Comparative study between the effect of first and second generation of anti-epileptic drugs on hepatorenal toxicity in female albino rats

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Abstract:

Sodium valproate (first generation) and oxcarbazepine (second generation) are common antiepileptic drugs used today. The study was conducted to evaluate and compare the toxicity of these two drugs on both renal and hepatic functions test. The experiment was done on 18 female albino rats in Kerbala University /animal house of Pharmacy College and lasted for about two months,and the research was done with the agreement of the animal rights in the college. The rats were divided into three groups, the first group represented healthy animals, second group was drenched orally with 500mg/kg/day of sodium valproate and the third one was drenched orally with100mg/kg/ day of oxcarbazepine. Blood was collected for lab analysis and the hepatic /renal tissues were excised for histological examination. The results showed a significant elevation in liver (AST and ALT) ,as well as parameters such as (urea and creatinine) in both second and third group in comparing with healthy group. histological examination of liver and kidney in animals drenched with valproate showed significant hepatocyte and tubular necrosis while in rats intoxicated with oxcarbazepine showed single hepatocyte necrosis and mild tubular necrosis. In this study, sodium valproate was suggested to have more hepatorenal toxicity than oxcarbazepine and this result was confirmed by both blood tests and histopathological examination.

دراسة مقارنة لادوية الصرع من الجيل الاول والثاني في التأثير السمي للكبد والكلية لإناث الجرذ المختبرية.

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الكلمات المفتاحية: صوديوم فالبروات ,اوكسكاربازبن, جرذان اناث بيضاء,وظائف الكبد والكلية, المقاطع النسيجية.

.الخلاصة :

تعد أدوية الصوديوم فالبروات (الجيل الاول) والاوكسكاربازيبن (الجيل الثاني) من اكثر أدوية الصرع المستخدمة شيوعا هذه الايام . كان الهدف من الدراسة هو مقارنة وتقييم التأثيرات السمية الناتجة عن استخدام كلا الدوائين في وظائف الكبد والكلية. أجريت هذه الدراسة على 18 أنثى من الجرذان المختبرية البيضاء في البيت الحيواني العائد لكلية الصيدلة / جامعة كربلاء واجري البحث بموافقة لجنة حقوق الحيوان بالكلية, وقد استغرقت التجربة مدة شهرين .قسمت الجرذان الى ثلاثة مجموعات المجموعة الاولى عدت كمجموعة السيطرة ,المجموعة الثانية جرعت (500 ملغم /كغم /اليوم من الصوديوم فالبروات بينما المجموعة الثالثة جرعت (100ملغم /كغم / اليوم من بالاوكسكابازبن. جمعت عينات من الدم للتحليل ورفع مقاطع من الكبد والكلية لغرض الفحص النسيجي. اظهرت التحاليل المأخوذة من المجموعتين الثانية والثالثة ارتفاع ملحوظ في نشاط انزيمات الكبد مقارنة مع مجموعة السيطرة.... (Creatinine and urea) والكلية وقد اظهرالفحص النسيجي للجرذان التي تم تجريعها بالصوديوم فالبروات ظهور نخر كبدي وكلوي انبوبي حاد بينما اضهرت العينات للجرذان المجرعة بالاوكسكابازبن نخرا طفيفا على مستوى الكبد والكلية. اثبتت الدراسة ان الصوديوم فالبروات هو الاكثر سمية على مستوى وظائف الكبد والكلية وقد دعمت هذه النتيجة بالتحاليل الدموية والمقاطع النسيجية.

introduction

Epilepsy is a chronic neurological disorder characterized by recurrent epileptic seizures [(1)]; it is not a single existence. Instead it is, a set of different seizure types and syndromes originating from several mechanisms that have in common the sudden, excessive, and concurrent discharge of cerebral neurons. This abnormal electrical activity may result in a variety of events, including loss of consciousness, abnormal movements, atypical or odd behavior, and distorted perceptions that are of limited duration but occur again if untreated[(2)].Sometimes it is caused by damage to parts of the brain which can be result from: difficult birth, brain infection, like meningitis, stroke, and serious brain injury [(3)]. Potentiation of glutamate or using a glutamate receptor agonist has been shown to promote seizure activity, while the use of glutamate antagonists decreases seizure activity [(4)].

Sodium valproate (sodium 2-propylpentanoate) which is the sodium salt of Valproic acid, which is old antiepileptic (first generation) drugs used in the present time. In addition to its wide use in both generalized and partial epilepsies, several new approved indications involving the treatment of bipolar disorders, neuropathic pain,. Several different mechanisms of action reflect this wide spectrum of activities. [(5)]

The mechanism ,it is thought to be related to a direct or secondary increase in concentrations of gamma-amino butyric acid (GABA) which is an inhibitory neurotransmitter [(6)].

