Evaluation of Dose Related Structural Changes in Sodium-Valproate-Induced Hepatotoxicity and a Possible Protective Role of Vitamin E in Adult Albino Rats

Original Article

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ABSTRACT

Background: Sodium valproate (SV) is a widely administered antiepileptic drug, although hepatotoxicity is a side effect. Vitamin E (vit. E) being a potent antioxidant agent and essential fat-soluble nutrient that can dramatically decreased this hepatotoxic effect.

Aim of the Work: To investigate the histopathological and ultrastructural changes caused by different doses of SV, observe their correlations with liver biomarker levels and assess the defensive role of vit. E against SV-hepatotoxicity.

Methods: Sixty male adult albino rats were randomly divided into six groups. Group 1 was treated with normal saline. Group 2 was treated orally with vit. E (100 mg/kg/day). Groups 3, 4 and 5 were treated intraperitoneally with SV at doses of 100, 300 and 500 mg/kg/day, respectively, for 8 consecutive days. Group 6 was treated intraperitoneally with SV (500 mg/kg/day) and orally with vit. E (100 mg/kg/day) for 8 consecutive days. On the ninth day, blood samples were collected to assess the biochemical markers of the liver, and the results were statistically analysed. The rats were deeply anaesthetized and sacrificed. Liver specimens were carefully dissected, and portions were fixed in 10% formalin solution for histopathological examination; others were fixed in 2.5% glutaraldehyde for ultrastructural study.

Results: The liver function among the different groups was found to be significantly changed in dose dependent manner. Histopathological examination showed gradual distortion of hepatic lobular architecture and infiltration of lymphocytic cells. Concerning hepatocyte ultrastructure, SV was a destructive compound for most intracellular organelles. This toxicity was most obvious in the groups treated with higher doses; however, concurrent administration of vit. E with SV provided some hepatoprotection.

Conclusion: SV is a destructive compound to the liver architecture, especially in high doses. However, SV damage can be attenuated by concurrent administration of vit. E, which considerably decreases most SV-induced hepatotoxicity.

Received: 29 Septmber 2019, **Accepted:** 07 November 2019

Key Words: Hepatoxicity, rats, valproic acid, vitamin E.

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ISSN: 1110-0559, Vol. 43, No.2

INTRODUCTION

The Disease Control and Prevention centres estimated that epilepsy affects around 65 million people around the world, so epilepsy is the third most popular chronic neurological problem[1,2]. As the surgical interference is not the usual choice for epileptic patients, medical therapy is now the most suitable choice for their treatment[3].

Valproic acid (VPA) is a the most common used drug for the treatment of epilepsy[4,5]. Also, it is also ascertained to be efficient in the treatment of neuropathic pain[6], bipolar disorder[7] and also in migraine prophylaxis[8]. It elevates the level of brain gamma amino butyric acid (GABA) which is a very important inhibitory neurotransmitter[9].

Sodium valproate (SV), the salt of VPA, is an eight-carbon branched-chain fatty acid, and its structure allows it to interact with the cell membrane, facilitating its therapeutic and toxic actions[10].

VPA has been reported to cause hepatotoxicity[11], nephrotoxicity[12], impaired fertility[13] and teratogenicity[14].

The mechanism by which VPA causes hepatotoxicity is poorly understood[15]. It was suggested that disruption in oxidation of mitochondrial fatty acid and reduction of ATP can play an important role in this toxicity[16]. However, oxidative stress also can be considered as the cornerstone in induction of VPA hepatotoxicity through many mechanisms including depletion of antioxidant enzymes, generation of reactive oxygen species and increases in lipid peroxidation[17,18,19]. As a consequence, liver tissue is damaged and releases its enzymes such as aspartate aminotransferase (AST) and alanine aminotransferase

 DOI: 10.21608/ejh.2019.16176.1161

(ALT) into the systemic circulation, suggesting the fact that the degree of liver tissue damage can be assessed by measurement of these enzymes in serum[20]. As VPA is still commonly used, development of new therapies that can counteract the hepatotoxic effects of this compound is important[21].

Vitamin E (vit. E) works as a potent antioxidant agent. As an essential fat-soluble nutrient, it must be provided by foods and supplements as the body is not capable to produce vitamin E[22]. Vit.E is proved to be effective in decreasing lipid peroxidation, oxidative stress, and reactive oxygen species that cause toxicity in human and animal bodies[23].

Hence, the present study was conducted to evaluate dose-dependent changes in VPA hepatoxicity and the possibility of a hepatoprotective effect of vit. E via light and electron microscopic examination along with analyses of biochemical markers, including AST, ALT, alkaline phosphatase (ALP), total protein (TP), albumin (A), total bilirubin (TB) and direct bilirubin (DB).

MATERIAL AND METHODS

1- Material

• Chemicals and reagents

1. Sodium valproate (SV) powder was obtained from Medical Union Pharmaceuticals (MUP; Giza, Egypt). The tested doses of SV (100, 300 and 500 mg/kg/day) were chosen based on previous studies by Shakya *et al*[24].
2. Capsule of vit. E was obtained from a pharmacy in the concentration of 100 mg (Cairo Pharm. & Chem. Ind. Co., Cairo, Egypt) and was given in a dose of 100 mg/kg/day[25].

• Animals

Sixty male adult albino rats (11-15 weeks) weighing 170-220 g were got and retained at the Animal House of the Faculty of Medicine, Zagazig University, Egypt. The animals were preserved for housing in a balanced temperature (23±1°C) and humidity (55±5%) and artificially illuminated room in plastic cages and (12:12 h light:dark cycle) They were fed balanced food and given free access to food and water totally free from chemical adulteration.

All experimental procedures were performed in accordance with the Institutional Animal Care guidelines and the requirements of the Ethical Committee of the Faculty of Medicine, Zagazig University, with ZU-IACUC committee approval under number ZU-IACUC/3/F/14/2019

• Experimental design

The animals were allocated to six groups, each of which included ten rats. The control group (group 1, G1) was given an intraperitoneal (i.p.) injection of 0.9% saline solution at a volume of 1 ml/kg daily for 8 days. The vit. E-treated group (group 2, G2) was given vit. E (100 mg/kg/day). Group 3 (G3) was given SV solution (100 mg/kg/day). Group 4 (G4) was given SV solution (300 mg/kg/day). group 5 (G5) was given SV solution (500 mg/kg/day). Group 6 (G6) was co-administered SV solution (500 mg/kg/day) with vit. E (100 mg/kg/day).

2- Methods

• Drug Administration

SV solution was prepared by weighing the purified SV powder according to the body weights of the rats and then dissolving it in normal saline to a final volume of 1 ml[24]. The SV was given via the i.p. route for eight consecutive days. Vit. E was dissolved in 1% Tween 80 solution at a dose of 100 mg/kg[25] and given orally via gastric lavage for eight consecutive days.

