

Evaluation of Hepatotoxicity of Two Famous Antiepileptic Drugs Depakine[®] and / or Epanutin[®] in Male Albino Mice *Mus Musculus*: Integrated Biochemical and Histological Studies

Nagla Zaky Ibrahim El -Alfy¹, Amany Ibrahim Alqosaibi², Mahmoud Fathy Mahmoud³, Ayman Mohammad Abdullah⁴

¹ Professor of Cytogenetics, ² Professor of Cytology and Histology, ³ PhD Researcher, Department of Biological and Geological Sciences, Faculty of Education, Ain Shams University, Cairo, Egypt, ⁴ Assistant Professor of Molecular genetics, Department of Biology, College of Science, University of Imam Abdulrahman bin Faisal, Dammam, Saudi Arabia

Abstract

Background: depakine and epanutin introduce useful tools in wide range of clinical issues. Liver is the local position for their metabolism and is susceptible for their influences.

Methods: forty-two male albino mice were divided into seven groups. control group injected with NaCl (0.9%) 1ml/kg, group 2 injected with depakine 25mg/kg/day, group 3 injected with depakine 50mg/kg/day, group 4 injected with epanutin 3mg/kg/day, group 5 injected with epanutin 6mg/kg/day, group 6 injected with (depakine 25 + epanutin 3) mg/kg/day and group 7 injected with (depakine 50 + epanutin 6) mg/kg/day. All animals were injected intraperitoneally, were fasted 12hours after last injection, were sacrificed via cervical dislocation and specimens were collected after one and two weeks for each dose.

Conclusions: human therapeutic doses range of depakine and/or epanutin produced elevation in the mean of liver aminotransferases enzymes levels in serum without dose or time depend and generate variable degrees of hepatotoxicity in mice according to dose and depend on time.

Keywords: aminotransferase, epilepsy, liver, phenytoin sodium, valproate.

Introduction

Epilepsy is an illness case targets a considerable percentage of total population without discrimination. It occurs as a result of abruptly and enormously depolarization of some or all encephalic neurons. This impact occurs in some neurons and causes local seizure or extends to all cerebral neurons cause generalized seizure. It is long-life curable disease [1]. valproate exists in several pharmaceutical forms to treat wide range of neurological disorders include epilepsy [2]. It generates several teratogenic effects [3] and psychiatric disorders [4]. Long term administration of valproate has adverse effects on brain mass [5], lower part of gastrointestinal tract [6], urinary system function [7], bone density [8] and liver enzymes [9]. Phenytoin is an effective antiepileptic drug [10], its effective dose differ individually from patient to another due to the polymorphism of hepatic

cytochrome responsible for its hepatic metabolism [11]. Phenytoin causes a group of teratogenic effects with defined phenotypes [12] and it enhances hepatotoxicity if co-administrated with paracetamol and its derivatives [13].

Materials and Methods

Current experiment was carried out on November 2019 at Ain shams university, faculty of education, biological and geological science department, Cairo, Egypt.

Drugs:

Depakine oral suspension 200 mg/1ml sodium valproate from Sanofi Aventis. Epanutin vail 250 mg/5ml phenytoin from El-Nile company for medicine

Industries. Drugs were diluted with saline to prepare all tested doses concentrations with fixed volume of 1ml/kg for each dose. All tested doses were derived from the human therapeutic dose [14].

Animals:

This study was applied on forty-two healthy and pure strain adult male mice (*Mus musculus*), (25 - 30 g) from animal house of research organization, Egypt. Mice were housed inside acrylic cages with base covered with clean sawdust under standard circumstances at 20°C, 12 hours of day/night cycle. Animals were nourished on commercial rodent grain and deionized water. Sawdust was changed daily to get rid of food and feces remnants and avoid contaminations. Mice handling and experimental steps followed the restricted guideline of Ain shams University ethics committee.

Experimental design:

Forty-two mice were equally distributed into seven groups (6 mice per group).

Control: injected with saline 1ml/kg.

2nd Group: injected with depakine 25 mg/kg/day.

3rd Group: injected with depakine 50 mg/kg/day.

4th Group: injected with epanutin 3 mg/kg/day.

