Evaluation of Hepatotoxicity of Valproic acid in albino mice, Histological and Histoistochemical studies

Ibrahim,M. A

Department of zoology, Faculty of Science, Helwan University
Basma_rw@yahoo.com

Abstract: Background: Epilepsy is an abnormal functional state of the central nervous system that is characterized by uncontrolled nerve cell activity and clinicaly by convulsive seizure with or without loss of consciousness. Valproic acid is widely used antiepileptic medication and has sever toxic effect on liver , this study aimed to investigate the histopathological and histochemical changes due to the effects of therapeutic dose (25mg/kg b.w.) of antiepileptic drug (Valproic acid) in albino mice. **Material and methods:** Forty mice were used, they were divided into four groups, one group serves as control group and the other three groups (A, B and C) administered the drug as follow: Group A received Valproic acid for 15 days, group B received Valproic acid for 30 days and group C, the recovery group (animals were administrated with drugs for 30 days then administration was stopped for another 10 days). The animals were sectioned 24 hours after the last dose, liver was taken for histopathological and histochemical studies. **Results**: The drug induces toxic effects on liver tissue which showed vacuolar degenerative changes, hypertrophied nucleus with fragmented chromatine, inflammatory cells aggregates and congested vasculature.**Conclusion:** Valproic acid has sever toxic effects on liver tissue and the toxicity was time related. Sort of recovery was recorded after discontinuation of the drug so its effect was reversible.

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Key words: liver- tissue- valproic acid- hepatotoxicity.

1. Introduction

Epilepsy affects 5 -10 per 1000 of the general population. It is due to sudden, excessive depolarization of some or all cerebral neurons. This may remain localized (focal seizure) or may remain generalized seizure, it may also affects all cortical neurons. Bromide (1857) was the first treatment of epilepsy, but it use now obsolete **(Bennett *et al*., 2003).**

Valproic acid is a branched –chain carboxylic acid similar in structure to endogenous fatty acid **(Bruni and Albright, 1993).** It was approved for treatment of epilepsy either as monotherapy or in combination with other anticonvulsant drugs **(Beatalden *et al*., 1979).** It is also used in the treatment of a variety of neuropsychiatric illnesses as mania, bipolar affective disorder, migraine, headache, prophylaxis and several anxiety disorders **(Owens and Nemeroff, 2003).** It elevates the whole brain gamma amino butyric acid (GABA) a major inhibitory neurotransmitter in the brain, and inhibits GABA degradation. **(DeVane, 2003).** Some severe side effect associated with Valproic acid treatment as encephalopathy which can be accompanied by hyperammonemia**, (Schmidt, 1984)** other side effects as Hepatotoxicity, thrombocytopenia, platelet aggregation and pancreatitis **(Kis *et al*., 1999).**

In **(1991) Siemes and Nau** found that the sever hepatotoxicity associated with Valproic acid is due to depletion of β-oxidation with increased synthesis of toxic unsaturated Valproic acid derivates. Another hypothesis lies in the possible Valproic acid induced depression of free radical scavenging enzyme activities.

Raza *et al*. (2000) studied the effect of Valproic acid on mouse liver, they found that this drug caused marked alteration in liver cell morphology, which was proportional to the period of treatment. Valproic acid induces fatty degeneration of hepatocyte, swelling and increased number of kupfer cells. Prolonged time of administration produce inflammation of portal tract, albumious degeneration and necrosis of septa, .precirrhotic condition, cirrhosis, degeneration of hepatocyte and glassy eosinophilic homogenous cytoplasm. When the time of administration increase the portal tract invaded by small, rounded inflammatory cell, hepatocytes were swollen with large nuclei and increased amount of condensed chromatin.

It was found that steatosis and necrosis of hepatocyte are the main histopathological changes in liver of albino rat induced by toxic dose of Valproic acid**. (khan *et al,* 2005).**

EL-Deeb, (2006) studied the effect of Valproic acid on the exocrine part of pancrease when used alone and when given concomitantly with L-cartnitine. When animals treated with Valproic acid alone the result obtained, showed variable degree of acinar degeneration and cellular infiltration between the acinar. The collagen fibers around the blood vessels were increased. Ultrastucturally, there were dilation of rough endoplasmic reticulum and Golgi appretus, focal destruction of mitochondria, increased number of secondary lysosome, decreased or even depletion of

zymogene granules and nuclear changes. Fat droplets in the basal part of acinar cells, cytoplasmic vacuoles as well as DNA damage were also noticed. On the other hand, pancreatic section of animals that were treated with Valproic acid and L- carnitine showed marked reduction of the pervious cellular change and cytoplasmic organelles were slightly affected. So L–carnitine minimizes the adverse effects of Valproic acid on the exocrine part of pancrease.