• Serum preparation

On the ninth day, venous blood samples were collected from tail of rats. The blood in tubes was left to coagulate at room temperature. Then, the samples were centrifuged for 20 min at 4000 rpm using a cooling centrifuge (Sigma 3-30 k). For evaluation of biochemical markers of the liver, the clear serum layer was removed and then stored at -80°C.

Serum alanine aminotransferase ALT (U/L) and aspartate aminotransferase AST (U/L) were measured by the method of Reitman and Frankel[26]. Serum alkaline phosphatase ALP (U/L) was measured based on the methods of Bessey *et al*.[27] and Lowry *et al*.[28]. TP total protein TP (g%) was measured by the method of Kingsley[29]. Albumin (g%) was measured based on the method of Pinnell and Northam[30]. The levels of bilirubin (total bilirubin TB and direct bilirubin DB) were measured based on the method of Perry *et al*.[31].

• Statistical Analysis

The collected data were carefully coded and analysed with a computer using Statistical Package for Social Sciences version 19 (SPSS) software. The testing methods included one-way analysis of variance (ANOVA) for comparisons among more than two groups of normally distributed data followed by least significant difference (LSD) tests for comparisons between two groups. *P-values* of ≤ 0.05 were considered to indicate statistical significance, while *P-values* of <0.01 were considered to indicate highly significant results. All the results are expressed as the mean ± SD.

• Histopathological Assay

The rats in all groups were deeply anaesthetized through i.p. injection of 75 mg/kg sodium thiopental[32] and then sacrificed. Liver specimens were carefully dissected, removed and prepared for histopathological examinations and ultrastructural study.

1- Preparation of paraffin section

Samples of the livers which were isolated from the

rats in the different groups were immediately fixed in 10% formalin solution in normal saline and embedded in paraffin. The thickness of paraffin tissue blocks was 4-5 microns. Sections of the samples were deparaffinised after collected on glass slides, then stained for routine examination with haematoxylin and eosin stain[33]. The examination was performed by an electric light microscope (Leica ICC50 W) in the Human Anatomy and Embryology Department, Faculty of Medicine, Zagazig University.

2- Ultrastructure preparations

After collection of liver specimens from different groups they were fixed in 2.5% glutaraldehyde for 24-48 h, then they were washed three to four times for 20 min in phosphate buffer (pH 7.2-7.4) and post-fixed for 2 h in a buffered solution of 1% osmium tetroxide, after which they were washed four times for 20 min in the same buffer. The fixed specimens then dehydrated by ethyl alcohol in ascending grades (30%, 50%, 70%, 90%, and 100%), and they were embedded in Epon resin after clearance in two changes of propylene oxide[34]. Then we remove the undesired tissue by trimming of the resin blocks. The semithin sections were cut and stained with toluidine blue and examined by light microscope to choose the selected areas. Ultrathin sections were cut with LKB ultramicrotome and contrasted with uranyl acetate and lead citrate[35]. After finishing the preparation of the grids they were examined and photographed using a transmission electron microscope (JEOL JEM-1010, Tokyo, Japan) at the Mycology and Regional Biotechnology Center, Al Azhar University for Boys, Cairo, Egypt

RESULTS

1- Biochemical Results

There were statistical significance difference between the different groups in ALT, AST, ALP, TP, Albumin, TB and DB. Using post-hoc least significant difference (LSD) to find the relation between each two groups, it was found that no significant difference between the control group (G1) and the vit. E group (G2) in any parameter. In addition, the control group and the vit. E group were significantly different from all other groups in all parameters. Regarding G3, it was significantly different from G4 in ALT, ALP, Albumin, TB and DB and from G5 in all parameters. No significant difference was found between G3 and G6 in any of the studied parameters. G4 was significantly different from G5 in AST, TP and ALP and from G4 in ALT, ALP, Albumin, TB and DB. G5 was significantly different from G6 in all parameters (Table 1 and Bar chart 1, 2, 3 and 4).

Therefore, serum ALT, AST, ALP, TB and DB increased with increasing SV dose; while, TP and Albumin are decreased. Concurrent administration of vit. E with SV improved liver biomarker enzyme activity as well as TP, Albumin, TB and DB levels.

2- Light microscopic examination (histopathological results)

The haematoxylin and eosin-stained sections of livers from the control group (G1) and the vit. E-treated group (G2) had the same histological structures. The sections from these groups revealed that each hepatic lobule was composed of central vein surrounded by tightly packed cords of hepatocytes in radiating manner. The hepatocytes in each hepatic cord were polygonal in shape with acidophilic cytoplasm and rounded vesicular nuclei. Binucleated cells were also observed with radiating blood sinusoids lined by endothelial cells between hepatic cords. (Figure 1). The portal area was formed of a portal vein with a thin wall and large lumen and a bile duct that was lined by single cuboidal cells with dark, rounded nuclei (Figure 2). These structures were still preserved in the rats of G3, but the blood sinusoids were dilated, the hepatocytes had slightly vacuolated cytoplasm and little inflammatory cell infiltration was present in the portal area (Figures 3, 4).

G4 and G5 showed marked loss of normal liver architecture with variable hepatocellular changes representing different degrees of lobular affection. The hepatic lobules contained some hepatocytes with ill-defined borders, condensed (dark-stained) nuclei, and vacuolated cytoplasm. There were dilated central veins, blood sinusoids and inflammatory cell infiltration around central veins (Figures 5, 7). The portal area showed dilated portal veins, marked cellular infiltration, increased bile duct wall thickness and the presence of more than one bile duct (bile duct proliferation) (Figures 6, 8).

In G5, some hepatic lobules showed focal area of infiltration (Figure 9). Some hepatic lobules showed marked intracellular vacuolations (Figure 10). Extravasated blood cells RBCs were also observed (Figure 11).

On the other hand, the liver sections of G6 showed variable degrees of improvement compared to the severity of changes observed in Group 5. Vacuolated cytoplasm and condensed nuclei were noticed in some hepatocytes, while other cells were more or less as in control group with acidophilic cytoplasm and rounded, vesicular nuclei. Many hepatocytes showed slightly dilated blood sinusoids and portal veins with some binucleated cells. Additionally, bile duct proliferation and minimal inflammatory cell infiltration were observed (Figures 12, 13).

Electron Microscopic Examination

No pathological changes in rat liver cell ultrastructure were observed in the control group (G1) or the vit. E-treated group (G2). The cytoplasmic organelles and the nuclei of the hepatocytes appeared normal. Numerous scattered mitochondria were observed throughout the cytoplasm (Figure 14 a, b and c). The mitochondria were oval or spherical with well-developed cristae, while the rough

endoplasmic reticulum (RER) appeared packed closely with flattened parallel cisternae studded with ribosomes. A distinct nuclear envelopes were noticed with rounded nuclei, and there were many aggregations of euchromatin and heterochromatin in the nucleoplasm (Figure 14 a). The Kupffer cells are present with the lining epithelium of the sinusoids between hepatocyte cords (Figure 14 d).