Many studies have supplied evidences for ROS generation during SV metabolism, including hydrogen peroxide and hydroxyl radical. [(7)]Many reports demonstrated that SV exposure elevates the formation of lipid peroxidation products in the liver [(8)] and reduce tissue levels of antioxidants, such as glutathione (GSH) [(9)] .the administration of SV in these experiments (intraperitoneal injection for 12 days

provoked hepatic damage revealed by increasing in serum glutamate oxaloacetate transaminases (AST) and glutamate pyruvate transaminases (ALT). Oxidative stress in liver was induced by SV since a decrease in GPx activities and increase in lipid peroxidation which could perforate the biologic membranes, were observed. Histopathological observations including cellular ballooning, microvesicularsteatosis, and hepatic necrosis also correlated with the biochemical parameters and are consistent with previous reports [10]

SV can induce renal tubular damage in children, and there are increasing reports of SV provoked Fanconi's syndrome where the renal tubules lose their ability to reabsorb electrolytes, glucose ,protein and urea .On the other hand, Histopathological changes of kidney tissue exposed to SV reveal the proximal and the distal convoluted tubules that show hydropic changes (small white vacuoles within the cytoplasm) and the glomeruli that appear hypercellularity. [11]

Trileptal:

OXC is a new drug (second generation)with range of anticonvulsant activity , it has an improved pharmacokinetic profile, is better tolerated and is related with few clinically significant drug-drug interactions.[12]

The primary mechanism of action is: blockade of voltage- sensitive sodium channels causing stabilization of hyperexcited neural membranes, inhibition of repetitive neural firing and inhibition of the spread of discharges., it has actions on NMDA receptors, but it has no action on serotonin, GABA or acetylcholine (Ach) receptors. [13]

Excretion of OXC primarily is done by the kidney. In most cases it is recommended to adjust the dosage in patients with Creatinine Clearance <50 mL/min to half the usual first dose and then increase slowly to achieve the required clinical response. The renal function should be monitored frequently in patients receiving therapy. [14]

Most antiepileptic drugs are primarily metabolized by the liver. In patients with liver disease metabolic activity of the liver may be decreased, causing elevated drug levels and increased risk of toxicity. [15].The occurrence of hepatitis is very rare (<1/10000), so that the liver function checking is recommended only when liver disease is suspected [16].

The aim of the study

This study was conducted to assess and compare the effects of sodium valproate (first generation AED) and oxcarbazepine (second generation AED) on both liver and kidney functions at biological and histological levels.

Materials and Methods

Chemicals:

Sodium valproate (Depakine) oral solution was obtained from the Essential Drug Company Sanofi and given orally at dose of 500mg/ kg[17] Oxcarbazepine (Trileptal) oral suspension was obtained from the drug company Novartis pharma AG, and given orally at dose of (100mg/kg/ day[18]).

Lab Animals:

18 females Wistar albino rats (230-250 g) were used and obtained from animal house of Karbala university/pharmacy college which were housed in groups in plastic cages, standard diet, tap water and placed on a 12-hour light/dark cycle for 60day (the experimental period).The animals were randomly divided into three groups, 6 animals per each group.

Experiment design:

1- Control group: was fed with only standard diet, tap water for 2 months.

2- Depakine group: rats were treated with Depakine with a dose of 500 mg/kg daily orally via needle gavage for 2 months.

3-Trileptal group: rats were treated with100 mg/kg Trileptal daily orally for 2 months. At the end of experiment, blood samples were collected from each rat in centrifuge tubes. Serum was separated from coagulant blood by centrifugation and then frozen for biochemical analysis.

Biochemical assay methods: Measurement of serum ALT[19], AST[19], Albumin[22], Creatinine[20], Urea[21].

Histopathological preparations:

The histological samples were preperd according to the procedure of [23]

.Statistic Analysis:

Data were expressed as mean ± SE. Differences between control and other experimental groups were tested for statistical significance using one-way analysis of variances (ANOVA). $P \leq 0.05$.

Results:

In case of rats drenched with SV, the liver (ALT and AST) and kidney (Creatinine and urea) function tests showed the following results; significant elevation in plasma ALT, AST, Creatinine and Urea levels relative to control healthy rats. Plasma albumin levels not significantly changed from the control group.In rats treated with OXC the results were obtained as following; significant elevation (to a lesser extent than SV) in plasma ALT, AST, Creatinine and Urea relative to control healthy rats. Plasma albumin levels not significantly changed from the control group.