According to **Khera (2005),** the teratogenic effects of Valproic acid on rats, was in form of abortions, reduction in the number of live fetuses and defects of the tail, rib phalanx, cytotrophoblasts and suppressed proliferation of fetal capillaries, reduced diameter nearing obliteration of umbilical vessels with or without karyorrhexis of embryonic tissue .the lesion in the placental labyrinth were specific but in the embryonic tissues they were generalized. It was postulated that the vascular lesion in the labyrinth and umbilicus may have influenced embryonic development by reducing maternoembryonic gaseous and nutritional exchange.

As Epilepsy is an abnormal functional state of the central nervous system that is characterized by uncontrolled nerve cell activity and clinicaly by convulsive seizure with or without loss of consciousness. Valproic acid is widely used antiepileptic medication, the aim of this work is to investigate the toxicity of Valproic acid on liver tissue .The drug was stopped for a period to study the recovery possibility of this toxicity.

2. Materials and methods:

Drug used:

Valproic acid is a white hydroscopic, crystalline powder very soluble in water. Each 100 ml depakine syrup contains 5.7 gm sodium Valproate. Produced by Global Napi pharmaceuticals Egypt. The experimental dose used in this study is the therapeutic dose of Valproic acid (25) mg/kg b.w. This dose was converted using conversion equation between different species **(Paget and Bernes, 1964).**

Experimental animals:

Forty adult female albino mice, each weighing 25-35 gm. were kept in the laboratory under standerdized condition of temperature and were maintained on a standerd diet and water *ad libtium* for at least one week before the expermintal study. They were divided into four groups as follow:

Group 1: The control group consists of ten mice.

Group 2: Ten animals were treated intraperitoneal with Valproic acid for 15 days.

Group 3: Ten animals were treated intraperitoneal with Valproic acid for 30 days.

Group 4: Ten animals were treated intraperitoneal with Valproic acid for 30 days then left to recover for another 10 days.

Methods:

1- Histopathological preparations:

Animals were sacrified by cervical dislocation, dissected and small pieces of liver were fixed, processed for light microscopic examination, sections were stained with Ehrlish H&E **(Bancroft and Gamble, 2002).** The cytoplasm appeared pink and nuclei acquire a blue color.

2- Histochemical preparation:

a- PAS-Reaction

The Periodic acid reaction (PAS), **(Hotchkiss and Marmu, 1954)** as applied for carbohydrate demonstration Fixation was carried out in 10% neutral buffered formalin, Paraffin section (5um thick) were brought down to water then placed in 1% periodic acid for 5 minutes, washed for minutes in running water, rinsed in distilled water and treated with Schiff's reagent for 20 minutes Section were then transferred through freshly prepared 0.5 % sodium bisulfate for 3 changes, followed by 5 minutes in running tap water, dehydrated, cleared in xylol and mounted in DPX. The positive materials appeared pink.

b-Total proteins:-

The total proteins were demonstrated by using the mercuric bromophenol blue method (BPhb) **(Mazia *et al*., 1953).** Small pieces of tissue were fixed in neutral 10 % buffered formalin. Staining was carried out in mercuric bromphenol blue stain and differentiated in 0.5% acetic acid. Tertiary butyl alcohol was used for dehydration the sections were then cleared in xylol and mounted in DPX Proteinic substance acquired deep blue color.

3. Results

1-Histopathological results

Control group:

A cross section of normal liver of mice shows that the hepatocytes are arranged in cords or plates, one or two cell thick, forming the normal liver cords radiating from the central vein. The space lying between the hepatic cords constitute hepatic sinusoid, which are lined with flattened endothelial cells and few phagocytic cells namely Von Kupffer cells. The nuclei of the later are spindle shape. The cytoplasm of the hepatic cells is pink in color with scattered basophilic granules. The nuclei of the hepatic cell are rounded in shape with granular chromatin material, some hepatocytes are binucleated. (Fig.1).

Treated groups:-

Histopathological examination of liver section in tissue of animals treated with Valproic acid for 15 days, revealed partial distortion of liver architecture, accompanied with vacuolar degenerative changes seen focally in hepatocytes.

