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RESEARCH ARTICLE

Protective effect of Vitamin C against valproic acid on liver: Histological and biochemical changes on local rabbits

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Objective: This research was designed to examine the impact of vitamin C on valproic acid-induced hepatotoxicity.
Methods: Male rabbits were separated into three groups, each with five animals. Control group: no treatment was provided. The valproic acid group received a dose of 400 mg/kg, while the valproic acid with vitamin C group received 400 mg/kg/day plus 10 mg/kg of vitamin C.
Results: The results showed the extent of the effect of valproic acid alone and with vitamin C alone on the levels of the liver enzymes AST (aspartate aminotransferase) and ALT (alanine aminotransferase) compared to the control group. The results of the AST levels showed a significant increase in the valproic acid group compared with the rest of the groups, while the group treated with vitamin C with valproic acid showed a significant decrease compared with the valproic acid group alone. Microscopic examination of liver tissue from the valproic group exhibited serious vacuolar degeneration with necrosis of hepatocytes, inflammatory cell infiltration in the portal area, recent thrombus, and congestion of the central vein. Liver samples from the valproic group displayed mild vacuolar degeneration of hepatocytes. Liver sections from the valproic + Vitamin C group showed a restoration of normal hepatocyte architecture.
Conclusion: Vitamin C can lessen the harmful oxidative effects of valproic acid in liver cells by acting as an antioxidant agent.

Keywords: Valproic acid, Antioxidant agent, Vitamin C, ALT, AST

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Introduction

Valproic acid is a medicine used to treat depression, mental diseases, epilepsy, and bipolar disorder [1, 2]. Valproic acid works to prevent and lessen epileptic seizures by delivering gamma aminobutyric acid, which aids in the reduction of seizures [3]. Valproic acid induces oxidative stress by altering cell membranes, resulting in the depletion of antioxidants in the body. These processes typically produce hepatotoxicity by harming liver cells [4]. Its adverse effects extend to the kidneys, resulting in nephrotoxicity, lower fertility [5], and some teratogenic alterations [6]. Previous studies have revealed that therapeutic levels of valproic acid cause a minor rise in liver function enzymes, as well as a unique type of hepatotoxicity in fetuses that presents as mild hepatic steatosis and necrosis [6]. The specific mechanism by which VPA produces hepatotoxicity is not well understood. According to Tony *et al*. (2002) [7]. VPA-induced hepatotoxicity is attributed to the oxidative stress resulted by increasing reactive oxygen species and free radical production [8]. This was attributed to the occurrence of glutathione depletion, as a study conducted on rabbits showed that valproic acid caused a significant depletion of the level of glutathione in the body [9, 10]. Vitamin C is considered a water-soluble vitamin and is one of the effective antioxidants in ridding the body of free radicals. Natural sources of vitamins include citrus fruits and green leafy vegetables [9]. Studies have indicated that vitamin C can protect against oxidative stress caused by acetaminophen [11], isoniazid, and fluorouracil [8]. This study was designed to examine the effect of vitamin C on valproic acid-induced hepatotoxicity.

Materials and methods

Medications

Valproic acid and ascorbic acid from Pioneer Company, Sulaymaniyah, Iraq were used. The purity analytical grade (p.a. or pro-analysis). The doses used were calculated according to the weights of the animals used in the experiment.

Ethical approval

Ethical approval Ethical approval number M.K. / 3818 F 3/7/2023 from Basic Dental Sciences Department / Dentistry College / Mosul University.

Animals

The study used healthy male rabbits (local rabbits) weighing 1–1.5 kg and aged 10–12 months. They were raised in the college's animal house, in the laboratory of animals, where the standard rearing conditions were taken into account in terms of lighting, humidity, and the provision of water and food necessary throughout the study period.

Experiment design

The 15 male rabbits were separated into three groups, each with five animals. Daily treatment lasted 14 days. They were given orally and sacrificed on the 14th day. Control group: No treatment was provided. The valproic acid

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group received a dose of 400 mg/kg, while the valproic acid with vitamin C group received 400 mg/kg/day plus 10 mg/kg of vitamin C.