The hepatocytes in G3 showed irregular nuclei, mitochondrial ballooning, few lipid droplets (Figure 15 a), slightly dilated sinusoidal spaces and few collagen fibres in pre-hepatic spaces with glycogen deposition (Figure 15 b).

Disintegration of most cellular contents were observed on examination of livers of G4, except for dark, pyknotic nuclei and a few mitochondria, RER and glycogen (Figure 16 a). Scattered lipid droplets were also noticed, and many vacuoles were observed near dilated, congested sinusoidal spaces (Figure 16 b).

On examination of group 5 which received the highest dose of SV (500 mg/kg) (G5) there was nearly complete destruction of most cellular components with cytoplasmic rarefaction and swelling of mitochondria with fragmented RER (Figure 17 a, b). Additionally, the cytoplasm of these cells showed large numbers of lipid droplets and irregular, pyknotic nuclei (Figure 17 a, b). In addition, the hepatic sinusoids, as displayed in (Figure 17 c), were markedly dilated, filled with blood and large amounts of collagen fibres depositions.

Examination of livers of G6 showed mitochondrial, nuclear and cell membrane structures and glycogen distribution in the cytoplasm were more or less as in control group, but few cytoplasmic lipid droplets were present (Figure 18 a). Additionally, the hepatic sinusoids were still slightly dilated and contained little cell debris. Some binucleated cells were also observed (Figure 18 b).

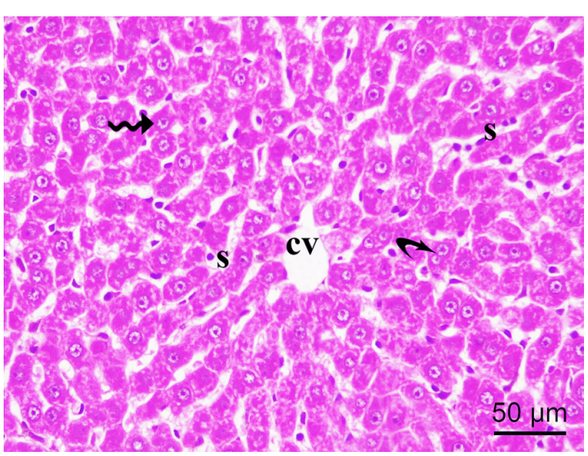


Fig. 1: A photomicrograph from a section of the liver of the control group showing a part of hepatic lobule with cords of hepatocytes polygonal in shape and radiating from the central vein (CV). These hepatocytes have acidophilic cytoplasm and rounded vesicular nuclei (zigzag arrows). Narrow blood sinusoids (S) are radiating in between liver cords. Binucleated cells are also visible (curved arrow).H&E; X 400

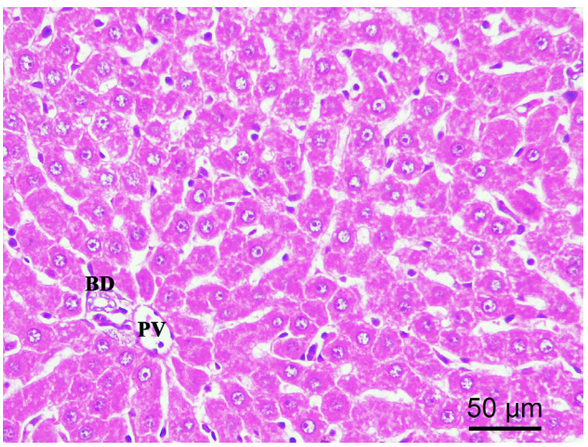


Fig. 2: A photomicrograph from a section of the liver of the control group showing the portal area containing the portal vein (PV) and a bile duct (BD) lined with single cuboidal cells. H&E; X 400

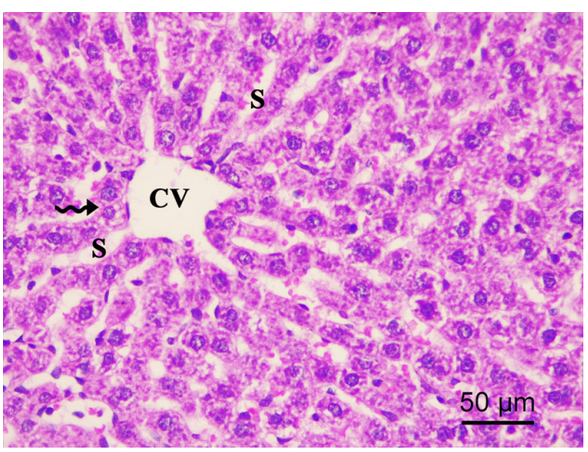


Fig. 3: A photomicrograph from a section of the liver of an albino rat given SV (100 mg/kg) (G3) showing dilated blood sinusoids (S) and hepatocytes are radiating from central vein (CV). The hepatocytes have rounded vesicular nuclei and slightly vacuolated cytoplasm (zigzag arrows) H&E; X 400

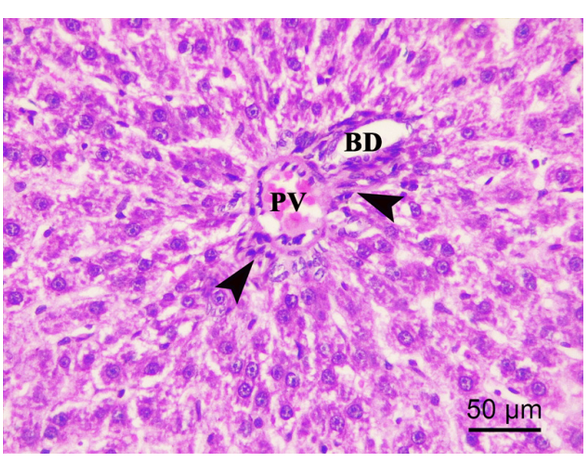


Fig. 4: A photomicrograph from a section of the liver of an albino rat given SV (100 mg/kg) (G3) showing the portal vein (PV), the bile duct (BD), and few inflammatory cell infiltration in the portal area (arrow heads). H&E; X 400

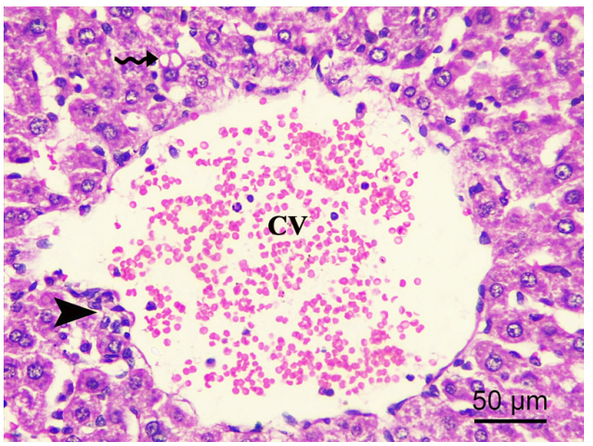


Fig. 5: A photomicrograph from a section of the liver of an albino rat given SV (300 mg/kg) (G4) showing inflammatory cell infiltration (arrow head) and vacuolated hepatocytes (zigzag arrow). H&E; X 400