Focal areas of necrosis could be also detected (Fig.2) scattered focal aggregates of inflammatory cells seen in portal areas(Fig.2&3) and between hepatocytes. (Fig. 4). Congested portal vein (Fig.5) with enlarged atypical cells could be recognized in areas. (Fig.6)

When animals were treated with Valproic acid for 30 days section in liver tissue showed, marked distorted hepatic architecture, In addition to scattered multifocal necrotic areas also were detected. Majority of hepatocyte showed vaculation, (Fig.7) accompanied with variation in nucleus regarding to size and shape, hypertrophied nuclei with fragmented chromatin could be also seen.(Fig.8) normal mitotic figure were occasionally seen (Fig.9)

Sections in liver of mice treated with Valproic acid for 30 days then left for 10 days to recover showed mild improvement where normal lobular architecture could be identified in areas, hepatocytes were intact with vesicular nuclei. However mild focal necrosis, with congested dilated central vein was observed in areas, in addition to hepatocellular vaculation. (Fig.10)

2-Histochemical Studies

1- Polysaccharide:-

Control:-

In the liver section obtained from control mice, a markedly strong PAS reaction was clearly illustrated indicating their richness in polysaccharide. The reaction product was demonstrated in the form of intensely red color particles condensed together into massive patches located exclusively in the cytoplasm of the individual hepatocytes, since the nuclei have not acquired any PAS positive reactivity. Such reactivity was comparatively stronger in the peripheral than the inner (centrilobular) areas. (Fig.11).

Treated groups:-

Inspection of the polysaccharide content in the liver tissue of Valproic acid treated group for (15 days) revealed mild to moderate loss of their content, where polysaccharide content appeared as small aggregates of fine granules dispersed in the cytoplasm of hepatocytes. Longer duration of Valproic acid administration (30 days) produced complete depletion of polysaccharide content. After 10 days recovery, revealed no significant restoration of polysaccharide content, however with differences within liver areas, where focal areas of hepatocytes appears with traces of polysaccharide.(Fig.12&13&14).

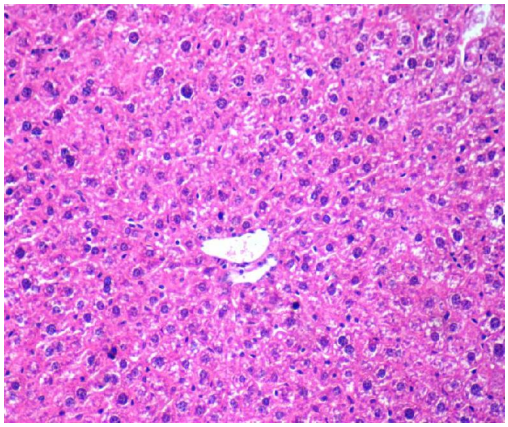
2-**Total protein:-**

Control group:-

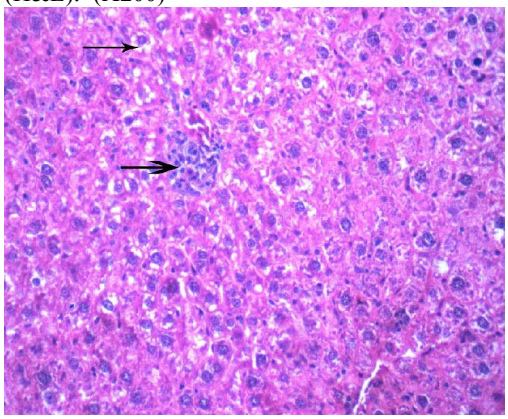
Using the mercuric bromophenol blue method, the protein content of liver cells of normal mice was demonstrated as numerous irregular blue particles against a lightly stained cytoplasm. These particles were scattered in all the cytoplasmic regions. The cells were limited by intensely stained plasma membranes. The nuclear envelops, the nuclei as well as some chromatin element were also positively stained. (Fig.15).