After the 14-day treatment period, blood specimens were collected from rabbits, and the animals were sacrificed humanely with minimal suffering by decapitation under ether anesthesia, depending on the ethical guidelines. To collect organs for the study, which were cleaned and washed from the blood before being placed in neutral formalin until histopathological sections were performed on the samples.

Statistically analysis

Average values were used in addition to standard deviations (SD). The ANOVA test was used to conduct statistical analysis. The Tukey's Honest Significant Difference (HSD) test was used as a post hoc test following the ANOVA. In addition, the significant values were at the p level of less than 0.05.

Results

Statistical Analysis of table 1:

– Sum of Squares:
  – Between Groups: The total variability in AST levels explained by the differences between the experimental groups is 213.733.
  – Within Groups: The variability in AST levels within each group is 171.600.
  – Total: The overall variability in AST levels is 385.333.
– Degrees of Freedom (df):
  – Between Groups: The number of groups minus one (3 - 1) is 2.
  – Within Groups: The total number of observations minus the number of groups (15 - 3) is 12.
  – Total: The total degrees of freedom is 14.
– Mean Square:
  – Between Groups: The mean variability between groups (213.733 / 2) is 106.867.
  – Within Groups: The mean variability within groups (171.600 / 12) is 14.300.
– F-Value: The F-value (106.867 / 14.300) is 7.473.This value constitute proportion of variation among groups to the variance within the groups.
– Significance (Sig): The p-value is 0.008, indicating that the results are highly substantial at the 0.01 level. This means there is a statistically substantial differentiate in AST levels between the experimental groups.

The analysis exhibit that there are substantial differentiate in the AST levels between the experimental groups, suggesting that the treatment or condition applied to the groups had a substantial effect (Table 1).

The valproic Acid group shows a substantial increasing in AST levels comparing to other groups. The group of valproic Acid + Vitamin C shows a substantial decreasing in AST levels comparing to the valproic Acid alone (Table 2).

The results of the ANOVA test show the differences in ALT levels between the different groups. If the probability is lower than the specified importance level (0.05), this point out that there are substantial differences between the groups. In this case, the p-value is 0.020, which is less than 0.05 (Table 3) and (Figure 1).

ANOVA analysis shows that are substantial statistical differentiate between the average levels of ALT among the different treatment groups.

Since the p≤ 0.05, the alternative hypothesis is that there is a substantial difference among the group means at the ALT level.

The results also show that vitamin C caused a decrease in the level of ALT compared to the group treated with valproic acid alone (Table 4, Figure 2).

Microscopic examination of liver tissue from the control group rabbits revealed typical histological features, including a central vein, sinusoids, hepatic cords, and Kupffer cells, observed at 100X magnification (Figure 3). In con-

Table 1. Statistical Analysis of AST Levels between and Within Experimental Groups

Source of Variation	Squares Sum	df	Mean Square	F	Sign.
Between Groups	213.733	2	106.867	7.473	0.008*
Within Groups	171.600	12	14.300		
Total	385.333	14			

* Highly Significant at P – value 0.01

Table 2. Statistical Analysis of AST Levels among Experimental Groups

Groups	N	Subset for alpha = 0.05	
		1	2
Control	5	30.8000	
Valproic Acid + VITC	5	31.2000	
Valprioc Acid	5		39.0000
Sig.		0.870	1.000

Table 3. statistically analysis between and within groups of experimental groups

ALT	Groups	Sum of Squares	df	Mean Square	F	Sig
	Between Groups	247.600	2	123.800	1.838	0.020*
	Within Groups	808.400	12	67.367		
	Total	1056.000	14			