5th Group: injected with epanutin 6 mg/kg/day.

6th Group: injected with (depakine 25 + epanutin 3) mg/kg/day.

7th Group: injected with (depakine 50 + epanutin 6) mg/kg/day.

All animals were injected via intraperitoneal route. Mice of all were selected randomly, sacrificed and

samples were collected after one and two week(s) for each dose.

A) Biochemical analysis

Blood serum is extracted after centrifugation of the blood at 2000 xg /10 minutes/ 5°C in tube without anticoagulant. Concentrations of AST (aspartate aminotransferase) and ALT (alanine aminotransferase) liver enzymes in serum were estimated by Randox kits (UK) with applying a specific methodology [15].

B) Histology

liver was isolated after dissection, rinsed with normal saline, fixed in 10% formalin/24 hours, dehydrated in ascending concentrations of ethanol, cleared in xylene, immersed in paraffin at 56°C/24hours, sectioned at 4 microns' thickness, mounted on glass slide, stained by hematoxylin & eosin and examined under light microscope [16].

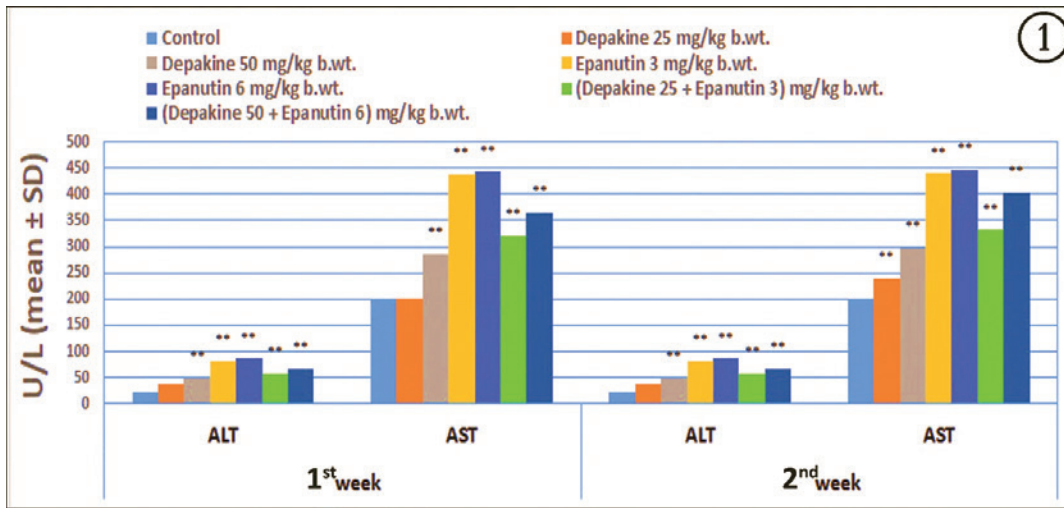
C) Statistical analysis:

Data were analyzed by version 16.0 of (SPSS). Results are expressed by means ± SD of three independent experiment. Statistical significance of difference was determined by T- test. A level of P <0.005 was defined as statistically significant.

RESULTS

A) Biochemical analysis:

As it was shown in figure 1 the mean of ALT or AST enzymes concentrations in serum exhibited high significant increase $P < 0.001$ in all treated groups except in groups that were treated with depakine 25mg/kg/day showed no significant results $P > 0.005$ in ALT concentrations even after one or two weeks of treatment and AST concentrations after one week only in comparison to control group.



** represented a highly significant $P < 0.001$.

Figure 1: Histogram represents the relationship between ALT and AST serum activity and the time of treatment in control group, groups treated with single doses of either depakine or epanutin and groups treated with combination doses of depakine in addition to epanutin.

