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ORIGINAL RESEARCH

Carnitine in Alleviation of Complications Caused by Acute Valproic Acid Toxicity; an Exprimental Study on Mice

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Abstract: **Introduction:** Hyperammonemia and hepatotoxicity are well-known complications of valproic acid (VPA) poisoning. The objective of this study is to evaluate the potential role of carnitine in mitigating the adverse effects of acute VPA toxicity in mice. **Methods:** 54 male mice (25-30 g) were randomly assigned to one of three categories, including acute, sub-acute, and chronic poisoning. Each category contained 3 groups, each consisting of 6 mice (Group 1: control, Group 2: VPA treated, and Group 3: VPA + carnitine treated). The animals were sacrificed 24 hours after the initial injection, and their blood, liver, and brain samples were compared between groups of each category regarding liver function biomarkers, oxidative stress markers, ammonia level, and liver histopathologic changes using one-way ANOVA followed by Tukey's multiple comparison test. **Results:** The administration of VPA increased the serum level of aspartate aminotransferase (AST) (p=0.003) and alanine aminotransferase (ALT) (p=0.001), as well as serum, and brain level of ammonia (p=0.0001 for both) in the intervention group. Elevated levels of lipid peroxidation and oxidative stress (p=0.0001 for both) in the liver tissue, decreased liver glutathione (p=0.0001) and ferric ion-reducing antioxidant power (FRAP) (p=0.0001), and histopathologic changes in the form of moderate to severe inflammation were observed. Administration of VPA + carnitine reduced AST (p=0.05) and ALT (p=0.01), increased the FRAP, reduced free oxygen radicals and liver lipid peroxidation (p=0.0001 for all), and decreased tissue damage in the form of moderate inflammation. The administration of carnitine was ineffective in reducing brain or plasma ammonia levels in acute VPA-treated animals (p = 0.0115). **Conclusion:** Although the administration of carnitine has been suggested as a protective remedy in cases of VPA toxicity, according to the present study, it did not have an antidotal effect and did not prevent encephalopathy or liver injury in acute VPA toxicity.

Keywords: Valproic acid; Chemical and drug induced liver injury; Hepatic encephalopathy; Carnitine

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1. Introduction

Valproic acid (VPA) is a medication that is frequently prescribed to manage mood disorders and prevent seizures. Of the known side effects are hyperammonemia and hepatotoxicity, which can occur both in therapeutic doses and in cases of poisoning. About thirty-five percent of patients who receive VPA experience hyperammonemia, which often occurs in the context of a decrease in serum L-carnitine concentration (1). The primary cause is believed to be the disruption of hepatic urea production or a minor elevation in renal ammonia production (2, 3).

Valproic acid causes a deficiency of carnitine and acetyl coenzyme A. Hence, the oxidation of all fatty oxides and, ultimately, energy production is reduced, and the formation of valproylcarnitine may impede the renal reabsorption of L-carnitine (4). A decrease in glutamate concentration leads to an impairment in the production of N-acetyl N-glutamate (NAGA), a cofactor of carbamoyl phosphate synthetase I (CPSi). CPSi is an enzyme that plays a crucial role in the urea cycle by catalyzing the conversion of ammonia and bicarbonate into carbamoyl phosphate, which is then used to produce urea in the liver (5). L-carnitine supplementation can, therefore, reduce ammonia concentration in patients who use VPA. However, the period to reach normal ammonia levels is unknown (6-8). The administration of VPA can lead to a temporary, dose-dependent elevation in liver enzyme levels, as well as idiosyncratic hepatotoxicity. This rare and potentially fatal condition shares similarities with Reye's

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syndrome (8). Microvesicular steatosis can be observed during histological examination of VPA-induced hepatotoxicity. The underlying cause seems to be the inhibition of beta-oxidation of fatty acids in the mitochondria and their accumulation in liver cells (9). A study conducted on rats showed that administration of L-carnitine can improve microvesicular steatosis (10).

Although VPA overdose rarely leads to hepatotoxicity (11), several studies suggest that prophylactic L-carnitine administration may be beneficial in patients with plasma concentrations exceeding 450 mg/L or those who suffer encephalopathy (12, 13).

Many studies have shown that the administration of VPA in clinical settings can be related to reduced carnitine levels, hyperammonaemia, hepatotoxicity, and encephalopathy. carnitine supplementation is efficient in managing hyperammonemia and hypocarnitinemia (14-20). To date, no studies have been conducted to investigate the protective effects of L-carnitine in cases of acute VPA poisoning, and the current treatment protocol is based on the prophylactic administration proposed in VPA-induced hyperammonemia in epileptic patients. The primary objective of this study is to explore the potential roles of carnitine in mitigating the adverse effects of acute VPA toxicity in mice.

2. Methods

2.1. Study design and setting

In this experimental study, 54 male BALB/c mice weighing 25-30 grams were obtained from the animal nest of Shiraz University of Medical Sciences, Shiraz, Iran. The animals were randomly assigned to one of three categories , including acute, sub-acute, and chronic poisoning. Each category contained three groups, each consisting of 6 mice (Group 1: control, Group 2: VPA-treated animals, and Group 3: VPA + carnitine-treated animals). The animals were sacrificed (thiopental 200 mg/kg, intraperitoneal (i.p)) 24 hours after the initial injection, and their blood, liver, and brain samples were collected.

The Ethics Committee of Animal Use at this University approved the study procedures (IR.SUMS.REC.1396.S595). The experiments were conducted in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No.80-23; revised 2011). They were fed with mice, water, and food. The animals were maintained under optimal conditions throughout the experiment (temperature at 23± 2° C and ambient lighting 12 hours of light and 12 hours of darkness). In all experiment groups, mice were sacrificed using sodium thiopental at the end of the treatment period.

2.2. Category 1: Acute poisoning model

The control group (n=6) received 0.9% saline (3.5ml/kg) 24 hours before the sampling. The VPA group (n=6) received valproic acid (350 mg/kg) via intraperitoneal injection 24 hours prior to the sampling. The VPA + carnitine group (n=6) received valproic acid (350 mg/kg) and carnitine (500 mg/kg) via i.p injection, followed by another dose of carnitine (500 mg/kg) 24 hours later.

2.3. Category 2: Subacute poisoning model

The control group (n=6) received 0.9% saline (1.75ml/kg) for seven days before the sampling. The VPA group (n=6) received valproic acid (175 mg/kg) via intraperitoneal injection for seven days before the sampling. The VPA + carnitine group (n=6) received valproic acid (175 mg/kg) and carnitine (500 mg/kg) via i.p injection for seven days.

2.4. Group 3: Chronic poisoning model

The control group (n=6) received 0.9% saline (0.875 ml/kg) for 21 days before the sampling. The VPA group (n=6) received valproic acid (87.5 mg/kg) via intraperitoneal injection for 21 days before sampling. The VPA + carnitine group (n=6) received valproic acid (87.5 mg/kg) and carnitine (500 mg/kg) via intra-peritoneal injection for 21 days.