* Substantial at P ≤ 0.05

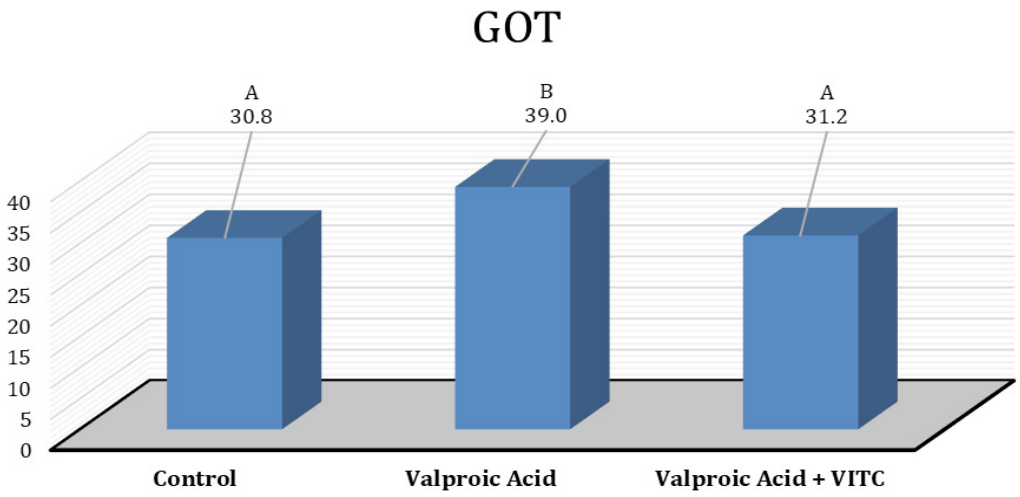

Fig. 1. Level of AST in different groups of treatment

Table 4. statistically analysis of ALT level between groups of experiment

Groups	N	Subset for alpha = 0.05	
		1	2
Control	5	72.0000	72.0000
Valproic Acid + VITC	5	68.6000	
Valprioc Acid	5		78.4000
Sig.		0.097	0.099