Treated groups:-

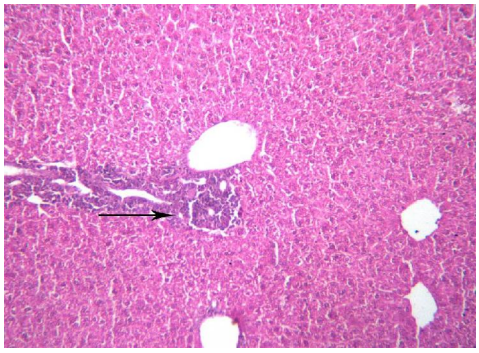
In Valproic acid treated mice, the liver manifest, mild to moderate loss in the protein content in their hepatocyte the loss was time dependent, where 30 days treatment induce further loss than 15 days treated time, whereas the group of animal treated for 30 days, followed by 10 days recovery showed restoration to great extant, in 15 days group, the cells membrane, nuclear membrane and nuclei attain high staining affinity, while some hepatocytes showed mild diffuse portentous material or few deeply stained large particles, meanwhile in 30 days moderate stain affinity were noticed in cell membrane, nuclear membrane and nuclei. (Fig. 16 & 17 & 18).



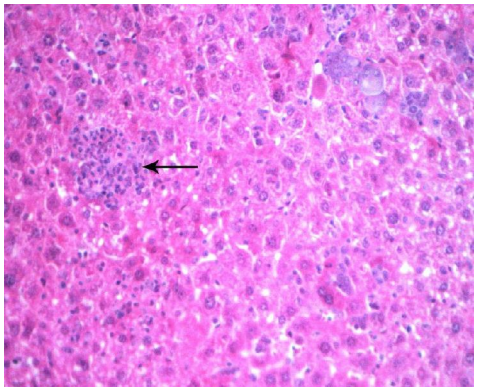
Fig(1):A photomicrograph of liver section in control animal showing normal lobular architecture (H&E). (X200)



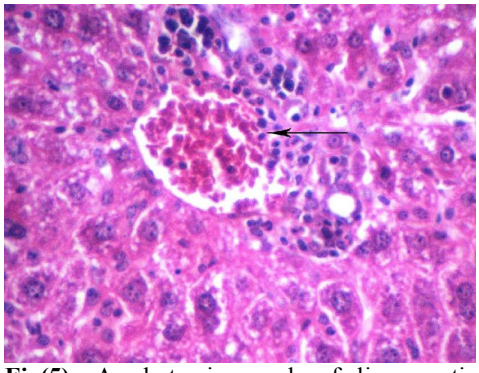
Fig(2): A photomicrograph of liver section in animal treated with Valproic acid for 15 days showing vacuolated hepatocyte (thin arrow) focal necrotic area (thick arrow) partial distortion of liver architecture (H&E). (X200)



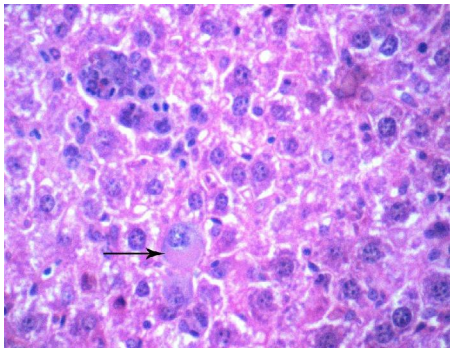
Fig(3): A photomicrograph of liver section in animal treated with Valproic acid for 15 days showing focal inflammatory cell aggregates in portalarea(arrow) (H&E). (X100)



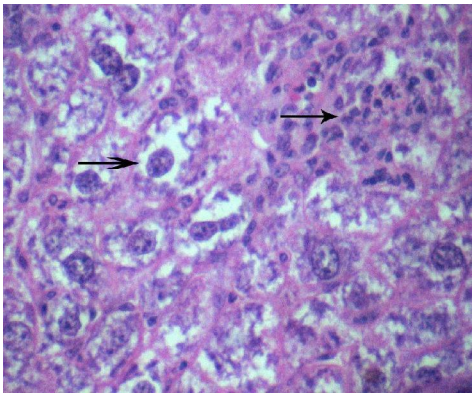
Fig(4): A photomicrograph of liver section in animal treated with Valproic acid for 15 days showing focal inflammatory cell aggregates between hepatocytes area (arrow) (H&E). (X200)



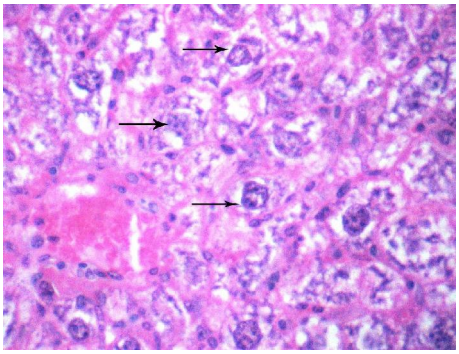
Fig(5): A photomicrograph of liver section in animal treated with Valproic acid for 15 days showing dilated congested portal vein (arrow) (H&E). (X400) (X400)