2.5. Data gathering

The dataset of this study incorporated variables including the prescribed dosage of valproic acid, aminotransferases (Serum glutamic oxaloacetic transaminase (SGOT), Serum Glutamic Pyruvic Transaminase (SGPT)), lactate dehydrogenase (LDH), Billirubin, serum and cerebral ammonia concentrations, lipid peroxidation in liver samples, liver glutathione content, and the formation of reactive oxygen species in the liver. Furthermore, various pathological alterations were documented through the histological examination of the liver.

2.6. Sample collection

After treatments (day = 22), animals were deeply anesthetized with thiopental (200 mg/kg, i.), and the abdominal cavity was opened.

Blood samples (≈1 mL) were collected from the inferior vena cava and transferred to a test tube containing EDTA. The samples were then centrifuged for 10 minutes and stored at a temperature of -20 °C until they were analyzed. Measurement of the levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), plasma bilirubin, and ammonia level were determined by use of a commercially available analytical kit (Pars Azmun, Tehran, Iran). Specimens of liver tissue were collected. The whole brain was also excised. Tissue samples were washed with a 0.9 % sodium chloride solution and used for further studies.

2.7. Brain tissue homogenate and ammonia levels

About 1 g of the forebrain tissue was removed and homogenated in 10 KCL (1.15% w: v solution). Then, 100 μL of trichloroacetic acid (TCA; 10% w: v) was used to precipitate

proteins. Samples were centrifuged at 12,000 g for 10 minutes at a temperature of 4°C.
Afterward, the pH of the supernatant as well as plasma samoles, were adjusted to 7.0 by adding a 2 M solution of bicarbonate potassium. Finally, ammonia levels was determined using a commercially kit based on phenate-perchlorate reaction (21, 22).

2.8. Liver tissue homogenate

The whole liver was excised and put in a container. A portion of the liver tissue (100 mg) was excised and preserved in 10% neutral formalin for histopathological analysis. The rest (≈8 g) was and used for biochemical tests.

2.9. Measurement of lipid peroxidation in liver sample

A 10% w: v of liver homogenate suspension was prepared by homogenizing a liver tissue sample in a cold solution of KCl (1.5% w/v, pH 7). Next, 3 milliliters of 1% phosphoric acid and 1 milliliter of 0.6% thiobarbituric acid were added to 0.5 milliliters of the mixture (23, 24). The mixture was then heated at 100°C for 45 minutes. After cooling to room temperature, n-butanol (4 mL) was added, and the absorbance of the organic layer was measured at 532 nm following centrifugation (25).

2.10. Liver glutathione content

A liver tissue sample (1 g) was homogenized in 10 milliliters of cold ethylenediamine tetraacetic acid (EDTA) 0.02 M. Then, 4 milliliters of water and 1 milliliter of 50% TCA were added to 5 milliliters of the mixture. The mixture was centrifuged at 3000 g (15 min) (26). Next, 2 milliliters of the supernatant were mixed with 4 milliliters of Tris-HCl buffer (0.4 M) and 0.1 milliliters of 0.01 M 5,5' - dithiobis (2-nitrobenzoic acid). Finally, the absorbance of the layer was measured at 412 nm (27, 28).

2.11. Measurement of Reactive Oxygen Species (ROS) formation in liver sample

A sample of liver tissue (1 g) was homogenized in 5 ml of 40 mM Tris-HCl buffer at pH 7.4 at a temperature of 4°C. Then, 100 μL of the resulting tissue homogenate was added to 1 mL of Tris-HCl buffer (40 mM, pH = 7.4), and 10 μL of 2', 7'-dichlorofluorescein diacetate (DCFH-DA), resulting in a final concentration of 10 micromolar (29). The samples were incubated in the dark at a temperature of 37°C for 15 minutes (30, 31). Finally, the fluorescence intensity was measured at the excitation wavelength of 485 nm and the emission wavelength of 525 nm using a fluorimeter.

2.12. Measurement of Ferric Reducing Antioxidant Power (FRAP) capacity in liver sample

A homogeneous mixture of liver tissue (100 μL) was added to a tube containing 3 ml of FRAP solution at room temperature for 4 minutes (protected from light) (32, 33). After centrifugation (10000 g for 1 minute), The spectrophotometer was used to measure the absorbance at λ = 593 nm.

2.13. Preparation of liver histology slides

The specimens were fixed in formalin and then embedded in paraffin wax. Subsequently, 5-micrometer sections were prepared and stained with hematoxylin and eosin.

2.14. Outcomes

To examine the alterations in the cycle of ammonia biodegradation, we conducted an assessment of the serum and brain ammonia levels. To assess the potential toxic effects on the liver, the concentrations of liver aminotransferases, lactate dehydrogenase and Billirubin were measured; additionally, we conducted a histopathological examination of liver tissue, to identify any microscopic signs of cellular damage across studied groups. In our investigation of the mechanisms underlying liver cell damage, we measured the levels of lipid peroxidation and oxidative stress in the liver tissues; and in order to evaluate the liver's antioxidant system and its detoxification potential, we measured the levels of glutathione in the liver. To evaluate oxidative stress, we measured the ferric reducing ability of plasma in stored blood samples.

2.15. Statistical analysis

Data are presented as mean ± standard deviation or frequency (%). The statistical analysis was conducted using SPSS 22 software (version 19.0, Microsoft, USA). The data were analyzed using one-way ANOVA followed by Tukey's multiple comparison as the post hoc test. A P-value of less than 0.05 was considered statistically significant.

3. Results

3.1. Characteristics of the included studies

Male BALB/c mice (n = 54; weight = 25-30 g) were randomly assigned to acute, sub-acute, and chronic poisoning groups.

3.2. Variation in liver biomarkers

Evaluation of liver biomarkers revealed that the levels of plasma AST (p = 0.003), ALT (p = 0.001) , and LDH (p = 0.001) were significantly higher in the animals treated with VPA compared to the control group in all experimental groups. However, the variations of plasma bilirubin levels in the 2nd and 3rd study groups were different (p = 0.0079). Administration of carnitine significantly decreased the level of AST (p = 0.05) and ALT (p = 0.01) in VPA-treated animals in all experiment groups. However, it only had a significant effect on reducing plasma LDH (p = 0.001) and bilirubin (p = 0.0001) levels in the 2nd and 3rd study groups. Figures 1a-1c show the variations of liver biomarkers in hepatotoxicity caused by VPA and the effect of treatment with carnitine.