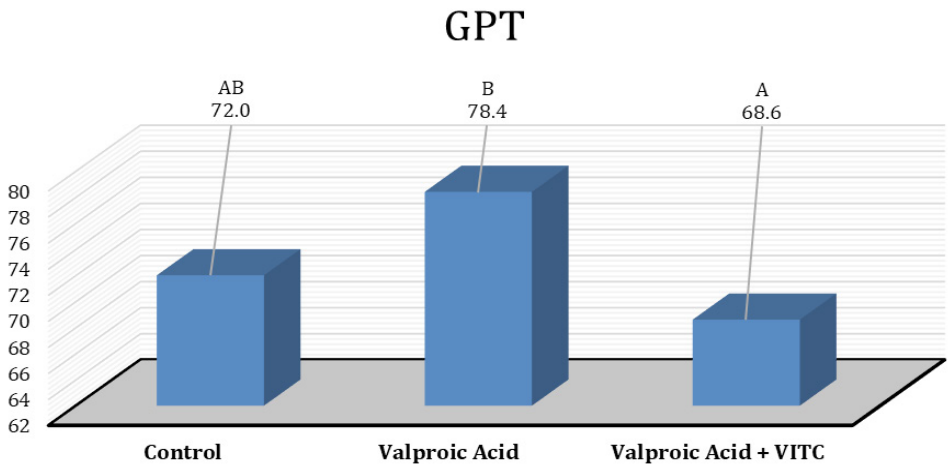

Fig. 2. Level of ALT in different groups of treatment

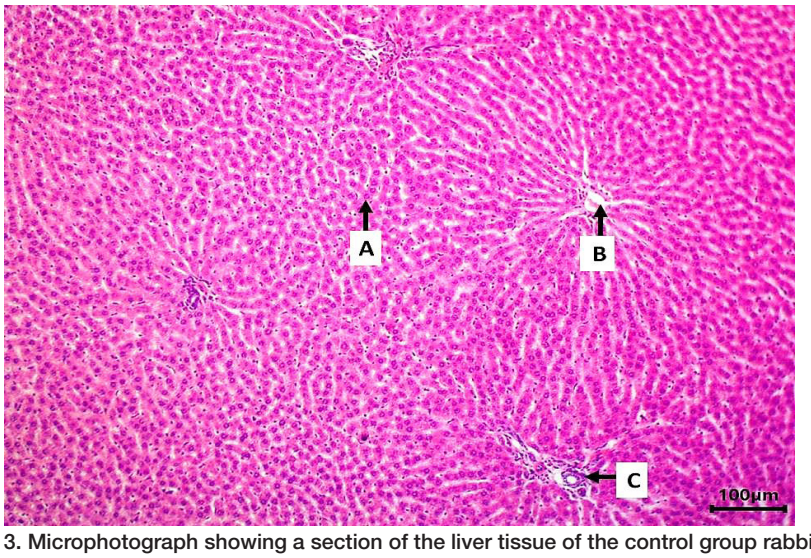

Fig. 3. Microphotograph showing a section of the liver tissue of the control group rabbits, note the hepatocytes (A), central vein (2), and portal area (C). 100 X.

trast, liver sections from the valproic group exhibited severe vacuolar degeneration (D) and necrosis (N) of hepatocytes, inflammatory cells infiltration in the portal area (i), recent thrombus (T), and congestion of the central vein (C) at 100X magnification (Figure 4), with similar findings observed at 400X magnification (Figure 5). Liver samples from the valproic group displayed mild vacuolar degeneration of hepatocytes (D), an intact central vein (C.V), and portal area (P) at 100X magnification (Figure 6), with preserved sinusoidal structures observed at 400X magnification (Figure 7). Notably, liver sections from the valproic + Vitamin C group showed a restoration of normal hepatocyte architecture (H), central vein (C.V), and portal area (P) at both 100X and 400X magnifications (Figure 8) and (Figure 9).

Discussion

This study used a high dose of 400 mg/kg of valproic acid in an animal model (rabbits) to replicate effects over a short period. Higher doses are used in animal research to ensure detectable and significant effects, to evaluate the substance's effects intensively on biological functions, and to simulate long-term effects to determine body tolerance and potential adverse effects. Valproic acid works in a way by affecting various aspects of brain function. It hinders voltage-gated sodium channels to stop the flow of sodium ions into neurons and ultimately regulates abnormal electrical activity to manage seizures effectively [10]. By boosting the levels of GABA (gamma-aminobutyric acid), it increases its presence in the brain, which helps decrease heightened activity and produces anti-seizure benefits [11].