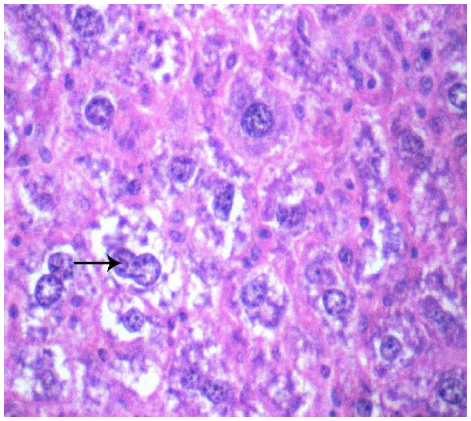
Fig(6): A photomicrograph of liver section in animal treated with Valproic acid for 15 days showing enlarged atypical hepatocytes (arrow) (H&E). (X400)



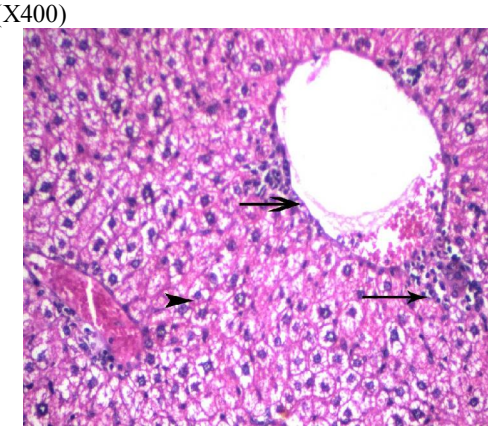
Fig(7): A photomicrograph of liver section in animal treated with Valproic acid for 30 days showing focal necrotic area (thin arrow), vacuolated hepatocytes (thick arrow) and marked distortion of liver architecture (H&E). (X400)



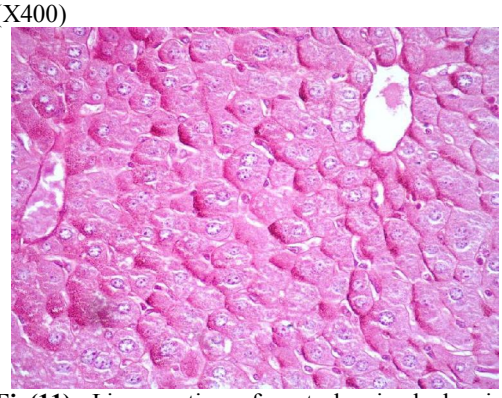
Fig(8): A photomicrograph of liver section in animal treated with Valproic acid for 30 days showing variation in nuclear size and shape,(thin arrow) enlarged nucleus with fragmented chromatin (thick arrow) (H&E). (X400)



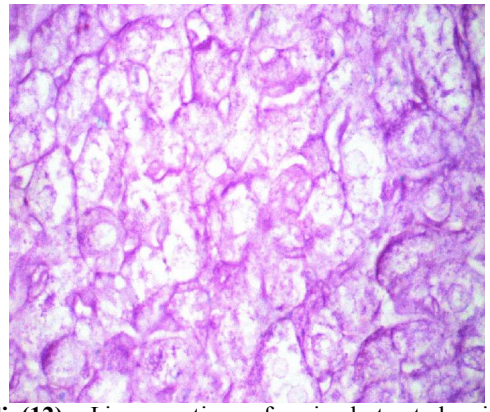
Fig(9): A photomicrograph of liver section in animal treated with Valproic acid for 30 days showing normal mitotic figure (thin arrow)(H&E). (X400)



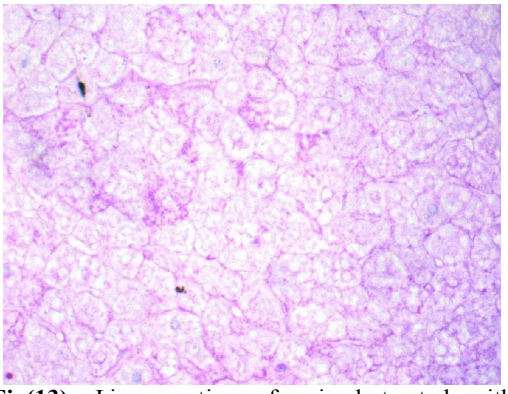
Fig(10): A photomicrograph of liver section in animal treated with Valproic acid for 30 days then left to recover for 10 days showing normal liver architecture, mild necrosis (thin arrow), dilated congested central vein (thick arrow) and vacuolated hepatocytes (arrow head)(H&E). (X400)