Table (1) the effect of antiepileptic drugs (Valproate and Oxcarbazepine) on liver and kidney function of female rats.

parameters / groups	ALT U/L	AST U/L	Albumin g/dl	Creatinine mg/dl	Urea mg/dl
Control	36.5^{a} ±0.61	65.5^{a} ±1.64	3.05 ±0.05	0.226^{a} ±0.01	35.833^{a} ±0.65
Valproate	76^{b} ±3.57	96.5^{b} ±1.72	3.033 ±0.07	0.333^{b} ±0.03	54.166^{b} ±2.48
Oxcarbazepine	44.833^{c} ±1.99	76.5^{c} ±1.91	3.066 ±0.06	0.3^{c} ±0.02	48.833^{c} ±1.30

-Different small letter means significant changing.-a, b, c means significant difference between control, valproate and oxcarbazepine groups.
– $P \leq 0.05$.

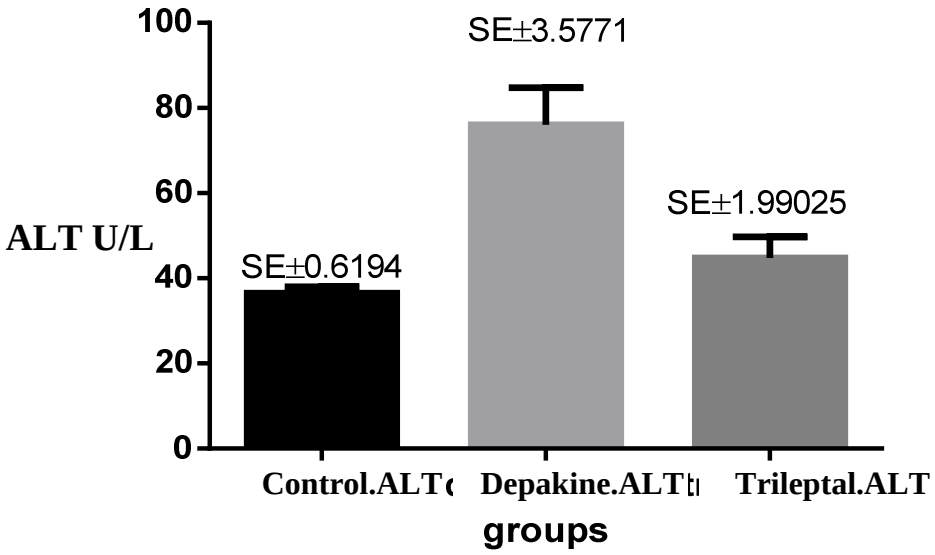

Figure (1) effect of valproate (Depakine) and Oxcarbazepine (Trileptal) on ALT in female albino rats.

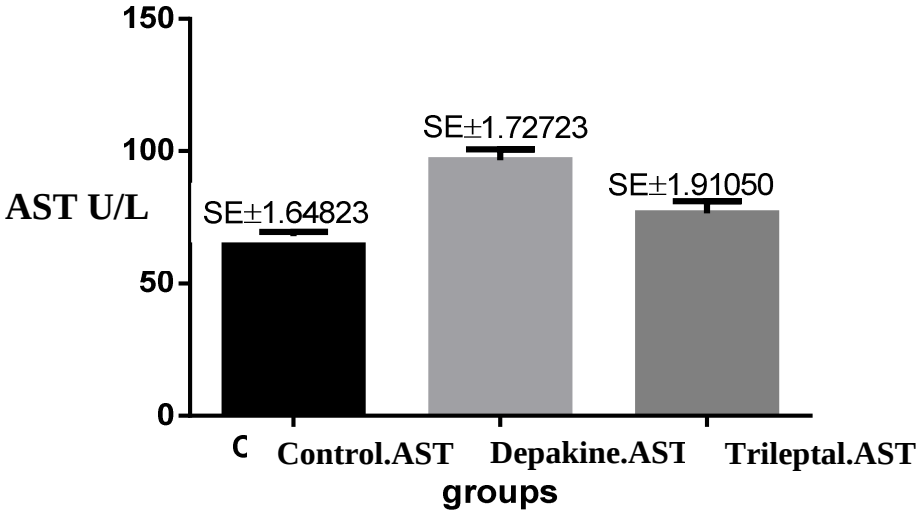

Figure (2) effect of valproate (Depakine) and oxcarbazepine (Trileptal) on AST in female albino rats.

