure 20: A photomicrograph of GFAP expression in the brain of set B weak positive (+) response (black arrow). Immunohistochemical stain, 400 x

and positive (++) immunoreactivity was detected in set C **Figure 21**, compared to set A as golden-brown fibers in neurons.

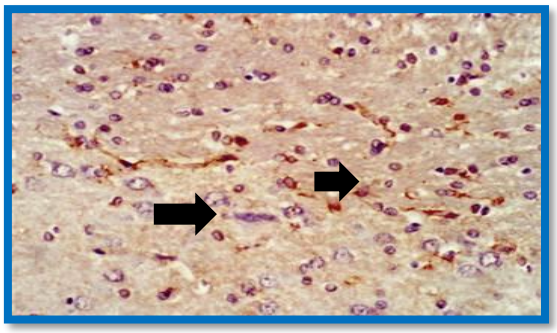

Figure 21: A photomicrograph of GFAP expression in the brain of set C revealed a positive (++) response (black arrow). Immunohistochemical stain, 400 x.

4. Discussion

Gabapentin's historic outlook emphasizes its anticonvulsant and antiepileptic actions. Gabapentin was first investigated in clinical trials at modest dosages and demonstrated to be beneficial as an additional medication. Despite the therapeutic benefits, it had and still has side effects that vary in severity depending on the doses **[22].** This is what the current study investigated and the results showed several lesions in the kidney at both studied doses like Malpighian corpuscles atrophy oedema and destruction of most urinary tubules the severity of histological changes was dose-dependent manner. The present findings were identical to the findings of **[23],** who discovered that treating male rats with gabapentin at high doses causes a lot of kidney lesions like vacuole degeneration and haemorrhage. The outcomes also were comparable to the outcomes of **[24],** who indicated that the selected drug could cause renal cell destruction.

The current findings were not consistent with the findings of **[25, 26].** The previous lesions' occurrence may be due to the that gabapentin affects the sympathetic nervous system, which controls the regulation of blood vessel functions and blood flow to the kidneys. This effect

could cause a change in blood pressure and its distribution in the renal blood vessels, which affects their functions **[27].** or perhaps because the studied drug causes an increase in the level of oxidative stress in the renal cells, which caused the above lesions (7). The medication alters the balance of calcium and ions inside the renal cells by acting as a blocking agent of calcium-transport pathways in the brain and spinal cord, which impacts the function of ion channels in the kidney **[28].**

The painkiller gab can occasionally generate DRESS disorder (drug response with eosinophilia and systemic signs). This is a severe allergic response that might damage essential tissues **[29].** The current outcome revealed some lesions in the brain at both doses such as necrosis and degenerative changes, and the severity of these lesions increases with increasing the concentration of the drug administered to the animals and that may be due to the that the drug easily passes through the selective semi-permeable membrane of the brain and affects the cortex layer, hippocampus, and spinal cord by affecting the calcium channels which may cause tissue lesions **[30].** The current outcomes were not consistent with the findings of **[31],** who found that the damage in the central nervous tissue may be due to the lack of Ache, and this condition is linked to memory and cognition deficits. The findings revealed that in significant increase in the body weight of both sets B and C those outcomes were not partially consistent with the findings of **[32],** who indicated a meaningful increase in the rat body weight after the administration of 300mg/kg for 40 days orally to albino rats.

The slight increase in the body mass in the present research could be explained by that the long-term GBP use may be linked to body mass gain because it may alter the activity of voltage-gated calcium channels in pancreatic β-cells that regulate the release of insulin. This depends on GBP's structure and mechanism of action, which is supposed to modulate neuron signaling by supplying the voltage-dependent calcium channels particular subunit **[33].** The findings were opposite to the results of **[14, 34 , 35].** The current findings showed a meaningful decrease at the dose of 21.8 mg/kg and an insignificant reduction at the dose of 43.6. mg/kg in the kidney weight. The results of the present study were corroborated by research, which claimed that excessive use of gabapentin may result in kidney damage **[32].**

The concentration levels of urea and creatinine in both doses revealed a non-meaningful change. The current outcomes were incomparable to the outcomes of **[36],** who found that gabapentin affects the regulation and flow of blood, in addition to its effect on the filtering and secretion process in the kidney, which causes an elevation in the urea and creatinine concentrations. The current results may be due to the drug concentration and dosing period. the

findings showed insignificant change in the level of cholinesterase of set B but there was an insignificant increase in the same enzyme level of set C. The present outcomes findings were comparable with the findings of **[31].** The slight increase in enzyme level maybe It may be because the drug affects the electrical channels in the brain, as it works too. modify the activity of these channels in the nerve cells, which affects the reception of nerve signals and thus the secretion of neurotransmitters **[37] and** affects the interaction of these transmitters with the nerve cells and their signalling **[38].** in addition to its effect on the secretion of the hormone's aldosterone and renin, which changes the balance of minerals and fluids in the body **[39].**

It is generally accepted that apoptosis triggers tissue dysfunction and that caspase -8 and apopain (caspase -3) are key factors in its development **[40].** The immunohistochemical assessment of the current study in kidneys showed positive and strong positive caspase 3 and caspase-8 at both selected doses, respectively in the basement membrane of the renal tubules. The current outcomes were comparable to those **[23, 41],** who found in contrast to Bcl-2, which had a much lower expression in the brains of gabapentin fetuses, Caspase 3 expression was significantly higher. Researchers found that gabapentin inhibits the levels of caspase-3 in the retinal cells of diabetic rodents **[42, 43].** which is opposite to the present results. The previous findings' appearance may be due to the destruction of the rough endoplasmic reticulum and mitochondria resulted in the release of Cyt c, which then activated caspase-3 and oxidative phosphorylation of caspase-8, initiating an irreversible stage of apoptosis. The over-expression in the caspase- 8 may be due to specific functions for reactive oxygen species in the external apoptotic process that have been discovered and identified. These functions involve facilitating apoptotic activation and death receptor activation via ROS-induced receptor aggregation and forming signalling pathways produced by rafts of lipids **[44].**

The results showed a weak positive and a positive immunoexpression of GAFP in the neuronal axons of damaged neurons in both selected doses respectively. The current findings consisted partially with the findings of **[45],** who found pregabalin reduced spinal GFAP activation and the medication did not lower spinal Iba1 expression. The current results are consistent with the findings of **[46].** The current findings are not consistent with the findings of **[47].** A rise in astrocyte activity corresponds to the removal of the overabundance of dopamine. It is well known that this neurotransmitter can be actively and specifically removed from outside of cells by astrocytes as well as neurons via dopamine transporters. It is then either transported into vesicles or degraded by the chemicals (enzymes) found in glial and nerve cells, namely monoamine oxidase B and catechol-O-methyl transferase **[48].** The increase in the GAFP

expression in the current study may be due to the Excessive dopamine that might promote the process of auto-to ortho-quinone resulting in damage from oxidation **[49].**

5. Conclusions

Gabapentin is a drug used to treat epilepsy and nerve pain. Through the results of the current research, we conclude that using double doses for one month caused pathological lesions in the kidneys and brain. It was observed that the drug induces programmed cell death in the kidney by activating gene expression of caspase proteins in the kidney. The immunohistochemical responses in the treated groups were moderate to severe compared to the control group. Positive responses appeared as a golden brown staining of the cytoplasm of kidney cells. In the brain, the axons of the nerve cells were stained with the same color, indicating excessive production of GAFP protein. This indicates that the drug causes an increase in the gene expression of a GAFP protein. Therefore, you must be careful when taking this drug, and you must adhere to the therapeutic doses specified by the doctor.

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