3.3. Variation in oxidative stress markers

Liver levels of oxidative stress markers were significantly increased in VPA-treated animals, e.g., increased lipid peroxidation (p = 0.0001) and an elevation in the levels of oxygen free radicals (p = 0.0001). In contrast, the liver's glutathione reserves and the ferric-reducing antioxidant Power (FRAP) were reduced compared to the control group (p = 0.0001). The administration of carnitine significantly increased the FRAP, thereby reducing oxygen free radicals and lipid peroxidation in the liver tissue across all experimental groups (p = 0.0001).

However, it only significantly affected the liver glutathione level in the 2nd and 3rd study groups (p = 0.0001). Figures 2a-2c show the changes in biomarkers related to oxidative stress and hepatotoxicity caused by VPA and the effect of carnitine treatment.

3.4. Variation in ammonia level

The first study group underwent an assessment of ammonia levels in both plasma and brain tissue. Compared to the control group, ammonia levels in plasma and brain tissue were significantly increased in VPA-treated animals (p = 0.0001). The administration of carnitine was ineffective in reducing brain or plasma ammonia levels in VPA-treated animals (p = 0.0115). Brain and plasma ammonia levels in acute VPA overdose and the effect of carnitine administration are depicted in Figure 3.

3.5. Liver histopathological changes

Table 1 illustrates the severity of histopathological changes of normal saline-control, VPA-treated, and VPA + carnitine in all experimental groups. Compared to the control group, histopathological examination of liver tissue revealed moderate (in groups 2 and 3) to severe (in group 1) inflammation, severe microvesicular steatosis, and mild congestion in VPA-treated animals in all experimental groups. In the group treated with VPA + carnitine, the histopathological changes were less severe, including moderate inflammation, moderate microvesicular steatosis, and moderate congestion of the liver tissue.

4. Discussion

Hyperammonemia and hepatotoxicity are well-known complications of valproic acid (VPA) poisoning. However, there is no investigation on the effect of high-dose carnitine on ammonia-induced hepato/neurotoxicity. Based on the data obtained from the current study, although carnitine provided a significant protective outcome in cases of VPA toxicity, this amino acid revealed no antidotal effect in preventing liver failure-associated encephalopathy in acute VPA toxicity.

Valproic acid is an antiepileptic drug with broad-spectrum efficacy that has been in use for over 40 years. It is effective in managing various types of partial or generalized seizures. This treatment is also utilized to prevent migraine headaches and neuropathic pains and control patients with bipolar disorder and schizophrenia (14, 15). The annual incidence of acute VPA poisoning is increasing, which could occur accidentally or intentionally with suicidal intent (16). The most frequently observed symptom of acute poisoning is a reduction in the level of consciousness, which can range from mild confusion to severe coma and even cerebral edema (17, 18). Interestingly, there is no correlation between the plasma levels of VPA and the severity of consciousness impairment (14). Acute poisoning can be accompanied by a brief and reversible increase in liver transaminases (19).

Although there is no reliable evidence, carnitine is routinely used as a preventive treatment in cases of acute VPA poisoning. According to the case report studies, carnitine administration can play a role in improving consciousness, preventing liver injury, and improving mitochondrial dysfunction (20, 34).

Compared to the control group, plasma AST, ALT, and LDH levels were significantly increased in all experimental groups. Hence, administration of carnitine significantly decreased AST and ALT levels. Accordingly, the study of Knapp et al. (9) and Ahangar et al. (35) revealed that 2-4 weeks of exposure to VPA increased the level of AST and ALT in the studied samples. Based on Knapp et al. (9), the reduction of carnitine reserves could cause hepatotoxicity after exposure to VPA. However, Laub et al. (36) reported a case of liver failure that resulted in death in a 3.5-year-old girl who was treated with VPA despite having normal serum carnitine levels.

Reduction in glutathione reserves and lipid peroxidation are known mechanisms of liver cell damage in VPA toxicity (37). This study has demonstrated that VPA causes an increase in the production of oxygen free radicals and lipid peroxidation in liver tissue. Additionally, liver glutathione reserves and FRAP were reduced in animals treated with VPA compared to the control group. Pourahmad et al. (38) have shown that the production of oxygen free radicals cause liver cell damage in VPA poisoning. The production of oxygen free radicals and reduction of mitochondrial membrane potential causes liver cell damage in VPA poisoning causes liver cell damage in VPA poisoning. Incubation of liver cells with VPA resulted in a rapid decrease of glutathione, which also indicates oxidative stress. The study conducted by Ahangar et al. (35) suggested the incidence of oxidative stress following 4 weeks of VPA administration in the form of an increase in lipid peroxidation and glutathione oxidation, as well as a general decrease in antioxidant capacity in liver cells.

Here, we showed that the administration of carnitine significantly increased the antioxidant power of ferric ion regeneration and decreased oxygen free radicals, and also decreased the incidence of liver lipid peroxidation after exposure to VPA. To date, no research has been conducted to assess the impact of carnitine in cases of acute hepatotoxicity caused by VPA. However, Shakoor et al. (39) indicated that treatment with carnitine can prevent fatty infiltration and liver necrosis after exposure to toxic doses of VPA in an animal

model. There are no controlled clinical trials, and there are conflicting reports about the effect of carnitine administration in preventing liver complications in patients with acute VPA poisoning (36, 40).

Compared to the control group, we found that VPA-treated animals had significantly increased ammonia levels in both plasma and brain tissue. Based on a case report and case series, plasma ammonia level increases briefly in patients with acute VPA poisoning (41, 42). Meanwhile, ammonia levels significantly increase in cases of severe VPA poisoning in patients who have been undergoing chronic treatment (43). Interestingly, Dealberto et al. (44) showed no correlation between the severity of encephalopathy symptoms and plasma ammonia levels.

Carnitine is an essential cofactor in fatty acid metabolism and energy production. When combined with VPA, it forms valproylcarnitine, which inhibits the transfer of extracellular carnitine into cells and mitochondria. VPA metabolites induce mitochondrial coenzyme A accumulation, and the palmitoyltransferase II enzyme system does not store carnitine. The decrease in carnitine leads to the production of 2-propyl-4-pentenoic acid, which is a toxic metabolite of valproic acid and prevents the excretion of ammonia through the carbamoyl phosphate synthetase I (45). The administration of carnitine has been suggested for patients with VPA-induced hyperammonemia based on these mechanisms. However, this study is the first structured survey to investigate the effect of carnitine treatment in acute VPA poisoning. According to a review of published articles, carnitine treatment has been shown to reduce ammonia levels in patients undergoing chronic VPA treatment (41, 46). Baddour et al. (47) showed that many patients who have VPA-induced hyperammonemia are clinically asymptomatic; they do not need any treatment, and only stopping the medication will resolve the problem. Despite the lack of sufficient evidence, some authors believe that carnitine may be helpful in cases of acute VPA poisoning with decreased levels of consciousness (14). In our study, the administration of carnitine did not significantly decrease brain or plasma ammonia levels in animals treated with VPA.