B) Histological observations:

Hepatic histological alterations of male albino mice were observed in variable degree according to dose and time in all treated groups in comparison to control group. Treatment with depakine 50 mg/kg/day for two weeks resulted in hepatocytes cytoplasmic changes in the form of cloudy swelling figure 2. While, treatment with epanutin 6 mg/kg/day for one week caused hepatic histopathological alterations in the form of inflammation and congestion in central vein figure 3 and after two weeks of treatment with the same dose of epanutin

hepatic histopathological alterations were in the form of hepatocytes nuclear change in the form of pyknosis developed to appearance of large area of necrosis and central vein congestion figure 4. Moreover, co-treatment with (depakine 25 + epanutin 3) mg/kg/day for one week induced hepatic histopathological alterations in the form of congestion in portal vein with inflammation figures 5. While co-treatment with (depakine 50 + epanutin 6) mg/kg/day for two weeks exhibit hepatic histopathological alterations expressed by hydropic degeneration and and nuclear karyomegaly figure 6.

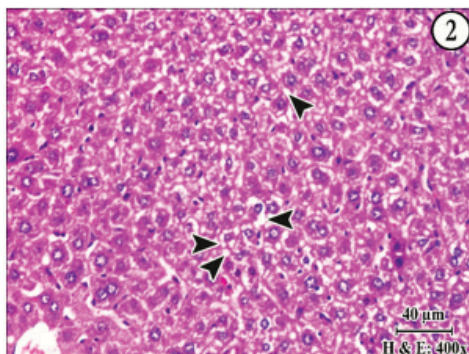


Figure 2: photomicrograph of C.S. in liver of male albino mouse treated with depakine 50mg/kg/day for two weeks showing hepatocytes cytoplasmic changes in the form of cloudy swelling with foamy appearance (arrows heads).

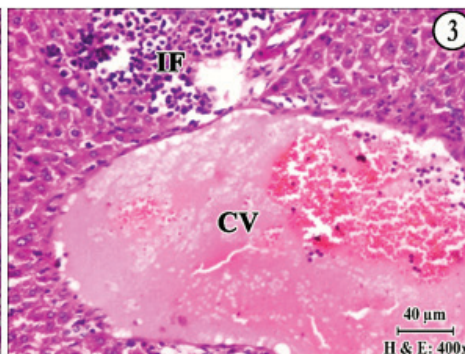


Figure 3: photomicrograph of C.S. in liver of male albino mouse treated with epanutin 6mg/kg/day for one week showing large area of inflammatory cells (IF), extensive congestion and dilation in central vein (CV).

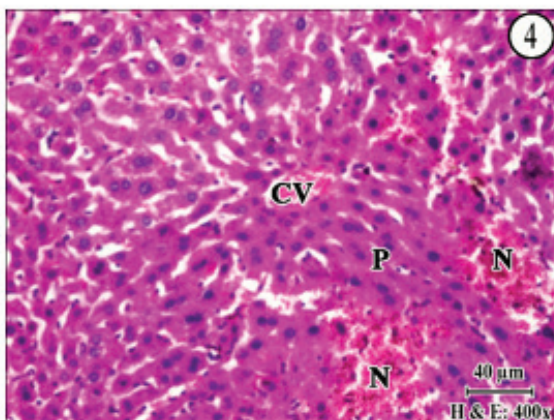


Figure 4: photomicrograph of C.S. in liver of male albino mouse treated with epanutin 6mg/kg/day for two weeks showing congestion in central vein (CV), large area of hepatocytes with pyknotic nuclei (P) and necrosis (N).

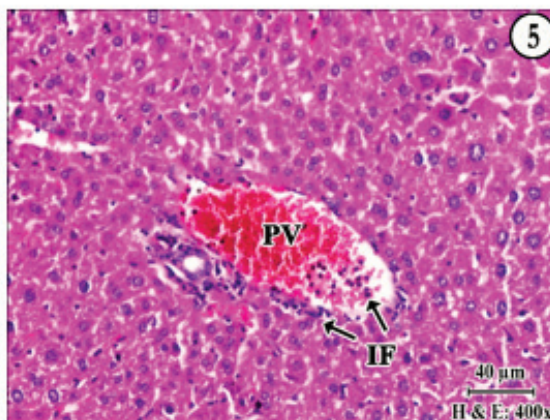


Figure 5: photomicrograph of C.S. in liver of male albino mouse treated with depakine 25mg/kg/day + epanutin 3mg/kg/day for one week showing extensive congestion in portal vein (PV) and inflammatory cells infiltration (IF).