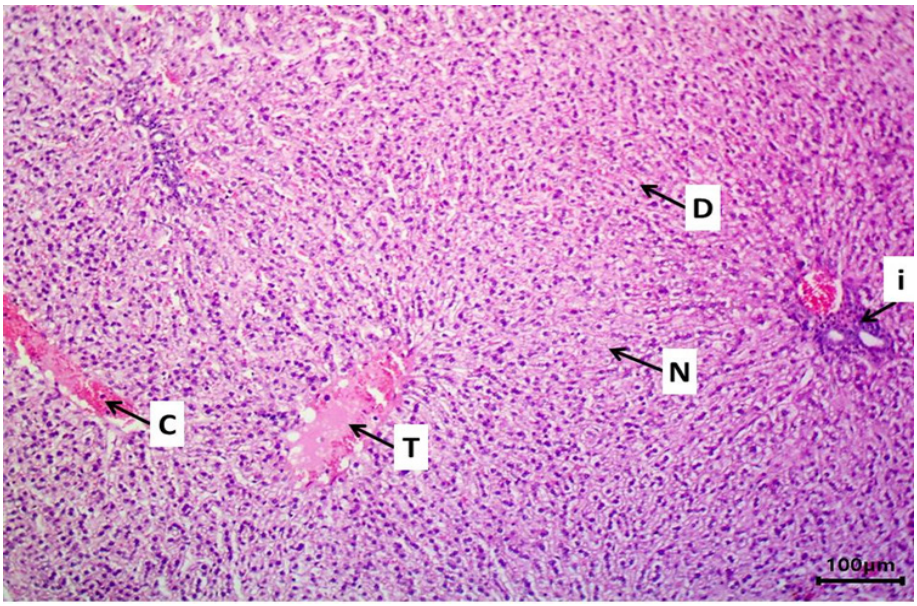

Fig. 4. Liver section of the valproic group viewing severe vacuolar degeneration (D) and necrosis (N) of the hepatocytes, inflammatory cells infiltration in portal area (i), recent thrombus (T) and congestion of the central vein (C). H&E stain, 100X.

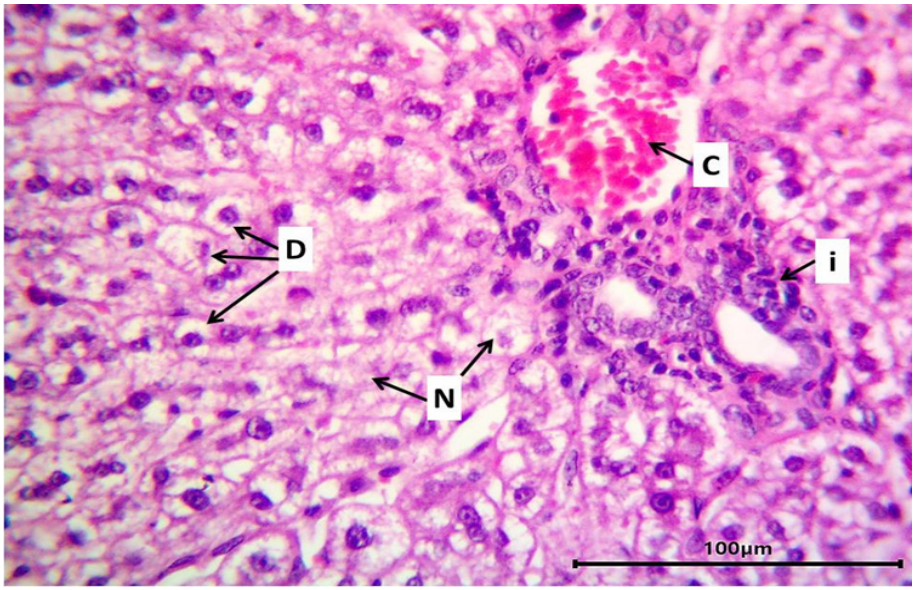

Fig. 5. Liver tissue of the valproic group animal exhibition severe vacuolar degenerate (D) necrosis (N) for hepatocyte, inflammatory cells infiltration in portal zone (i), and congestion of the central vein (C). 400 X

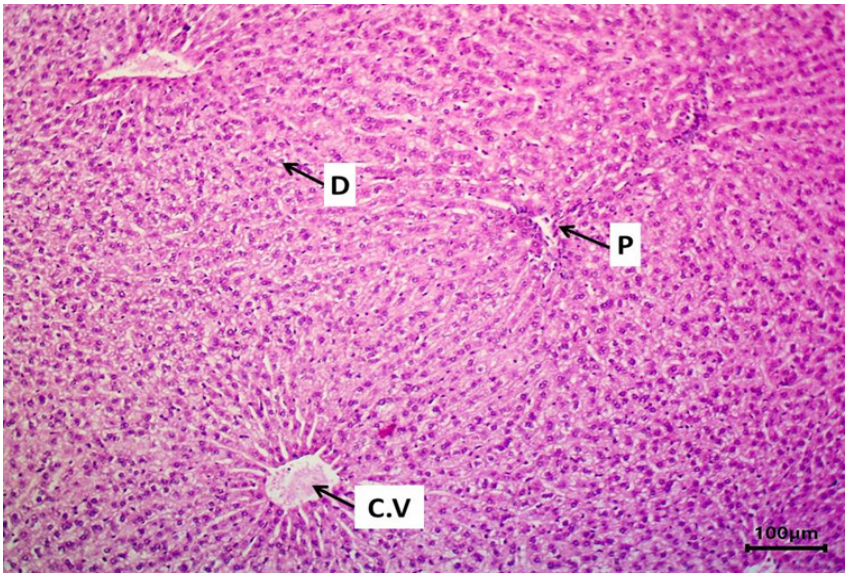

Fig. 6. Liver section of the valproic group viewing mild vacuolar degenerate of hepatocyte (D), intact central vein (C.V) and portal zone (P). 100 X.