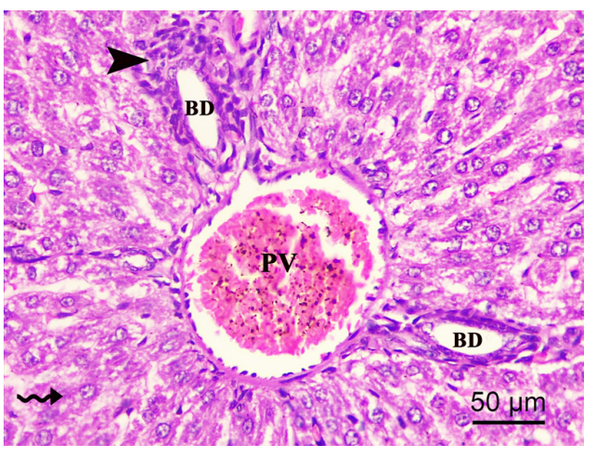


Fig. 6: A photomicrograph from a section of the liver of an albino rat given SV (300 mg/kg) (G4) showing dilated portal vein (PV), bile duct (BD) proliferation, inflammatory cell infiltration in the portal area (arrow head) and vacuolated hepatocytes (zigzag arrow). H&E; X 400

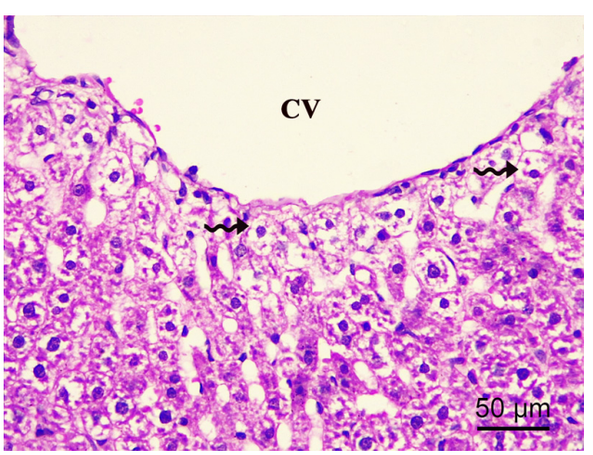


Fig. 7: A photomicrograph from a section of the liver of an albino rat given SV (500 mg/kg) (G5) showing marked loss of normal liver architecture, marked dilatation of the central vein (CV) and highly vacuolated hepatocytes with dark stained nuclei (zigzag arrows). H&E; X 400

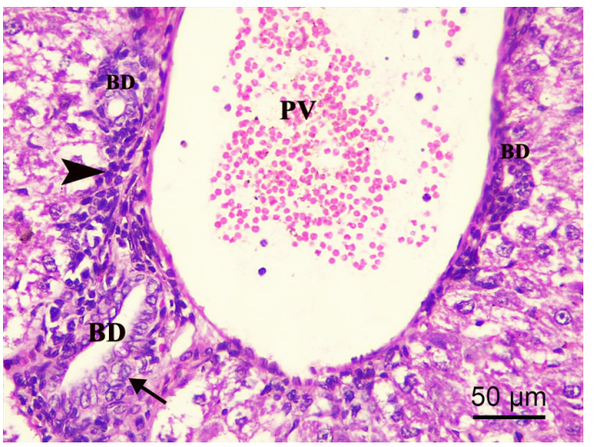


Fig. 8: A photomicrograph from a section of the liver of an albino rat given SV (500 mg/kg) (G5) showing marked dilatation of the portal vein (PV), inflammatory cell infiltration in the portal area (arrow head), increased bile duct wall thickness (arrow) and bile duct (BD) proliferation. H&E; X 400

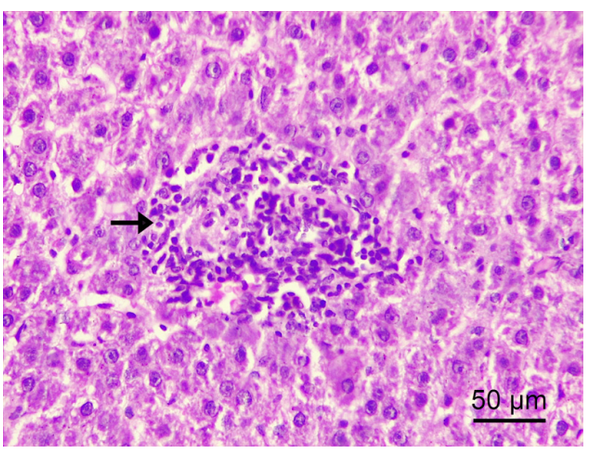


Fig. 9: A photomicrograph from a liver section of an albino rat given SV (500 mg/kg) (G5) showing a focal area of infiltration (arrow). H&E; X 400

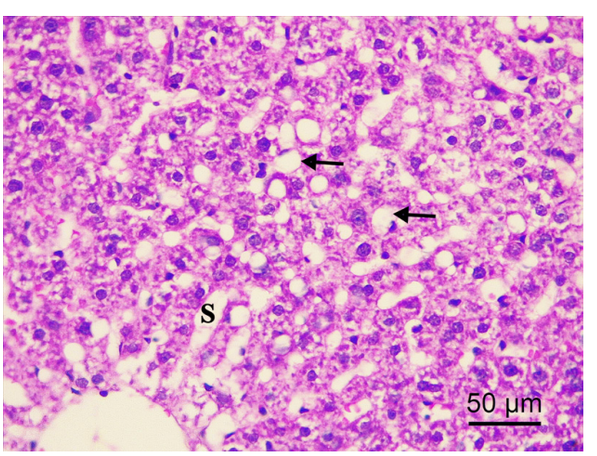


Fig. 10: A photomicrograph from a section of the liver of an albino rat given SV (500 mg/kg) (G5) showing marked intracellular vacuolations (arrows) and dilated blood sinusoids (S). H&E; X 400

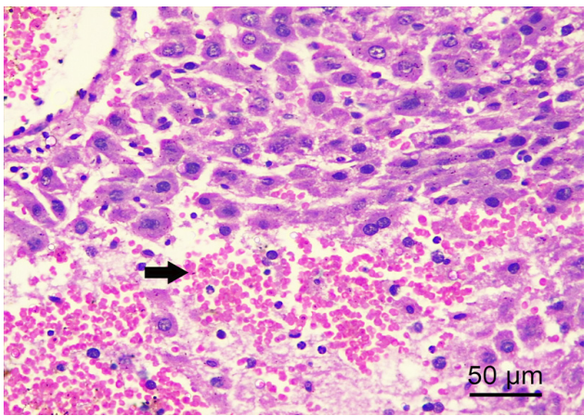


Fig. 11: A photomicrograph from a section of the liver of an albino rat given SV (500 mg/kg) (G5) showing extravasated blood cells RBCs (thick arrow). H&E; X 400