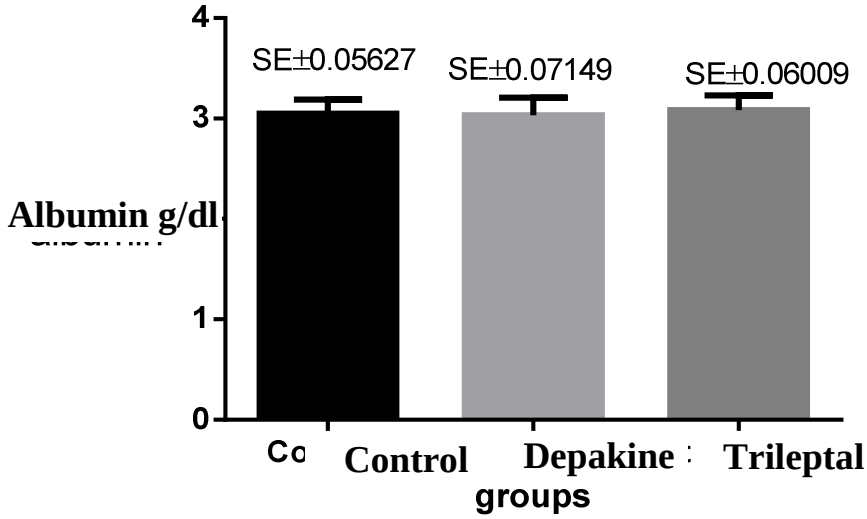

Figure (3) effect of valproate (Depakine) and oxcarbazepine (Trileptal) on albumin in female albino rats.

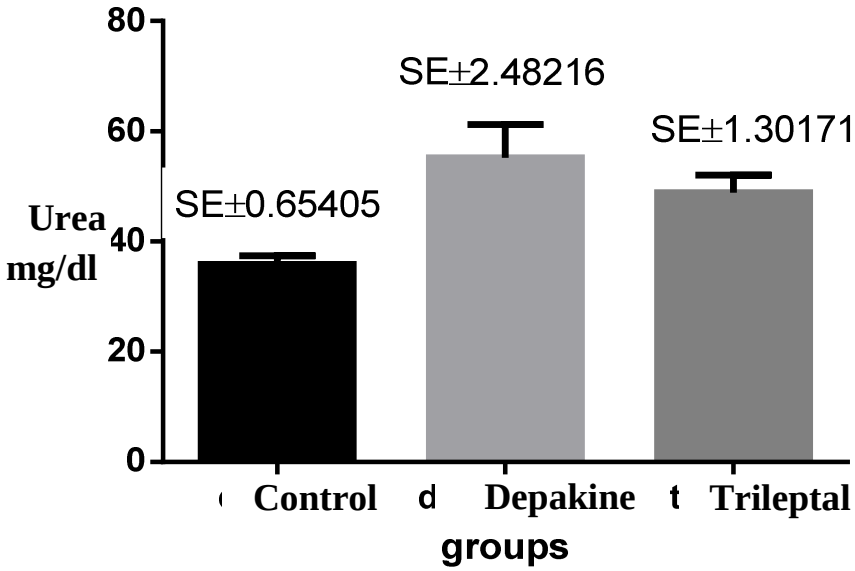

Figure (4) effect of valproate (Depakine) and oxcarbazepine (Trileptal) on urea in female albino rats.

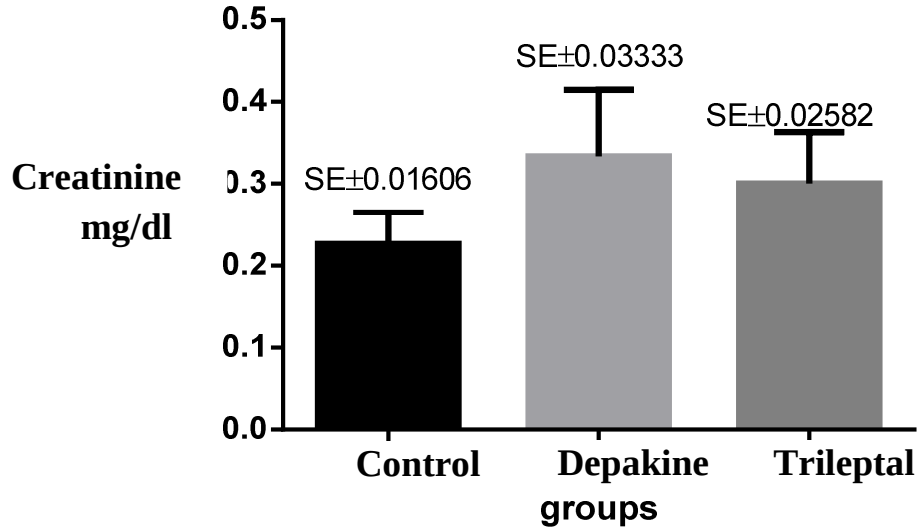

Figure (5) effect of valproate (Depakine) and oxcarbazepine (Trileptal) on Creatinine in female albino rats.