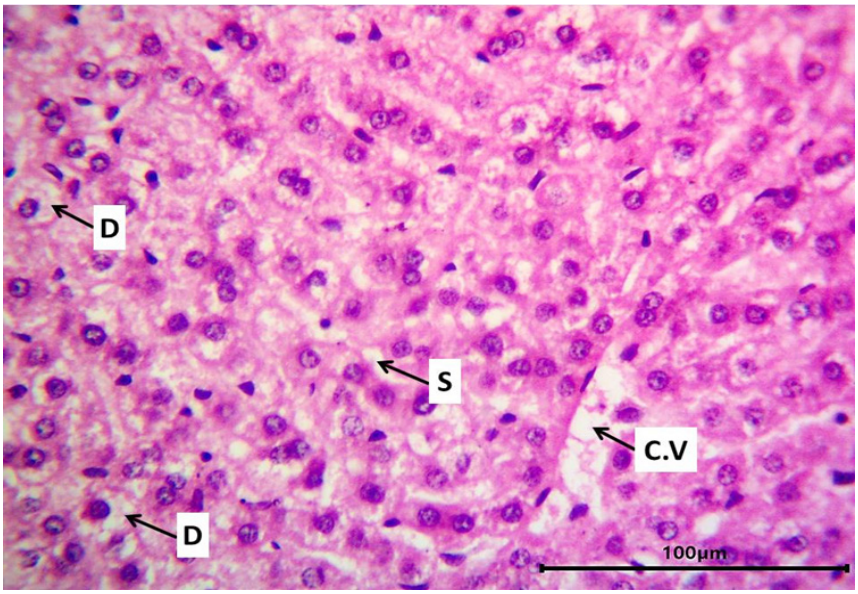

Fig. 7. Liver section of the valproic group showing mild vacuolar degeneration of the hepatocyte (D), intact central vein (C.V) with sinusoids (S). 400 X.

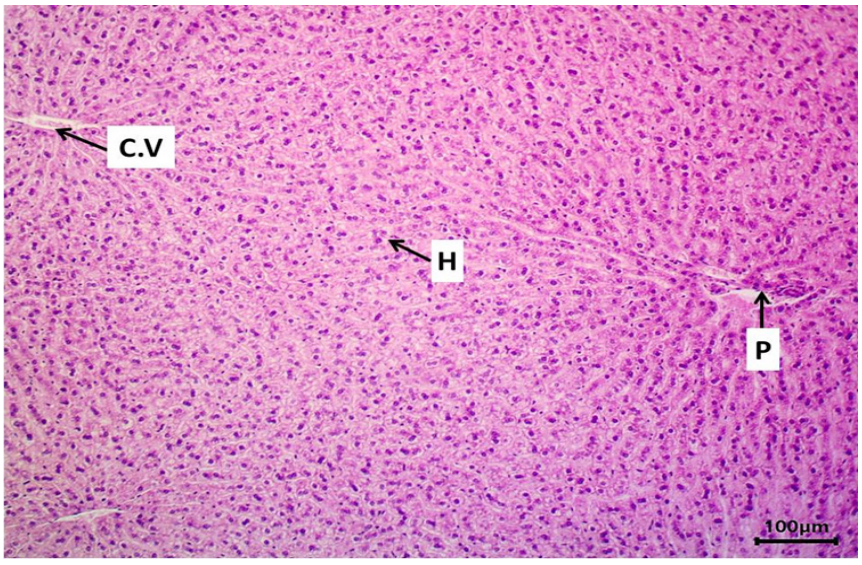

Fig. 8. Liver section of the valproic + vitamin C group showing ordinary structure of the hepatocytes (H), central vein (C.V) and portal zone (P). 100 X.