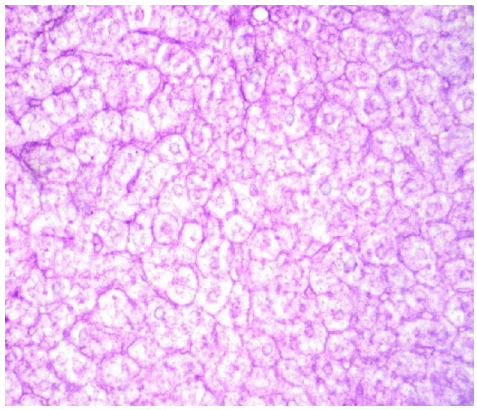
Fig(11): Liver section of control animal showing normal polysaccharide content in hepatocytes (PAS) (X400)



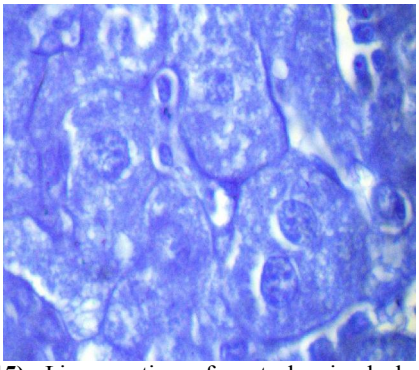
Fig(12): Liver section of animal treated with Valproic acid for 15 days showing moderate loss in polysaccharide content in many hepatocytes. (PAS) (X400)



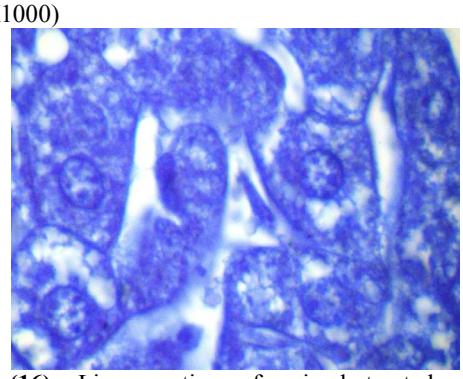
Fig(13): Liver section of animal treated with Valproic acid for 30 days showing sever loss in polysaccharide content in liver hepatocytes (PAS) (X400)



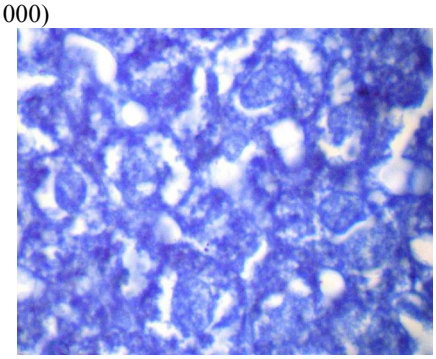
Fig(14): Liver section of animal treated with Valproic acid for 30 days then left for 10 days to recover showing sever depletion in polysaccharide content in most of hepatocytes (PAS) (X400)



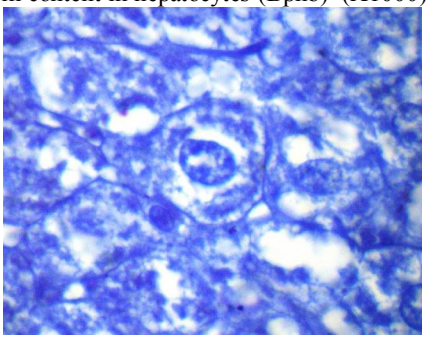
Fig(15): Liver section of control animal showing normal protein content in hepatocytes(Bphb). (X1000)



Fig(16): Liver section of animal treated with Valproic acid for 15 days showing mild loss in protein content In hepatocytes (Bphb). (X1000)



Fig(17): Liver section of animal treated with Valproic acid for 30 days showing moderate loss in protein content in hepatocytes (Bphb) (X1000)



Fig(18): Liver section of animal treated with Valproic acid for 30 days then left for 10 days to recover showing mild loss in protein content in hepatocytes (Bphb). (X1000)

4. Discussion:

1. Histological studies

The liver tissue of Valproic acid treated mice showed histopathological changes which include partial to sever distortion of liver architecture, congested vasculature, scattered to multifocal necrotic areas with focal aggregates of inflammatory cells, vacuolar degenerative changes, enlarged atypical hepatocytes, also variation in nucleus regarding to size and shape and hypertrophied nucleus with fragmented chromatin. However after a period of recovery mild improvement, where normal lobular architecture could be seen, with massive vacuolated hepatocytes.