Histological:

On histological examination of control healthy rats the following observation were obtained:

1. **Liver:** normal portal tract morphology and hepatic plate orientation, with normal central nerve vein.

2. **Kidney:** normal medulla and normal cortex.

While in rats treated with SV the following histological alterations were obtained:

1. **Liver:** significant hepatocyte necrosis, degeneration hydropic loss hepatic plate orientation, congestion and focal hemosiderin deposition.
2. **Kidney**: acute tubular necrosis, degeneration, intraluminal debris and sever congestion.

In case of rats treated with OXC the following histological alterations were obtained:

1. **Liver:** diffuse hepatocyte hydropic degeneration and single cell necrosis, mild biliary stasis, no significant inflammation or fibrosis.
2. **Kidney:** diffuse congestion, mild tubular necrosis mainly in proximal tubules, intraluminal debris, no significant inflammation or glomerular change.

1-Control:

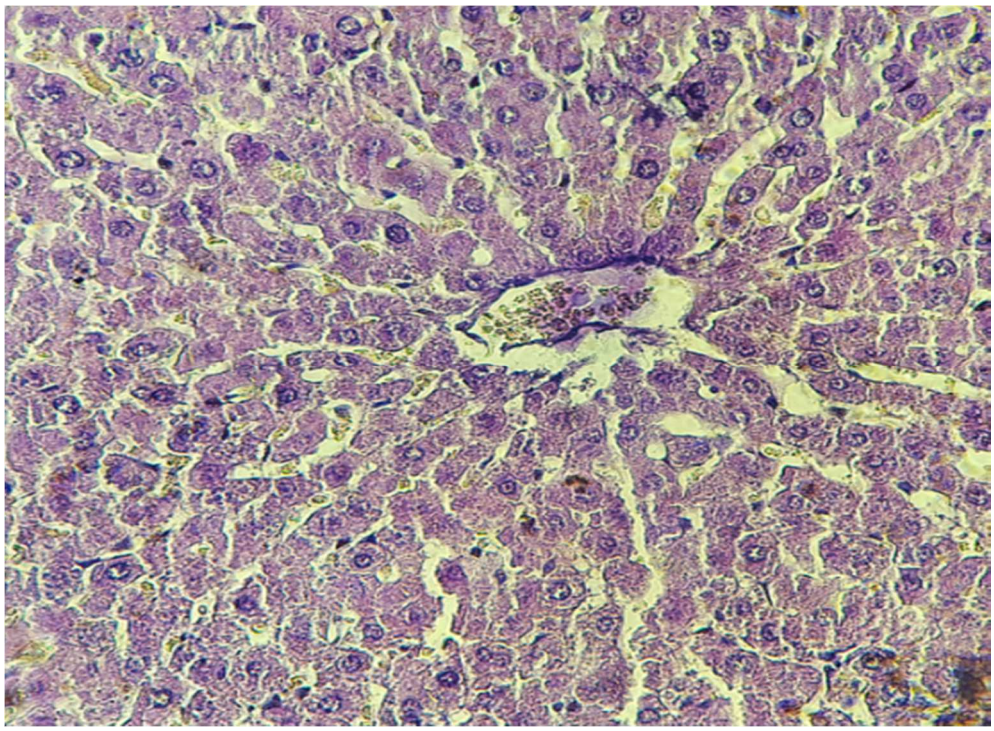

Image (1) transverse section of liver in control group (400X, H&E stain)