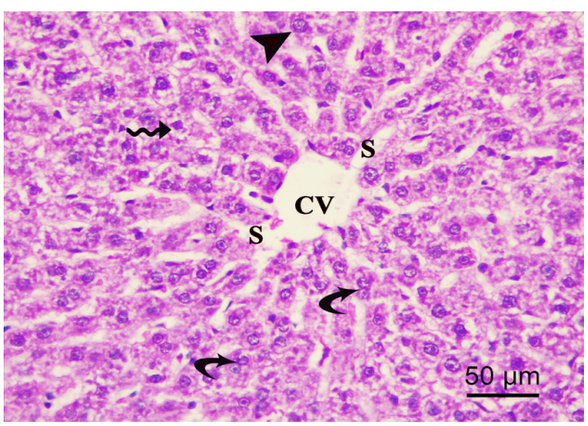


Fig. 12: A photomicrograph from a section of the liver of an albino rat given SV (500 mg/kg) and vit. E (100 mg/kg) (G6) showing hepatocytes radiating from the central vein (CV). Some hepatocytes show vacuolated cytoplasm and dark-stained nuclei (zigzag arrow), while others have acidophilic cytoplasm and rounded vesicular nuclei (arrowhead). Many hepatocytes are binucleated (curved arrows) with slightly dilated blood sinusoids (S). H&E; X 400

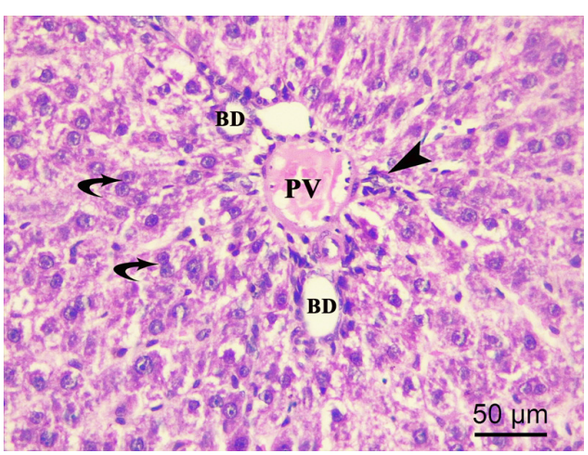


Fig. 13: A photomicrograph from a section of the liver of an albino rat given SV (500 mg/kg) and vit. E (100 mg/kg) (G6) showing a slightly dilated portal vein (PV), bile duct (BD) proliferation, minimal inflammatory cell infiltration (arrowheads) and many binucleated hepatocytes (curved arrow). H&E; X 400

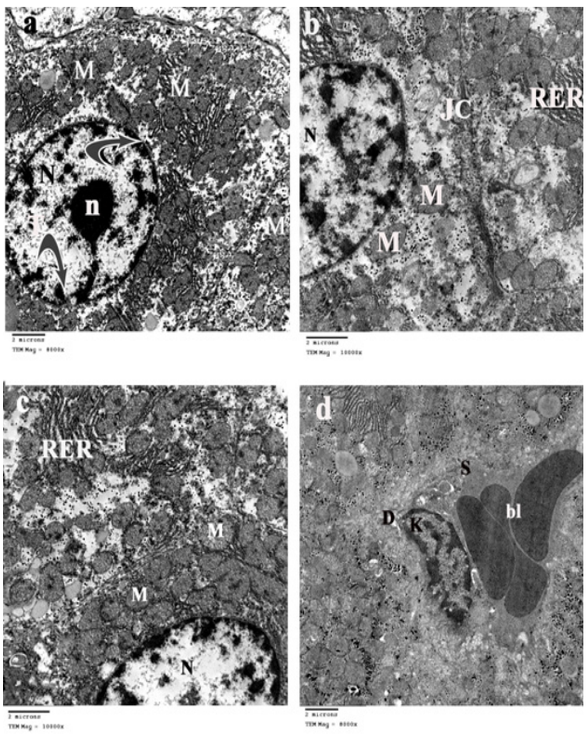


Fig. 14: Electron micrographs of an albino rat liver (control group). a- A hepatocyte showing mitochondria (M) and a nucleus (N) surrounded by a nuclear membrane with chromatin masses (curved arrows) and a nucleolus (n). b- A hepatocyte showing a nucleus (N), mitochondria (M), a rough endoplasmic reticulum (RER), microvilli and junctional complexes (JC). c- A normal hepatocyte showing a nucleus (N), mitochondria with well-developed cristae (M), and a rough endoplasmic reticulum (RER) that is closely packed with parallel flattened cisternae studded with ribosomes. d- A hepatic sinusoid (S) containing blood (bl); the space of Disse (D) contains Kupffer cells (k). a- TEM X 8000 b-TEM X10000 c-TEM X 10000 d-TEM X 8000

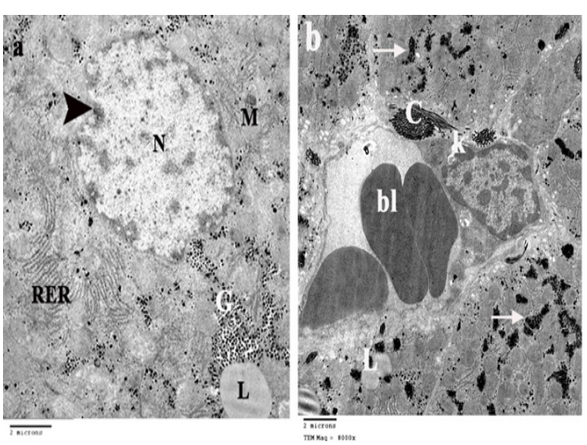


Fig. 15: Electron micrographs of hepatocytes of an albino rat given SV (100 mg/kg) (G3). a- A hepatocyte showing a nucleus (N) surrounded by an irregular nuclear membrane (arrowhead), mitochondrial ballooning (M) and few lipid droplets (L). Rough endoplasmic reticulum (RER) and glycogen deposition (G) are more or less as in control. b- A slightly dilated sinusoidal space with blood (bl) and few collagen fibres in the pre-hepatic space (C) near Kupffer cells (k) with extensive glycogen deposition (white arrows) and few lipid droplets (L).). a-TEM X 10000 b-TEM X8000