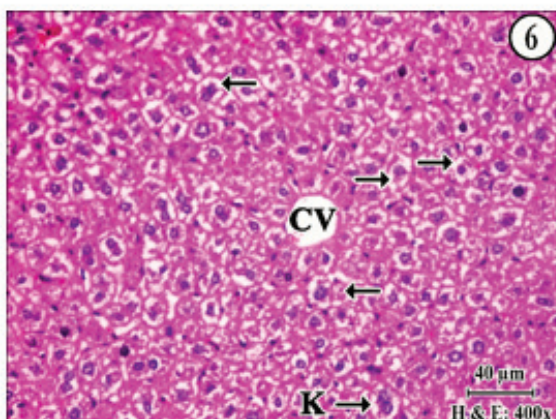


Figure 6: photomicrograph of C.S. in liver of male albino mouse treated with depakine 50mg/kg/day + epanutin 6mg/kg/day for two weeks showing normal central vein (CV), hepatocytes cytoplasmic changes in the form of hydropic degeneration (arrows) and hepatocytes nuclear change in the form of karyomegaly (K).

Discussion

Because liver is the target organ of many drugs metabolism [17], present work gave special attentions to hepatic histopathological changes in male albino mice resulted from treatment with depakine and / or epanutin to explain the results of ALT and AST concentrations in blood serum which are normally situated inside hepatocytes [18]. Current study observed that treatment with depakine and/or epanutin resulted in variable degree of histopathological alterations in the liver of male albino mice depend on dose and time compared to control group. Hepatic histopathological changes in depakine treated groups were in accordance with Ibrahim, (2012) [19] found that depakine induced reversible hepatotoxicity in albino mice depend on time. Hepatic histopathological changes in epanutin treated groups

were agreed by Saraswathy *et al.*, (2015) [20] declared that phenytoin causes injury in rat liver indicated with significant increase in concentrations of liver enzymes in serum. In the present work hepatic histopathological changes in groups treated with depakine and epanutin together are due to the toxic metabolites of either depakine [21] or immunoreactions of epanutin [22] in consideration with that depakine inhibits epanutin metabolism by removing it from the plasma binding site [23]. Inflammatory reactions that were observed in all treated groups are due to oxidative stress [24] which produced after the hepatic metabolism of either depakine [25] or epanutin [26]. Finding of biochemical analysis is occurred because inflammatory cells secret lipolytic enzymes that have a destructive effects on phospholipids of hepatocytes membrane [27] causes extracellular leakage of hepatocytes cytoplasm includes

aminotransferase enzymes. In addition, inflammatory reactions cause hepatocytes membrane permeability dysfunction through inhibition of energy metabolic enzymes [28] caused abnormal intracellular entrance of sodium ions with water and extracellular leakage of potassium ions resulted in observed cloudy swelling and hydropic degeneration in hepatocytes cytoplasm. In epanutin treated groups appearance of necrosis in the form of dark eosinophilic areas within parenchyma is due to the oxidative stress generates intracellular membranes damage and leakage of hydrolytic enzymes resulted in digestion of cytoplasmic contents [29]. Also, the oxidative stress resulted in hepatocytes nuclear changes in the form of karyomegaly and pyknotic nuclei through inhibition of spindle fiber formation and block anaphase [30] resulted in karyomegaly that is described as polyploidy [31]. also oxidative stress induces chromatin condensation and pyknosis [32]. In present study all treated groups showed variable degree of congestions in all parts of hepatic vascular system even portal vein or drain part of blood sinusoid and central vein. These congestions are due to the direct degenerative impacts of oxidative stress on the endothelial wall of hepatic sinusoids [33] leads to congestion in blood sinusoid extended to central vein [34] previous conditions collectively act as an obstacle for portal vein perfusion and portal congestion [35].

Conclusion

Administration of therapeutic doses of depakine drug and / or epanutin drug adversely induced hepatic injuries in male albino mice *Mus musculus* by dose and by time for each single or combination doses. It is recommended to utilize in human patient under restricted precautions.

Ethical Clearance: Taken from Ain shams University ethics committee.

Source of Funding: Self.

Conflict of Interest: Nil.

Refeerances

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