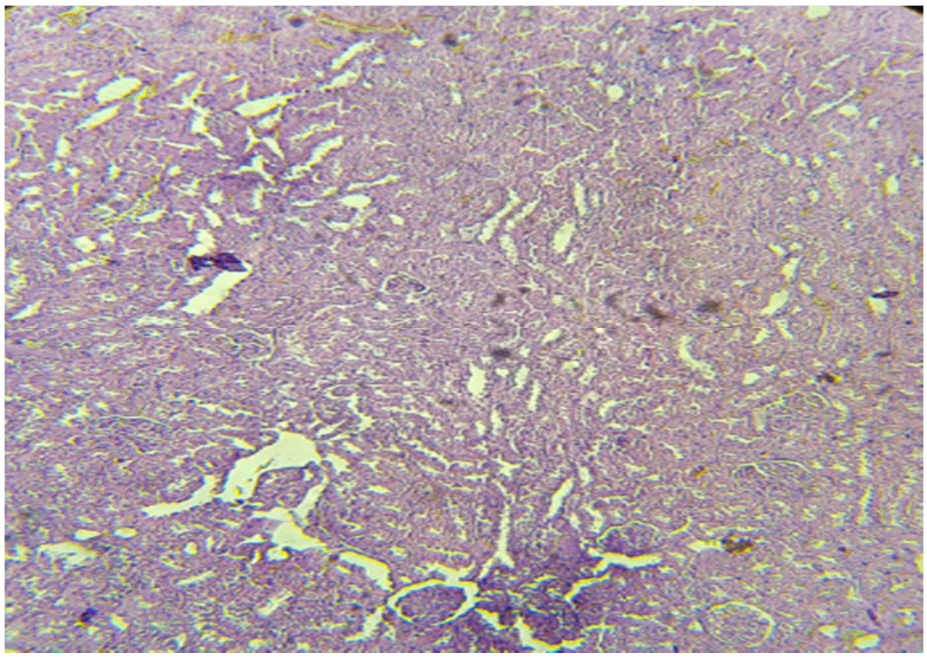

Image (2) transverse section of kidney in control group (400X, H&E stain)

2- Sodium valproate:

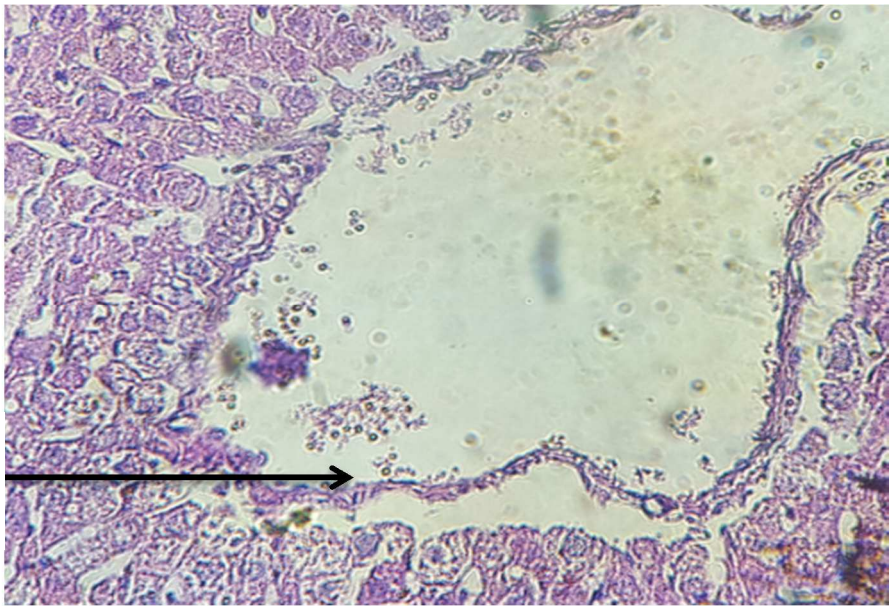

Image (3) transverse section of liver in Sodium Valproate group (400X, H&E stain)