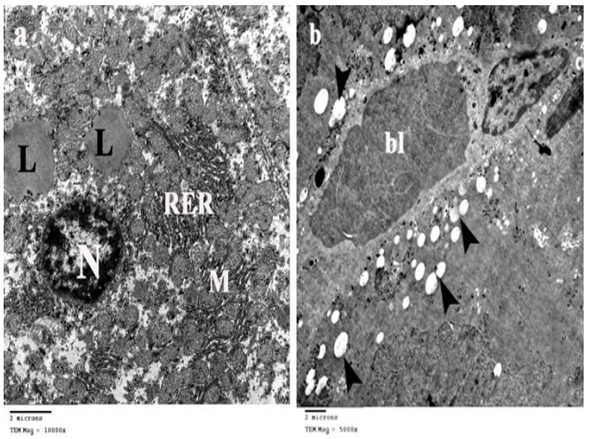


Fig. 16: Electron micrographs of hepatocytes of an albino rat given SV (300 mg/kg) (G4). a- A hepatocyte with a dark pyknotic nucleus (N), few mitochondria (M), rough endoplasmic reticulum (RER) and lipid droplets (L). (b) A dilated sinusoidal space with blood inside (bl) and a large number of vacuoles in the nearby hepatocytes (arrowheads). a- TEM X10000 b- TEM X5000

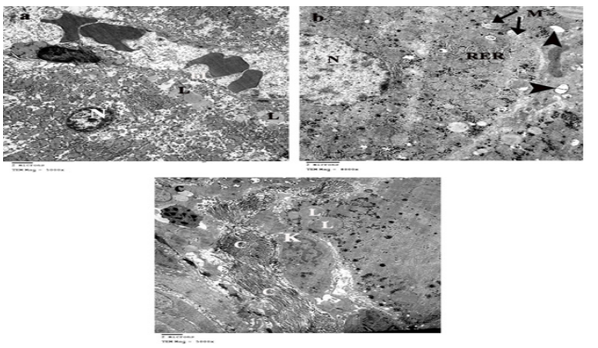


Fig. 17: Electron micrographs of hepatocytes of an albino rat given SV (500 mg/kg) (G5). a- A hepatocyte showing an irregular, pyknotic nucleus (N) and complete dissolution of most cellular components except a large number of lipid droplets (L). b- A hepatocyte showing an irregular nucleus (N), a fragmented rough endoplasmic reticulum (RER), and swollen mitochondria (M). Additionally, the cytoplasm of these cells shows vacuolation (arrowheads). c- A hepatic sinusoid that is markedly dilated and filled with very large numbers of collagen fibres (C) around Kupffer cells (K) with lipid droplets (L). a TEM X 5000 b- TEM X 8000 c-TEM X 5000

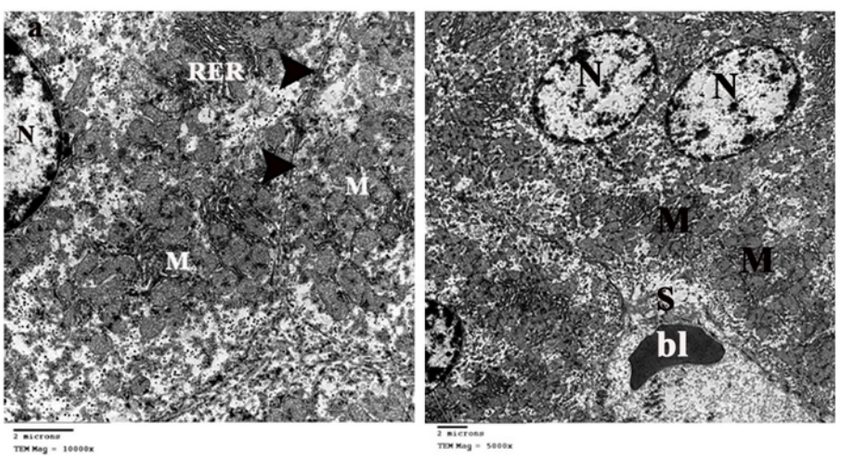


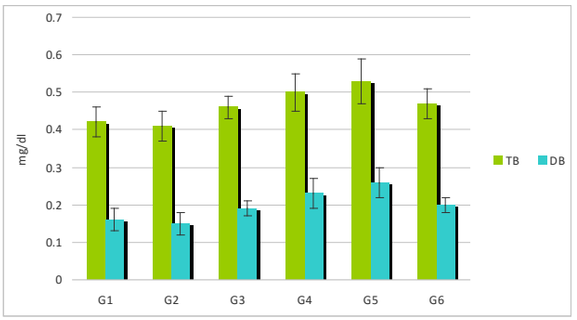
Fig. 18: Electron micrographs of hepatocytes of an albino rat given SV (500 mg/kg) and vit. E (100 mg/kg) (Group 6) a- A hepatocyte with nearly a normal structure of mitochondria (M) with aggregations near the cell membrane. Nucleus (N) and cell junctions (arrow heads(are more or less as in control b - A slightly dilated hepatic sinusoid (S) contained little or no cell debris with blood inside (bl) some binucleated cells (N) also observed with aggregations of mitochondria (M) . a- TEM X 10000 b-TEM X 5000

Table 1: Effects of different doses of SV with or without vit. E on liver function parameters in rats (n=60)

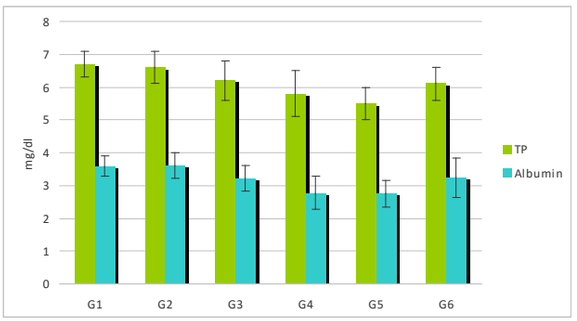
Variable \ Group	G1 Mean ± Sd	G2 Mean ± Sd	G3 Mean ± Sd	G4 Mean ± Sd	G5 Mean ± Sd	G6 Mean ± Sd	F	*P*
SGOT (AST) U/L	171±16.3^{a}	173±15.4^{a}	196±16.7^{b}	201±17.2^{b}	240±19.4^{c}	210±17.5^{b}	22.32	<0.001**
SGPT (ALT) U/L	84±6.5^{a}	86±5.9^{a}	107±8.7^{b}	118±9.2^{c}	125±9.3^{c}	109±8.9^{b}	41.41	<0.001**
AlP (U/L)	1522±6321^{a}	1514±60^{a}	1608±75^{b}	1685±83^{c}	1825±87^{d}	1640±41^{b,c}	27.30	<0.001**
Total Protein (gm/dl)	6.7±0.4^{a}	6.6±0.5^{a}	6.2±0.6^{b}	5.8±0.7^{b}	5.5±0.5^{c}	6.1±0.5^{b}	7.19	<0.001**
Albumin (gm/dl)	3.59±0.3^{a}	3.61±0.4^{a}	3.22±0.4^{b}	2.78±0.5^{c}	2.75±0.4^{c}	3.24±0.6^{b}	11.17	<0.001**
T Bilirubin (mg/dl)	0.42±0.04^{a}	0.41±0.04^{a}	0.46±0.03^{b}	0.50±0.05^{c}	0.53±0.06^{c}	0.47±0.04^{b}	10.73	<0.001**
D Bilirubin (mg/dl)	0.16±0.03^{a}	0.15±0.03^{a}	0.19±0.02^{b}	0.23±0.04^{c}	0.26±0.04^{c}	0.20±0.02^{b}	17.97	<0.001**