In comparison to the control group, the pathological examination of liver tissue showed severe inflammation, severe microvesicular steatosis, and moderate congestion in VPA-treated animals. The impairment of mitochondrial fatty acid and VPA beta-oxidation, which leads to microvesicular steatosis, is caused by the accumulation of liposomes in hepatic cells due to the lack of carnitine and acetyl CoA (9). Our study showed that milder injuries in the form of inflammation, microvesicular steatosis, and congestion in the hepatic cells were seen in all experimental groups. Sugimoto et al. (10) showed that carnitine administration improves microvesicular steatosis in the case of hepatotoxicity related to VPA in rats. According to some studies, hepatotoxicity is caused by the reduction of carnitine plasma levels, which leads to an imbalance between beta-oxidation and omega oxidation and the accumulation of 4-en-VPA metabolite (48, 49). In addition, carnitine deficiency can cause mitochondrial dysfunction due to CoA-SH deficiency (50).

In a study by Krahenbuhl et al. (51), a 39-year-old woman who was treated with valproic acid died due to liver damage. She suffered from a decrease in plasma and liver carnitine. In another study, Laub et al. (36) reported a case of hepatic failure in a patient who had normal plasma carnitine levels and died despite treatment with carnitine. So, they concluded that carnitine deficiency alone cannot be considered the cause of VPA-induced hepatotoxicity. Zimmerman and Ishak (50), in the study of fatalities in patients with liver injury caused by VPA, showed that a significant increase in liver aminotransferases is infrequent. However, microscopic examination showed microvesicular steatosis, often with necrosis, and some patients had developed cirrhosis despite a short period of treatment. Therefore, death following VPA poisoning was attributed to idiosyncratic liver injury in exposure to VPA metabolites. Stewart et al. (52) showed that rare mutations in the PLOG gene, which is responsible for coding mitochondrial DNA polymerase γ, can be responsible for fatal cases of hepatotoxicity in VPA poisoning.

VPA at therapeutic doses inhibits cell proliferation, while high doses cause cell death. Our study showed that carnitine administration effectively ameliorates liver cell damage in all experiment groups and reduces plasma aminotransferases in animals treated with VPA. Carnitine administration significantly improved the antioxidant capacity and reduced lipid peroxidation and free radicals. However, in VPA-treated animals, the ammonia levels in plasma and brain tissue were significantly increased compared to the control group. Carnitine administration did not significantly decrease the levels of ammonia in the brain and plasma. Here, we sought to explore the potential advantages of L-carnitine in managing the toxicity of VPA.

Using real-time ammonia levels of the blood and brain with sophisticated procedures could enhance our understanding of the protective effect of carnitine on liver frailer-associated hepatic encephalopathy. Moreover, techniques such as magnetic resonance imaging (MRI) could also improve the effectiveness of such therapeutic interventions. The effects of carnitine in other experimental encephalopathy models could also enhance our understanding of its action and finally pave the way for its clinical applications.

5. Limitations

It's crucial to acknowledge that our study had some limitations. For example, in a usual clinical situation, there's often a delay of several hours before treatment can be given following a sodium valproate overdose, which our study design did not consider. Moreover, the metabolic process in mice may not be identical to those in humans, particularly after an overdose. Despite these constraints, we believe that more trials are required. These should include administering L-carnitine at extended intervals after a VPA overdose to gain

a better understanding of its potential application in actual clinical environments.

6. Conclusions

Based on the data obtained from the current study, although carnitine provided a significant protective outcome in cases of VPA toxicity, this amino acid revealed no antidotal effect in preventing liver failure-associated encephalopathy in acute VPA toxicity.

7. Declarations

7.1. Acknowledgments

This article has been extracted from a study performed by the 3rd and 4th authors as an MD and doctorate thesis in pharmacy, No. 12838.

7.2. Conflict of interest

The authors have no conflicts of interest to declare.

7.3. Funding

We did not receive any funding for this work.

7.4. Authors' contribution

SMM is responsible for the study conception and design, and finalizing of the manuscript. AJ, RH, MS and ME participated in the study research, gathering data and took part in writing the manuscript. AJ, RH and SMM supervised the whole project. All authors have reviewed and approved the final manuscript.

7.5. Using artificial intelligence chatbots

We employed an artificial intelligence model (GPT-4) to assess the quality of English writing within the aricle.

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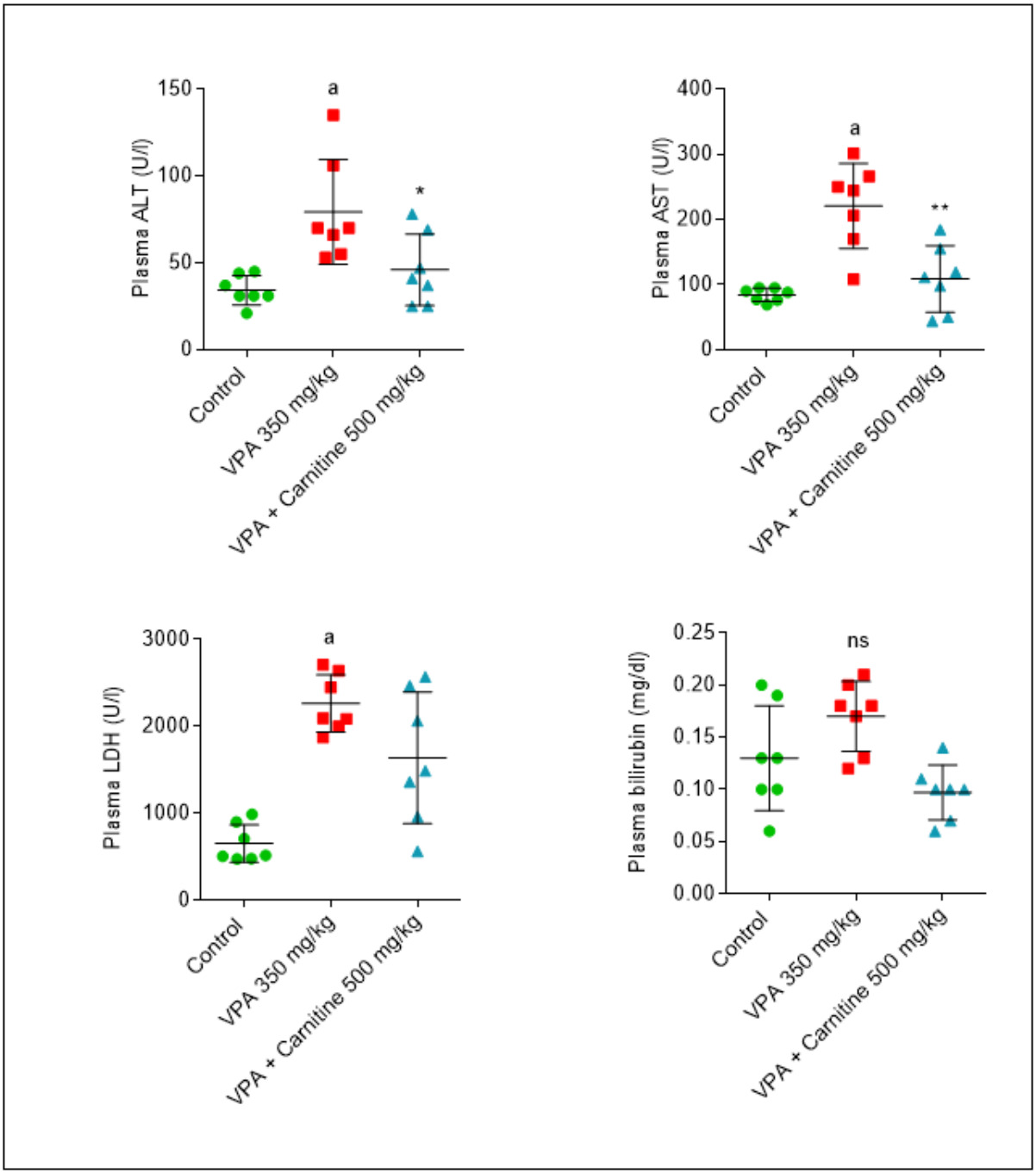