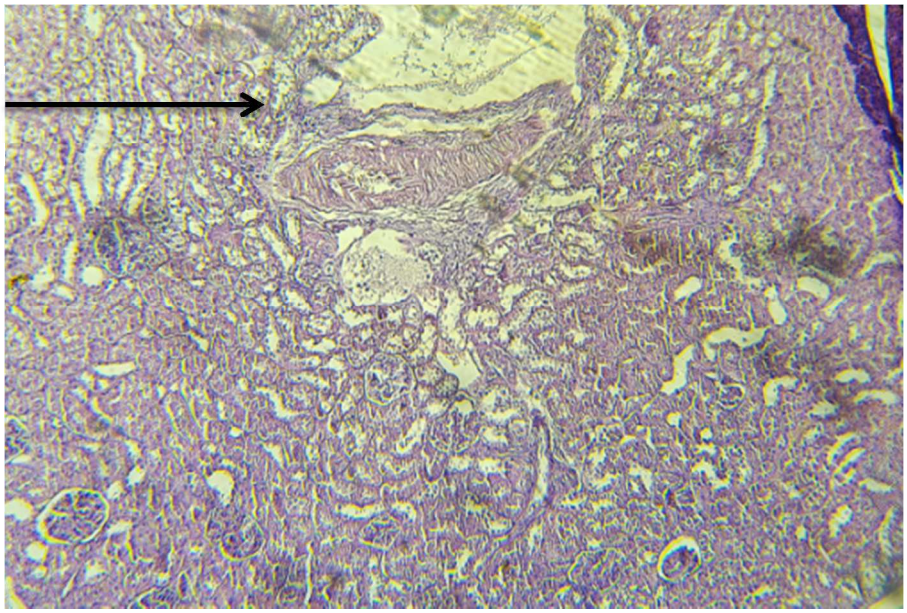

Image (4) transverse section of kidney in Sodium Valproate group (400X, H&E stain)

3- Oxcarbazepine:

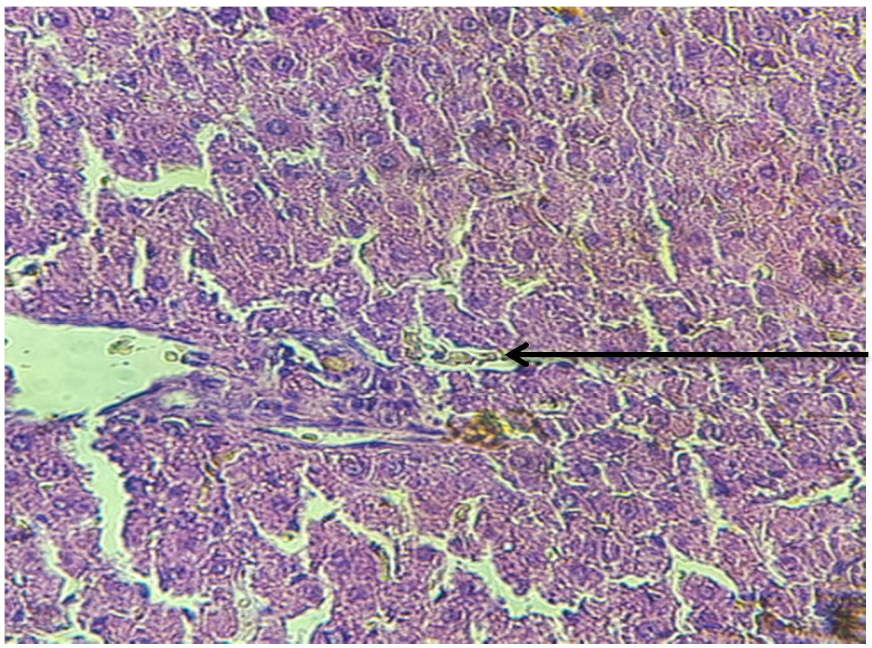

Image (5) transverse section of liver in oxcarbazepine group (400X, H&E stain)

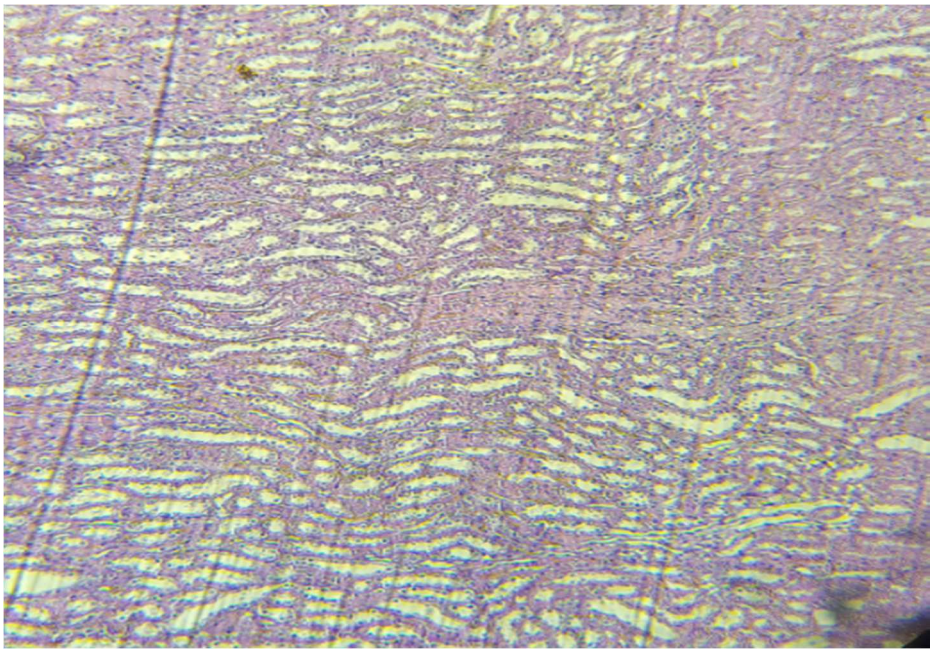

Image (6) transverse section of kidney in oxcarbazepine group

(400X, H&E stain)

Discussion:

The present study was focused on the effects of SV and OXC Antiepileptic drugs on both kidney and liver functions. In case of liver function, the parameters include ALT, AST and albumin activities to check liver function in the treated animals relative to healthy rats.