These results were in agreement with the finding of Khan *et al*., 2005who reported the histopathological changes of toxic dose of Valproic acid in liver of albino rats, these changes represented in partly distorted in the lobular architecture, in addition to foci of inflammatory cell infiltrate focal necrosis and congestion in the portal areas. It was postulated that Valproic acid aberrant metabolites or mediation of lipid per-oxidation might be the underlying mechanism of serious hepatic reaction.

Vacuolar degenerative changes and enlarged nucleus with condensed clumped chromatin material in hepatocytes due to administration of Valproic acid in our study have also been reported by Raza *et al*., 2000who found that when mice given sodium Valproate 0.71% weight / volume in drinking water for 7, 14, and 21 days caused alteration in liver cell morphology, in the form of degeneration of hepatocytes and glassy eosinophilic homogenous cytoplasm, also hepatocytes were swollen ,with large nuclei and increased amount of condensed chromatin. These changes were proportional to the period of treatment, so prolonged use of this drug should be carefully assessed.

After 30 days administration of Valproic acid, hepatocytes revealed variation in shapes and size of nucleus. This may be confirmed by Isharwal *et al.,* 2009 who reported that Valproic acid treatment caused significant nuclear alterations in normal drug-filtering organs (liver and kidney tissue), this due to the fact of Valproic act as Histone deacetylase inhibitors and promising anticancer agents that change the acetylation status of histones and loosen the chromatin structure.

Wiegand *et al*., (2009) reported that Valproic acid may cause impairments in fatty acid metabolism and disrupt the urea cycle leading to hyperammonemia which is considerd as marker of liver disease.

2-Histochemical Studies

1-polysacchride:-

In the liver tissues, carbohydrate inclusions have been proved to be almost formed of glycogen.

These inclusions were markedly reduced as a consequence of Valproic acid treatment, being more pronounced with increase time of administration, after recovery period there were mild restoration of carbohydrate content in the liver tissue.

These results were in agreement with the results of (Turnbull *et al*., 1983), who found that Valproate administration inhibit gluconeogenesis by 30-50% in isolated rat hepatocytes lead to decrease glucose deposition in hepatocytes, this was explained in terms of the accumulation of Valproyl –CO A and its further metabolites in the matrix of hepatic mitochondria .

Similar results were obtained by Thurstone *et al*., 1985, who reported that even a single dose of Valproate in the therapeutic range for man caused significantly reduction of the plasma glucose concentration.

The present results were agreed to great extent with the result of Kesterson *et al*. (1984) who found that PAS positive material was not observed in the hepatocytes of the rats treated with Valproic acid and its metabolites.

2-Total protein:-

The present study indicated that protein content was depleted significantly in liver tissue and this depletion was directly proportional with duration, but after recovery period there was restoration of protein content.

The pervious changes in the liver tissue may be coincide to great extent with the result of Thurstone *et al*., 1985 who observed that concentration of aspartate, glutamate and glutamine (amino acids) were reduced. All of this changes induced by valproate metabolite which can be explained by decrease of acetyl CO-A due to the accumulation of acid –soluble (non-acteyl) CO-A esters which would limit the activities of one or more enzymes in the pathway of fatty acid oxidation and the Krebs citric acid cycle. Decreases in acetyl Co-A would limit both ketogenesis and gluconeognesis.

It can be concluded that the administration of Valproic acid produce hepatic injury. The results suggest that histopathological and histochemical changes are dependent upon the duration of exposure to the drug so prolonged use of these drugs should carefully assessed.

All described changes induced by Valproic acid, were partially reversible, so kind of recovery could be detected after discontinuation of these drugs

Corresponding author
Ibrahim,M. A
Department of zoology, Faculty of Science, Helwan University
Basma_rw@yahoo.com

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