figure1a : Variations in plasma biomarkers in acute VPA toxicity (group 1) and the effect of carnitine administration. ns: not significant compared with control group. a: Indicates significant difference compared with control group (P<0.001). Asterisks indicate significant difference compared with VPA group (*P<0.05; **P<0.01). VPA: valproic acid; ALT: alanine aminotransferase; AST: aspartate aminotransferase; LDH: lactate dehydrogenase.

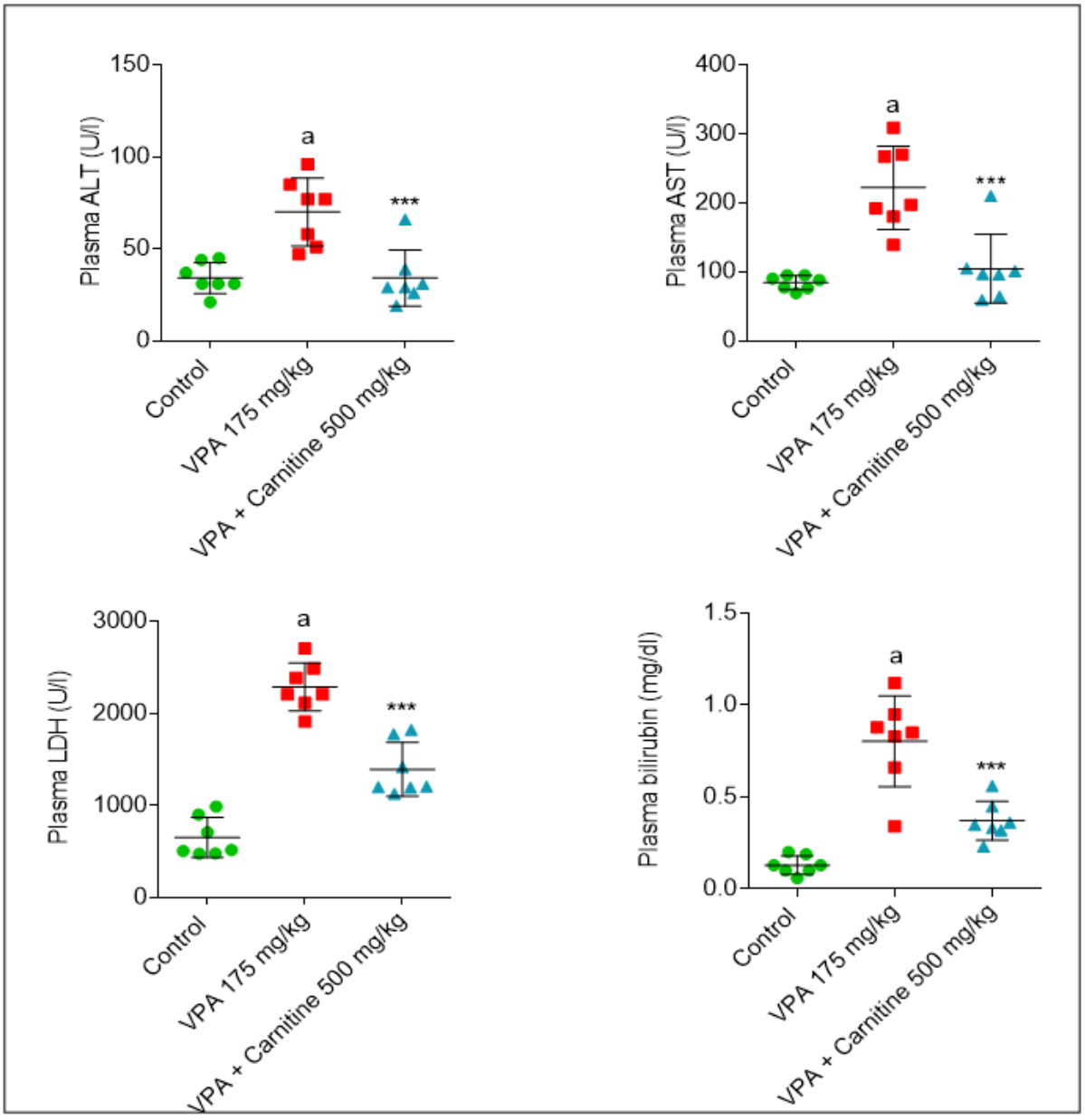

figure1b : Variations in plasma biomarkers in subacute VPA toxicity (group 2) and the effect of carnitine administration. a: Indicates significant difference compared with control group (P<0.001). Asterisks indicate significant difference compared with VPA group (***P<0.001). VPA: valproic acid; ALT: alanine aminotransferase; AST: aspartate aminotransferase; LDH: lactate dehydrogenase.