The results showed that animals treated with SV for two months (experimental period) highly stimulated the activity of AST and ALT in treated rats compared with controll. This stimulation was Dose-dependent and caused gradual damage in liver function.

In agreement with our study, some researchers reported that Valproic acid-mediated hepatotoxic injury is associated with a dose-dependent rise in serum liver enzymes. [24] Moreover, retrospective studies have demonstrated a transient elevation of liver ALT and AST in an epileptic patient on Valproic acid. [25] The hepatotoxicity of SV is suggested to be accompanied with excessive generation of free-radical intermediates, possibly as a consequence of VPA biotransformation [26], alterations in glutathione homeostasis [27], and/or depletion of cofactors required for antioxidant defense. [28]

Rats treated with OXC also showed marked elevation in liver enzymes (ALT and AST) compared with healthy rats. However, this elevation is not as significant as those treated with SV. This is consistent with the finding of many studies which reported that OXC has not been associated with hepatotoxicity except for anecdotal case reports, but it can cause a modest elevation of liver enzymes. [29] The mechanism

of OXC hepatotoxicity appears to be hypersensitivity or an immunological response to a metabolically generated drug-protein complex. [30]

In the study of kidney function, plasma Creatinine and urea levels were significantly higher in SV administered rats than controlled healthy group.

The SV-induced injury was associated with a dose dependent rise in serum renal markers. Altered glomerular filtration rate by SV, could be related to free radical injury since the redox status was affected. [11] The cytotoxic activity of SV is the result of the generation of hydrogen peroxide and the production of highly reactive hydroxyl radicals. [31]

Renal parameters (plasma Creatinine and Urea) were also elevated significantly in rats treated with OXC. However this elevation is less marked than those with SV treated. The results were agreed with the evidence of nephrotoxicity noted in the repeated dose toxicity in rat studies but not in dog or mice studies. [32]

The albumin level in both SV and OXC intoxicated rats wasn't significantly changed from healthy rats.

The histological results of this study showed that SV ingestion produced pronounced hepatic damage include significant hepatocyte necrosis, degeneration hydropic, loss hepatic plate orientation, congestion and focal hemosiderin deposition. These findings were agreement with a study showed that chronic administration of SV produced inflammation of portal tract and necrosis [33], vacuolar degenerative changes, inflammatory cell aggregates and congested vasculature. [34]

Renal tissue also showed acute tubular necrosis, degeneration, intraluminal debris and sever congestion. These finding correspond with the observation of proximal and the distal convoluted tubules that show hydropic changes (small white vacuoles within the cytoplasm) and the glomeruli that show hypercellularity. [11]

In the case of hepatic tissue of OXC treated rats, the results exhibited diffuse hepatocyte hydropic degeneration and single cell necrosis, mild biliary stasis but no significant inflammation or fibrosis. This result was agreed with a minor changes occurred during OXC toxicity such as dilation and congestion of the central vein and blood sinusoids of the liver. [35]

The renal tissue also showed no significant inflammation or glomerular change .However it had diffuse congestion, intra luminal debris and mild tubular necrosis mainly in proximal tubules . All the results in this study has demonstrated that the chronic use of SV is more likely than OXC to induce significant elevation in liver and kidney markers and to produce pronounced Histopathological changes.

Conclusion and recommendations:

1-Sodium valproate was suggested to be more toxic than OXC on kidney and liver functions.

2-Because AEDs treat the symptoms (seizure) and not the cause of epilepsy, epileptic patients need to take AEDs for a long period of time; consequently there is substantial need to develop better and safer AEDs.

3-Oxcarbazepine (second generation) was developed to improve tolerability and pharmacokinetic profile of the first generation AEDs with reducing drug interactions but without reducing the antiepileptic potency.

4-Due to this modification and improvement of AED profile, OXC appears to have less of some of the serious side effects associated with SV (first generation) such as hepatic and renal toxicity.

5-N-acetyl-β-D-glucosaminidase (NAG) as a parameter of renal function is also recommended to be measured in future studies.

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