Number of rats in each group = 10 rats Sd: standard deviation F: ANOVA test P: *P value* **: Highly significant (*P*<0.01)

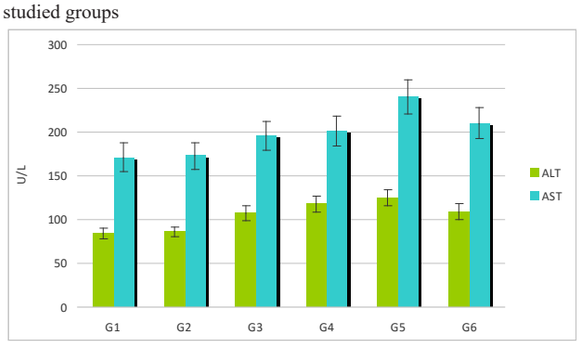
- Groups with the same letter in each parameter are statistically insignificant with each other (P>0.05), while groups with different letters are statistically significant with each other (*P*<0.05).



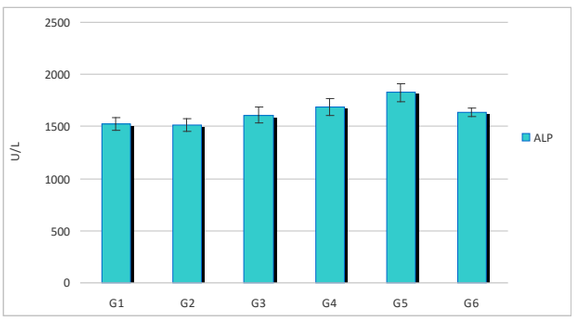
Bar chart 1: Mean ± SD levels of TB and DB among the different studied groups



Bar chart 2: Mean ± SD levels of TP and Albumin among the different studied groups



Bar chart 3: Mean ± SD levels of ALT and AST among the different studied groups



Bar chart 4: Mean ± SD levels of ALP among the different studied groups

DISCUSSION

SV is a broad-spectrum anticonvulsant with a normal therapeutic range reaching to 60 mg/kg/day. However, higher doses must be used in complicated and emergency cases to control various situations in the brain even though they can be toxic to other organs in the body. As the SV is metabolized in the liver, one of the most feared reactions is being highly toxic to the liver[36,37].

Adult rats were chosen in this study because they are similar to other mammalian species in organization of liver cells; hence they are useful animal models for studding liver function and structure[38].

Male albino rats were preferentially used in this study because they have constant hormone levels, unlike female rats. The variability in females should not be ignored, and because hormones can play roles in many inflammatory responses, the cells of the females are more sensitive for certain exposure times. Recent experiments have shown that there are significant differences in nuclear condensation, mitochondrial injury, plasma membrane permeability and RER status between the sexes[39].

In this study, rats were treated with SV at doses of 100 mg/kg/day, 300 mg/kg/day and 500 mg/kg/day. They were treated through a parenteral route (i.p.) to ensure that they received the full dose of SV.

Release of liver intracellular enzymes such as AST and ALT is considered as one of the most sensitive and dramatic indicator of hepatocyte injury after VPA administration. Elevated levels of these enzymes point to hepatocellular damage[40].

In this study, SV-treated rats had significantly higher levels of ALT, AST, and ALP levels compared with control group. Shaat *et al*.[41] and Hussein *et al*.[42] described that VPA-induced hepatic damage is usually linked to increases in serum liver transaminases in dose dependent manner in patients as well as in rats and that was in agreement with this study

The elevated levels of plasma transaminases in the SV-treated rats may have been due to either direct hepatocytic damage or oxidative overload leading to hepatocyte programed cell death[43].

Bilirubin was assessed to evaluate hepatocyte conjugation and excretory functions and was found to be significantly increased after administration of SV, supporting the findings of other studies[44,45].

Significant decreases in albumin and TP associated with administration of different doses of SV is reported in several studies[46, 47]. The depletion of ATP due to mitochondrial dysfunction induced by VPA leads to decreased protein formation as well as decreased gluconeogenesis[47].

In this study, histological assessment of the rats in G3 (treated with 100 mg/kg/day SV) showed preservation of the hepatic lobular architecture; however, the blood sinusoids were dilated, the hepatocytes had slightly vacuolated cytoplasm, and little inflammatory cell infiltration was present in the portal area. These finding are in agreement with those found by Shakya *et al*.[24], who reported that structural changes/damage in the liver did not occur even at higher SV doses of up to 200 mg/kg/day.

However, the study done by Abdella *et al*.[25] found that administration of VPA 100 at a dose of mg/kg/day for three weeks leads to inflammatory cell infiltration, hepatocyte degeneration, highly dilated congested central and portal veins, haemorrhage in hepatic parenchyma, fatty changes in bile ductules in hepatocytes which formed newly.

On the other hand, histological evaluation of the rats in G4 and G5 (treated with doses of 300 mg/kg/day and 500 mg/kg/day SV, respectively) showed distortion of the hepatic lobular architecture. Similar results were found in a study performed by Shakya *et al*.[24].

The hepatic lobules of rats from G4 and G5 also revealed some hepatocytes with ill-defined borders, condensed nuclei and vacuolated cytoplasm. Dilated central veins, blood sinusoids and inflammatory cell infiltration around the central veins were also present. The portal area showed dilated portal veins, marked cellular infiltration, increased bile duct wall thickness and the presence of more than one bile duct (bile duct proliferation), in agreement with the findings of Abdella *et al*.[25].

Alison *et al*.[48], Roskams[49] and Richardson *et al*.[50] reported that a ductular reaction, evidenced by the presence of duct-like structures, is a regenerative response to different types of liver injuries in humans. Hepatic stem cells can proliferate and differentiate into both mature hepatocytes and biliary epithelial cells under situations of chronic and remarkable hepatic injury caused by toxins, viruses and drugs.

In G5, some hepatic lobules showed focal area of infiltration. These findings could indicate a process leading towards necrosis. Moreover, some hepatic lobules showed marked intracellular vacuolations, which supports findings reported in other studies[11,51,52,53]. However, James W. Keterson *et al*.[54] did not observe such effects even after treatment with nearly lethal doses of 700 mg/kg/day. Moreover, Shakya *et al*.[24] did not observe any such specific pathological lesions, although aggregations of nuclei at certain intervals were observed in rats treated with 300 mg/kg and above.

Concerning cytotoxic effects on hepatocyte ultrastructure, VPA has been shown to be highly destructive substance to most of intracellular organelles[55,56].