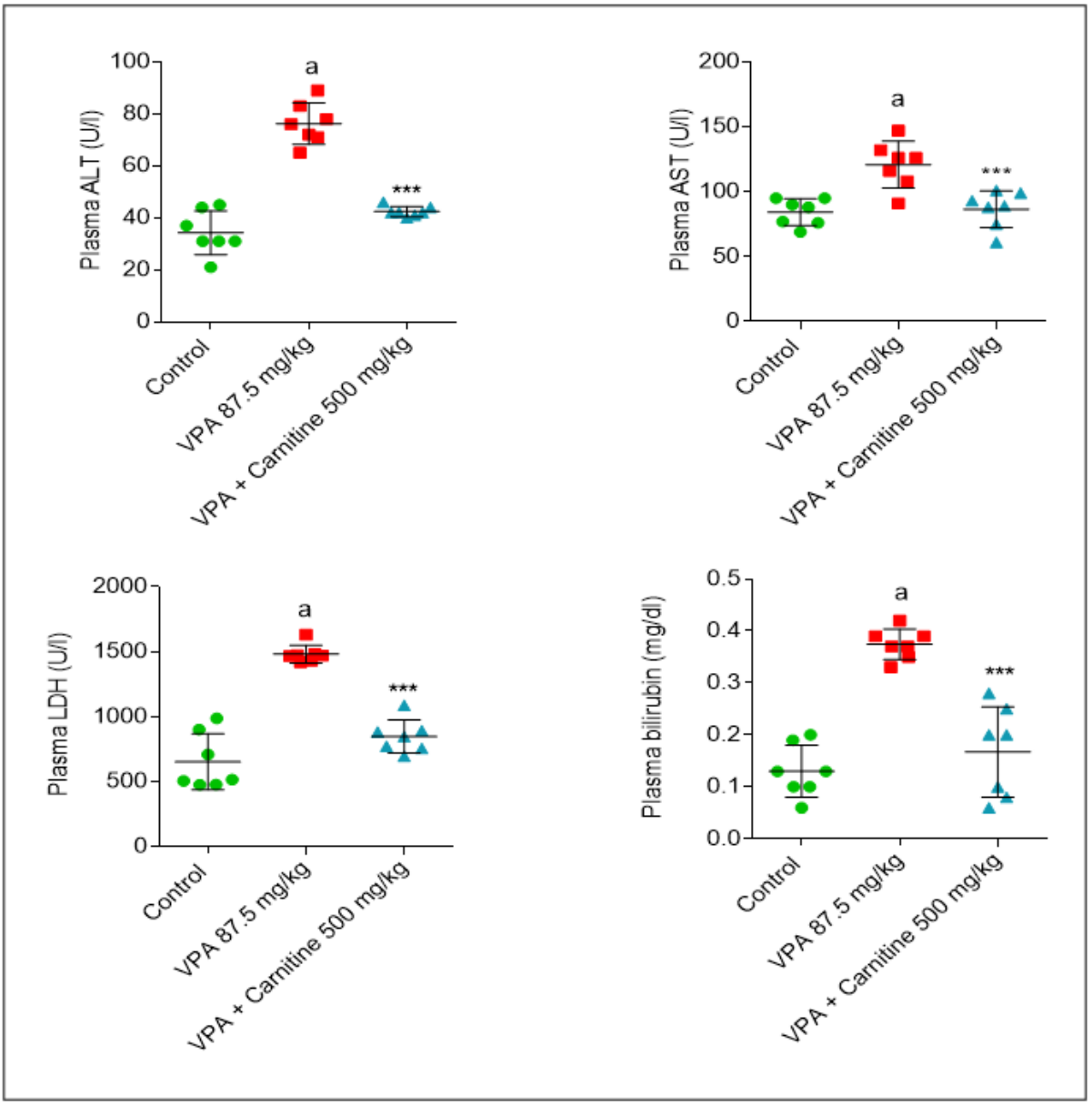

figure1c : Variations in plasma biomarkers in chronic VPA toxicity (group 3) and the effect of carnitine administration. a: Indicates significant difference compared with control group (P<0.001). Asterisks indicate significant difference compared with VPA group (***P<0.001). VPA: val-.proic acid; ALT: alanine aminotransferase; AST: aspartate aminotransferase; LDH: lactate dehydrogenase

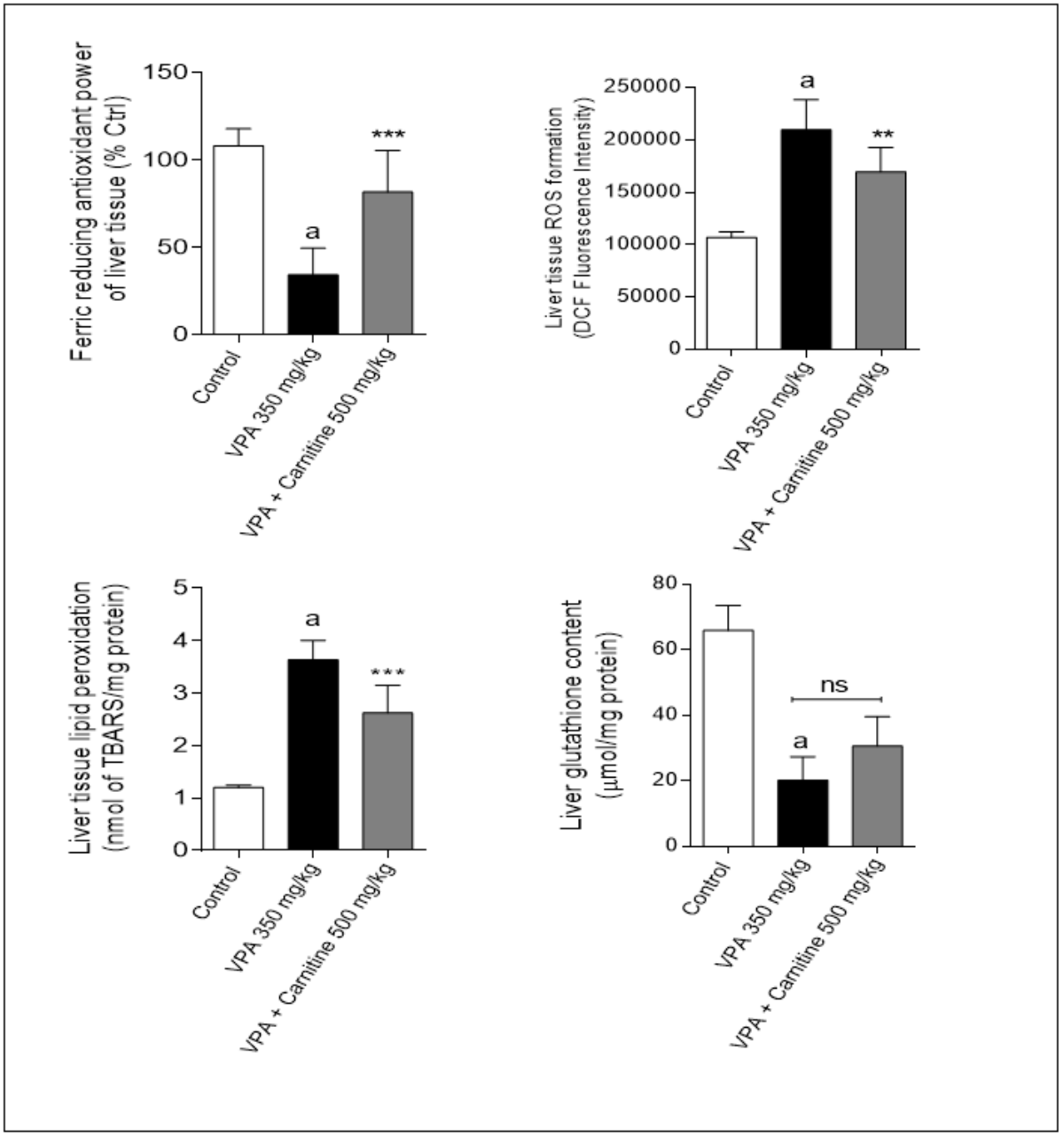

figure2a : Changes in biomarkers related to oxidative stress and hepatotoxicity caused by valproic acid (VPA) in acute toxicity (group 1) and the effect of carnitine treatment. ns: not significant compared with control group. a: Indicates significant difference compared with control group (P<0.001). Asterisks indicate significant difference compared with VPA group (**P<0.01; ***P<0.001). ROS: reactive oxygen species; DCF: .dichlorofluorescein