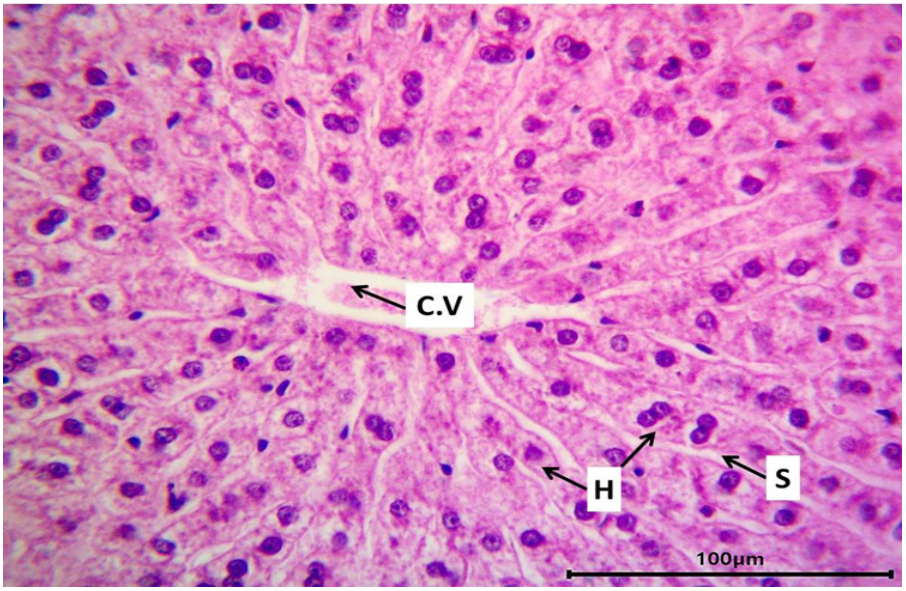

Fig. 9. Histological section of liver of the valproic + vitamin C group viewing normal architecture of the hepatocytes (H), central vein (C.V) and sinusoids (S). H&E stain, 400X.