The present study showed that treating rats with SV at different doses resulted in gradual deterioration of intracellular organelles with swelling of mitochondria and abnormalities of cristae especially at high doses associated with collagen fibers depositions, lipid droplets accumulations and vacuolization of the cytoplasm. These findings are in agreement with those of Tein *et al*.[57], who observed ultrastructural changes characterized by the presence of lipid droplets, vacuoles of the cytoplasm and abnormalities of mitochondria, and those of Abdella *et al*.[25], who observed that SV is highly destructive component for most of cellular contents except for a few mitochondria and the RER, in treated mice.

The altered mitochondrial structure in VPA-treated cells may be resulted from VPA inhibition of β-oxidation. This mechanism may also clarify the slight reduction in the cell viability observed in VPA-treated hepatocytes[56].

Fatty infiltration has been proposed to be due to inhibition of some enzymes in the β-oxidation cycle by VPA and its metabolites, which leads to interference with the oxidation of fatty acids and ultimately results in accumulation of lipids inside hepatocytes; additionally, prolonged administration of VPA causes mobilization of fatty acids from their stores to the liver[58].

In the present study, concurrent administration of vit. E with SV in G6 caused hepatoprotection. Compared with those in G5, the levels of liver biomarkers in G6 were greatly decreased, and the histopathology of the liver was greatly improved. These results are consistent with previous reports showing a protective role of vit. E against SV-induced inflammatory dysfunction of the liver[25,53]. Abdella *et al*.[25] demonstrated that the most potent dose of vit.E that decrease the chromosomal aberrations and hepato-pathological lesions towards negative control levels and increasing the mitotic index were 50 and 100 mg/kg. In contrast a higher dose (200 mg/kg) of vit. E was less effective with regard to these parameters

In the present study, electron microscopic examination of G6 (receiving concurrent administration of vit. E with SV) the nuclei were more or less as in control (with some binucleated cells) and intact RERs; however, the mitochondria were still damaged, with abnormal aggregation in the cytoplasm and deposits. In addition, normal microvilli and intact junctional complexes were observed. The hepatic sinusoids of the same group were intact with slight dilatation. This was in agreement with Yassa *et al*.[59], who concluded that vit. E, by inactivating generated free radicals, exerts an apparent protective effect against the genotoxicity of diazinon by. Moreover, it was reported that vit. E able to inhibit DNA destruction caused by ciprofloxacin in time and dose-dependent manner[60]. Additionally, furthermore many studies have suggested that the hepatic toxicity mutagenic effects of VPA is mainly caused by production and presence of toxic metabolites and this toxicity can be dramatically decreased or even disappear upon treatment with potent antioxidant agents such as vit. E[60,61,62].

CONCLUSION

This study showed that vit. E coadministration with SV leads to fewer histopathological changes in the rat liver than SV alone, suggesting that vit. E cotreatment may help the amelioration of side effects of SV.

RECOMMENDATION

We recommended that patients on chronic use of sodium valproate must co-administrate vitamin E to protect their livers, do periodic investigations of liver function and they must not increase dose of medications without doctor's prescription.

ACKNOWLEDGEMENTS

Special thanks to the Anatomy Department, Faculty of Medicine, Zagazig University, and many thanks to the Zagazig University Animal House.

CONFLICTS OF INTEREST

There are no conflicts of interest.

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الملخص العربى

تقييم للتغيرات التركيبية المتعلقة بجرعة فالبروات الصوديوم في سمية الكبد والدور الوقائي الممكن لفيتامين ه في الفئران البيضاء البالغة

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الخلفية: فالبروات الصوديوم هو دواء يستخدم على نطاق واسع ضد الصرع على الرغم من انه يسبب تسمم الكبد كأثر جانبي. لقد ظهر فيتامين ه (فيتامين E) كمغذٍ أساسي قابل للذوبان في الدهن يعمل كمضاد للأكسدة في الجسم البشري.
الهدف من العمل: تهدف هذه الدراسة إلى استكشاف التغيرات النسيجية والتركيبية التي تحدثها جرعات مختلفة من فالبروات الصوديوم ، ومراقبة ارتباطها بمستويات المؤشرات الحيوية للكبد في الدم وتقييم الدور الوقائي لفيتامين ه (فيتامين E) في سمية الكبد الناجم عن الجرعة الزائدة من فالبروات الصوديوم
الطريقة: تم تقسيم ستين من ذكورالفئران البالغين البيضاء الي ست مجموعات، عشر في كل منها. تمت معالجة المجموعة الأولى (المجموعة الضابطة) بالمحلول الملحي الطبيعي. تم علاج المجموعة الثانية بفيتامين ه بجرعة ١٠٠ ملغ / كغ / يوم ، عن طريق الفم. تمت معالجة المجموعات الثالثة والرابعة والخامسة داخل الصفاق باستخدام فالبروات الصوديوم ١٠٠ و ٣٠٠ و ٥٠٠ ملغم / كغم / يوم على التوالي لمدة ثمانية أيام متتالية. تمت معالجة المجموعة السادسة داخل الصفاق مع فالبوات الصوديوم ٥٠٠ ملغ / كغ / وفيتامين ه (١٠٠ ملغ / كغ) ، عن طريق الفم لمدة ثمانية أيام متتالية. وفي اليوم التاسع تم جمع عينات الدم لتقييم العلامات الكيميائية الحيوية للكبد ثم تم إجراء التحليل الإحصائي للنتائج. تم تخديرعميق للفئران وتم تشريحهما بعناية لأخذ عينات الكبد. تمت إزالة أجزاء من الكبد وتثبيتها في ١٠٪ من الفورمالين.وكذلك تم وضع أجزاء أخري من الكبد من العينات الطبيعيه وغيرها من العينات المعالجه في غلوتارالدهيد بنسبة ٢,٥ ٪ للدراسة التركيبية.
النتائج:عند مقارنة وظائف الكبد بين المجموعات ، وجد أنه قد تغيرت بشكل كبير اعتمادا علي الجرعه. وأظهر الفحص الهستوباثولوجي التشوه التدريجي لخلايا الكبد ووجود مجموعة من حطام الخلية مع تسلل الخلايا اللمفاوية. فيما يتعلق بالتأثيرات السامةفي البنية التحتية للخلايا الكبدية، تبين أن فالبروات الصوديوم مركب مدمر لمعظم العضيات داخل الخلايا. كان أكثر وضوحًا في مجموعات الجرعات الأعلى (٣٠٠ مجم / كجم / يوم و ٥٠٠ ملغم / كجم / يوم) ولكن العلاج المتزامن لفيتامين ه مع فالبروات الصوديوم تسببت في بعض الحماية.
الخلاصة: فالبروات الصوديوم مركب مدمر لهندسة الكبد خاصة في الجرعات العالية ويمكن منعه إلي حد ما بالعلاج المتزامن بفيتامين ه لأنه قلل معظم تسمم الكبد الناجم عن فالبروات الصوديوم.