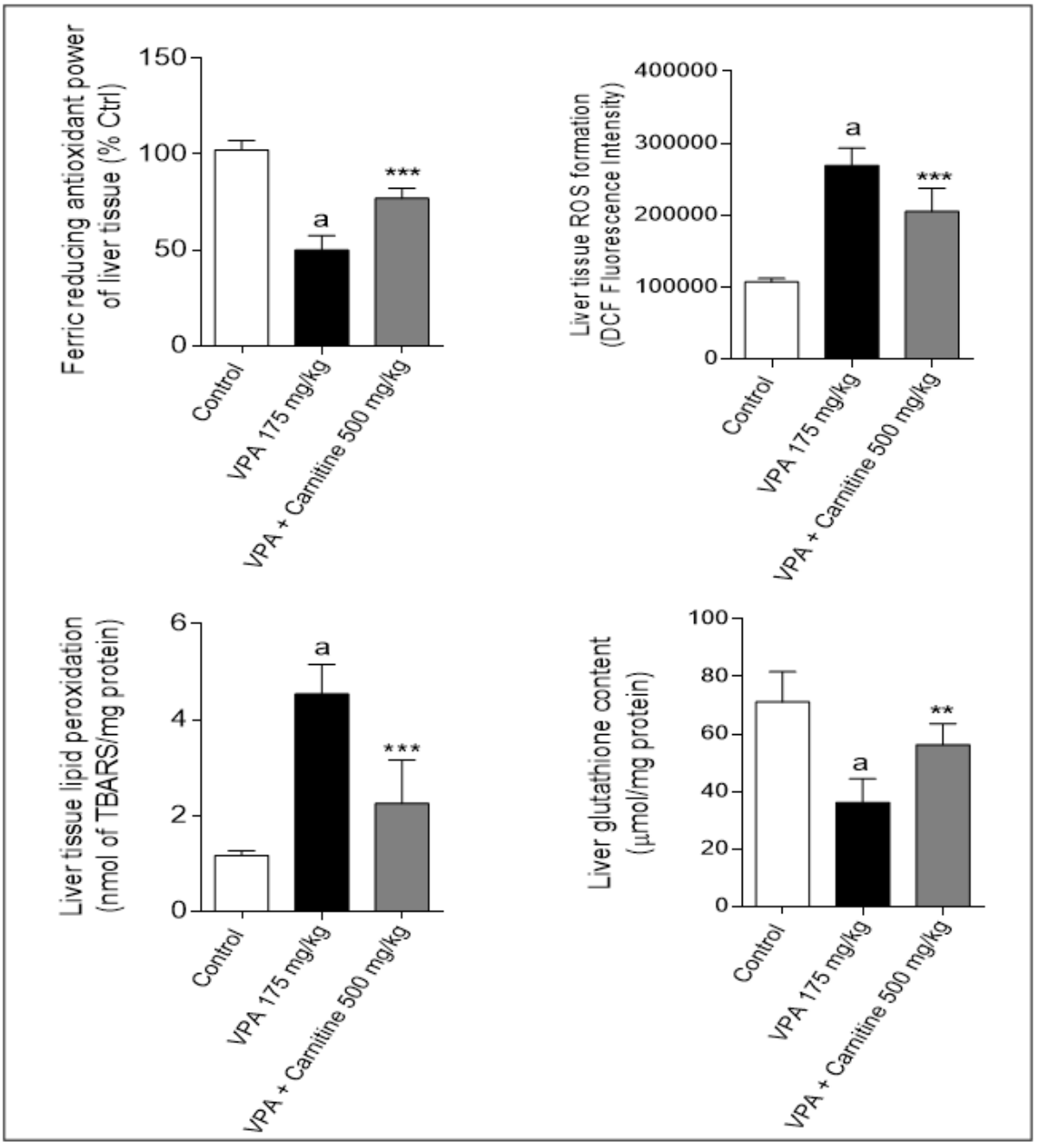

figure2b : Changes in biomarkers related to oxidative stress and hepatotoxicity caused by valproic acid (VPA) in subacute toxicity (group 2) and the effect of carnitine treatment. a: Indicates significant difference compared with control group (P<0.001). Asterisks indicate significant .difference compared with VPA group (**P<0.01; ***P<0.001). ROS: reactive oxygen species; DCF: dichlorofluorescein