Valproic acid works with voltage-gated calcium channels and alters how neurons function to impact neurotransmission [12]. It impacts how genes are expressed by blocking histone deacetylases, which results in changes in gene expression linked to seizures and inflammation [12]. The liver mainly breaks down acid using pathways, like the cytochrome P450 pathway which creates reactive compounds such as 4-en-VPA and free radicals causing oxidative stress [13]. A specific metabolite called 4 -en-VPA prevents the breakdown of fatty acids in mitochondria leading to build up, in liver cells. Raised levels of triglycerides termed as fatty liver disease [14] High levels of oxygen species (ROS) are produced during the metabolism of acid, in the body and can cause harm to proteins, DNA and fats in liver cells leading to an increased risk of cell death [15]. Moreover toxic byproducts like 2-en-VPA and DHP also play a role in liver damage by blocking enzymes in mitochondria such as aspartate transaminase. Valproic acid and its breakdown products also reduce levels of antioxidants, in the liver, glutathione (GSH) which worsens the damage caused by ROS [16]. The current study demonstrated that liver tissue showed a clear effect in groups treated with valproic acid; this effect was accompanied by an increase in liver function enzymes, namely AST and ALT, and these enzymes are among the indicators of liver cell damage [17]. These consequences are coherent with prior studies that recorded such effects [17, 18]. One study discovered that valproic acid leads to a reduction in glutathione levels in liver cells, and this decrease is linked to the metabolism of the substance in the liver as well as its toxic effects on the liver cells. The onset of toxicity is seen with decreased glutathione levels, which suggests that low glutathione levels contribute significantly to valproic acid's harmful effects on the liver [19]. The metabolism of valproic acid by cytochrome p 450 leads to the formation of many toxic metabolites and free radicals that cause an increase in the harmful effects on the liver. Valproic acid is extensively processed in the liver by cytochrome P450 enzymes. In the metabolic phase of valproic acid 4-en-VPA, and other reactive intermediates are produced. These metabolites have the potential to disrupt function by blocking β oxidation, which results in the buildup of fatty acids and the creation of reactive oxygen species (ROS). The body counters these effects with glutathione, which causes its depletion and deficiency in the body [14]. Depletion of glutathione from hepatocytes has a severe toxic effect on liver cells, as has been proven in many studies. The buildup of oxygen species (ROS) causes oxidative stress. A state in which the equilibrium between ROS generation and antioxidant protection is disturbed. This oxidative stress results in harm to cells by means of lipid peroxidation, oxidation, and DNA impairment. Glutathione (known as GSH), an antioxidant compound, is depleted when ROS levels rise, consequently worsening the damage to the liver [8, 13, 18]. Several research studies have discussed the impact of valproic acid on different cellular activities like oxidative stress levels and mitochondrial function, as well as cell survival rates [20]. They emphasize how valproic acid can be harmful by impeding biological processes such as mitochondrial functioning and cellular energy generation. This interference can result in heightened stress and disturb the metabolic equilibrium within cells. These discoveries provide insights into the influence of acid on cells, which can help evaluate its possible risks and ensure its safe application in therapy [21-23]. Valproic acid has the ability to cause stress by changing the properties of cell membranes. This results in a rise in the generation of radicals and superoxide anions that can result in harm to cells [24]. When there are more free radicals in the body's system than usual levels can handle effectively and antioxidants like Vitamin C get used up to fight them off, it can lead to a drop in the body's antioxidant levels. Antioxidants are important for shielding cells from oxida-

tive effects, so when they run low, cells could be more vulnerable to oxidative harm [25]. The current study showed liver histological effects represented by inflammatory infiltration, liver cell degeneration, and central vein dilatation. These effects agreed with previous studies that showed such effects [26]. It has been proven that valproic acid has the effect of causing damage to mitochondria and damage to the plasma membrane, which causes energy deficiency in cells and a decrease in the level of ATP in particular in hepatic cells and in the body in general, as well as causing an unprecedented increase in free radicals that assault cell membranes and cause oxidative damage, which has a prominent role in causing degeneration and cellular necrosis, as shown by a previous study in 2015 [13], Hamza *et al*. According to prior research, the usage of antioxidants and radical scavengers, which are considered preventive chemicals, prevents and decreases oxidative stress while also protecting against its adverse effects [8, 27]. A study found that ascorbic acid has antioxidant effects and is a potent (reactive oxygen species) ROS scavenger. It reduces lipid peroxidation by donating electrons [15, 28]. According to Adikwu and Deo (2013) [16, 29], the importance of Vitamin C is evident in its ability to combat the effects caused by valproic acid by restoring GSH levels and actively eliminating ROS to minimize cellular harm. This antioxidant property of Vitamin C may help alleviate the liver damage caused by valproic acid and be an element in safeguarding liver health during treatment with valproic acid. Vitamin C can boost antioxidant status while decreasing MAD lipid peroxidation. These findings are consistent with our previous findings, which showed that vitamin C plays a beneficial function in counteracting the detrimental effects of valproic acid on liver cells [30]. Finally, the effect of ascorbic acid is due to its contact with oxidizing chemicals via a redox reaction, which reduces the oxidative effect of hazardous compounds on cells [31, 32].

Conclusion

The research shows that valproic acid has an impact on liver enzymes AST and ALT, pointing to liver stress or injury. Interestingly, when Vitamin C is taken together with valproic acid, the heightened enzyme levels decrease significantly, hinting at Vitamin C's role against valproic acid-related liver damage. The statistical evaluations, such as the ANOVA, test up the variations in enzyme levels across various treatment groups.

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Conflicted interest

None to declare.

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