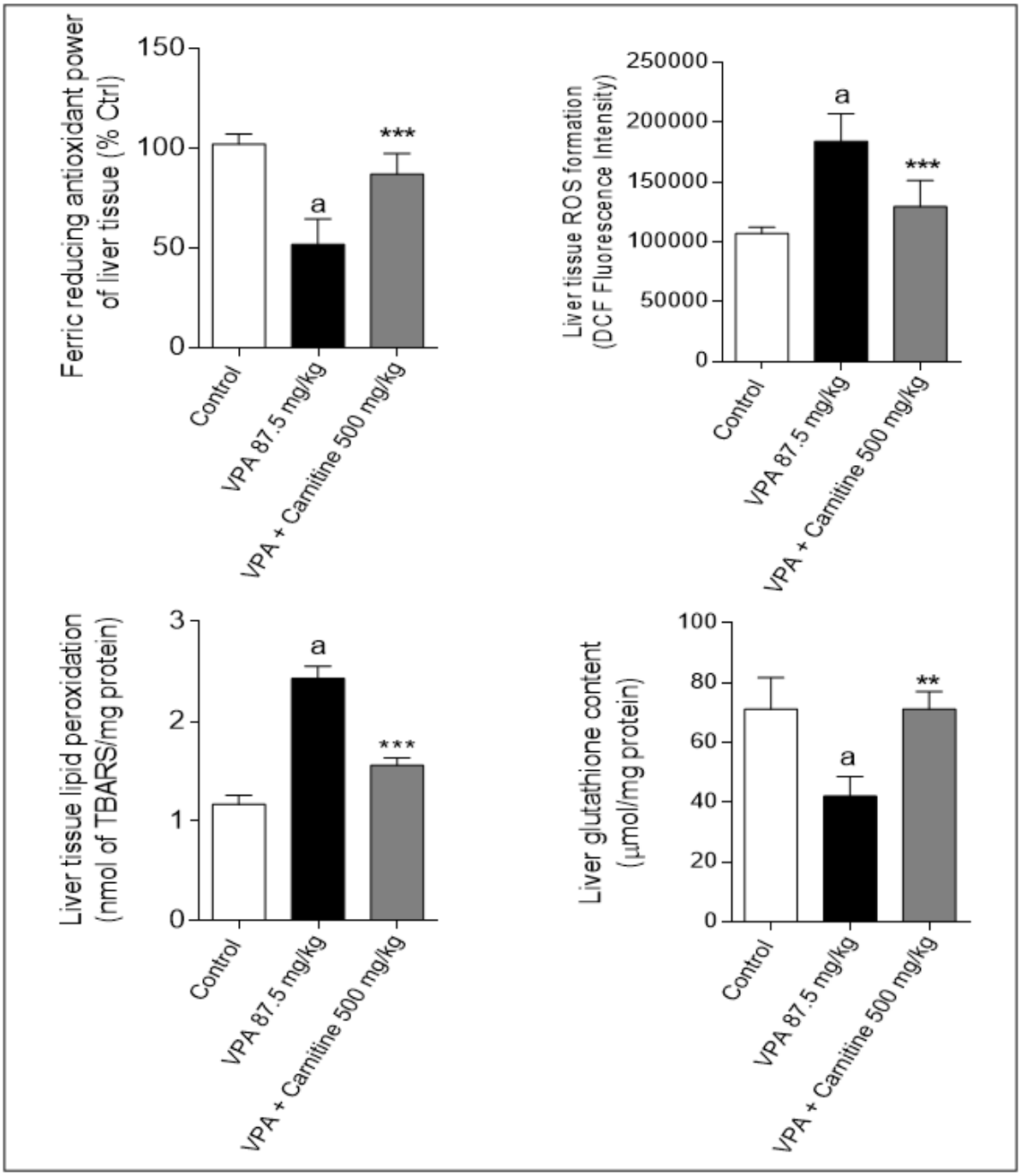

figure2c : Changes in biomarkers related to oxidative stress and hepatotoxicity caused by valproic acid (VPA) in chronic toxicity (group 3) and the effect of carnitine treatment. a: Indicates significant difference compared with control group (P<0.001). Asterisks indicate significant .difference compared with VPA group (**P<0.01; ***P<0.001). ROS: reactive oxygen species; DCF: dichlorofluorescein

Table 1: The severity of histopathological changes of the liver in normal saline-control, VPA-treated, and VPA + carnitine groups

Poisoning model	Microvesicular steatosis	Congestion	Inflammation
Acute			
Control	Negative	Negative	Negative
VPA	Severe	Moderate	Severe
VPA + Carnitine	Moderate	Moderate	Moderate
Subacute			
Control	Negative	Negative	Negative
VPA	Severe	Moderate	Moderate
VPA + Carnitine	Moderate	Mild	Mild
Chronic			
Control	Negative	Negative	Negative
VPA	Severe	Moderate	Moderate
VPA + Carnitine	Moderate	Mild	Mild

VPA: valproic acid.

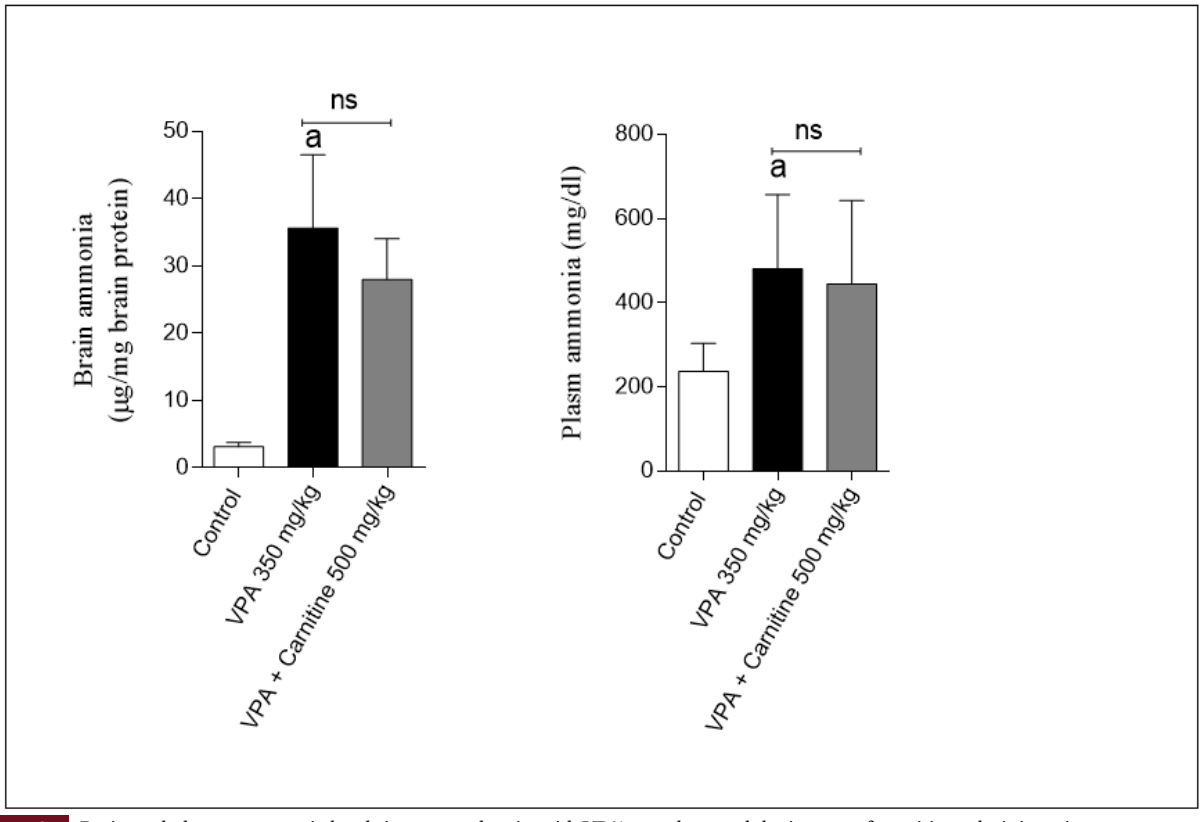

figure3 : Brain and plasma ammonia levels in acute valproic acid (VPA) overdose and the impact of carnitine administration. ns: not .(signif-icant compared with control group. a: Indicates significant difference compared with control group (P<0.01