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Evaluation of Rheum aqueous extract effects on liver function in valproic acid-induced hepatotoxicity in rat model

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Abstract:
Introduction: Valproic acid (VPA) is the major drug used to treat epilepsy and neurological disorders. However, liver damage is a negative side effect associated with VPA. The liver function plays a significant role in hepatotoxicity induced by VPA.
Objectives: The current study was conducted to evaluate if L-carnitine and aqueous extract of rheum (Rhubarb) reducing agents have any benefits against hepatotoxicity induced by VPV.
Methods: Animals were divided into five groups and cytotoxicity markers were assessed. Moreover, animals in the first group received 1 ml distilled water per animal for 15 days, the second group received VPA orally at a dose of 250 mg/kg/day for 20 consecutive days, the third group received L-carnitine orally at a dose of 250 mg/kg/day for 15 consecutive days) after induction by VPA, the fourth group received Rhubarb aqueous extract 80mg/kg /day; orally for 15 consecutive days) after induction by VPA, and the fifth group received a mixture of L-carnitine and Rhubarb extract after induction by VPA.
Results: A dosage of 250 mg of VPA significantly elevated markers of liver function like ALT, AST, and ALP. Using an aqueous extract of Rhubarb elucidates a hepatoprotective effect compared to the standard drug of L-carnitine.
Conclusions: Using L-carnitine, Rhubarb aqueous extract, and the mixture of L-carnitine and Rhubarb aqueous extract decreases ALP, ALT, and AST levels. Histopathological changes in the oxidative stress and liver function markers were observed in VPA-treated animals. The findings from this study highlight the potential therapeutic activities of Rhubarb aqueous extract in mitigating VPA-induced liver injury
Keywords: Hepatotoxicity, Hepatoprotective, Rhubarb, Epilepsy, Valproic acid

Introduction:

The diseases of the liver may not be noticed by clinicians, leading to a delay in the initiation of effective therapies (Salih et al., 2018). Hepatotoxicity refers to liver damage or injury caused by exposure to medicines or other non-pharmacological items. Although considered a drug adverse reaction that may be rare, hepatotoxicity is a severe condition and a leading cause of drug withdrawal from the pharmaceutical market.,. Two hepatotoxicity types: intrinsic reaction, which is dose-dependent and predictable but uncommon, and idiosyncratic reaction, which is not predictable, not dose-dependent but more frequent (Alejandra Cano & Pedro, 2017).

Drug-induced liver injury (DILI) is the major cause of drugs being removed from drug development and the market. DILI accounts for 3–5 % of jaundice cases in humans and is a major contributor to approximately 50% of acute liver damage cases in Western countries (Ezhilarasan & Mani, 2022). the most common problems of liver cellular damage are liver function and oxidative stress disturbances (Ridha & Taher, 2013). In 1962, the clinical properties of VPA were identified during tests as a khellin solvent derivative being studied for its anticonvulsant properties. The initial clinical medicine on epilepsy involving valproic acid sodium salt was documented in 1964. Depakine was first time introduced to clinical use in France in 1967, and in the United

Kingdom UK in 1973, and later got approval from the Food and Drug Administration (FDA) of the US in 1978 (Meseguer et al., 2021). While various mechanisms underlying the mood-stabilizing and antiepileptic effects of VPA, its exact mode of action remains unclear. Valproate enhances the presence of synaptic neurotransmitter gamma-aminobutyric acid (GABA) through presynaptic and postsynaptic mechanisms. Enhanced GABA inhibitory activity promotes GABA-mediated responses in specific brain regions associated with regulating seizure generation and propagation (Romoli et al., 2019).

VPA therapy is generally safe and effective, but a limited subset of patients may experience fatal reactions of hepatotoxicity, particularly in children under two years old and those receiving multiple medications (Zhu et al., 2017). Valproic acid has been reported to induce idiosyncratic DILI (Teschke & Uetrecht, 2020). Although the exact mechanism of hepatotoxicity is not completely understood, VPA-induced hepatotoxicity involves various mechanisms, including oxidative stress, glutathione depletion, microvesicular hepatic steatosis, lipid peroxidation, apoptosis, and hyperammonemia (Koroglu et al., 2021). The imbalance between reactive oxygen species (ROS) and antioxidants leads to physiological and biochemical dysfunctions. VPA has been demonstrated to stimulate the generation of ROS (Kandemir et al., 2022).

Traditional Chinese medicine as a rich medical heritage, has enormous development potential, and its multi-approach, multi-component, multi-target functions offer new opportunities for the treatment of acute liver injury. The Liver Disease Professional Committee of the Chinese Association of Integrative Medicine developed the "Guidelines for the Diagnosis and Treatment of Liver Fibrosis with Integrated Traditional Chinese and Western Medicine," and rhubarb is considered the primary Chinese medicine for the disease's treatment and prevention (Gong et al., 2023).

The most widely used medicine to treat valproic acid hepatotoxicity is L-carnitine, Rhubarb (Rheum rhabarbarum L.), belonging to the family Polygonaceae, is a perennial herbaceous plant highly valued for its abundant nutraceutical properties. The medicinal properties of rhubarb are attributed to its bioactive compounds, including anthraquinones, hydroxyanthraquinone, emodin, aloe-emodin, and Rhein. These compounds have demonstrated a wide range of therapeutic activities as antioxidant, anticancer, antidiarrheal, antidiabetic, anti-inflammatory, diuretic, antimicrobial, and hepatoprotective effects, among others. (Bhat, 2021). In rats, the active components of anthraquinone in rhubarb can significantly reduce serum ALT, AST, MDA, and ROS levels while increasing superoxide dismutase (SOD) activity (Zhuang et al., 2020).

In this study, using an aqueous extract of Rhubarb in vivo and comparing its effect on liver function to L-carnitine was assessed.

Materials and methods:

Plant material

Rheum stems were available in the Iraqi market with medicinal herbs of Baghdad bureau. These stems were ready to be crushed and used in research trials. The contaminants were air-dried for two weeks at ambient temperature. Subsequently, the stems were crushed using an electric grinder and stored in opaque containers sealed at -4°C until the extraction procedure was initiated.

Aqueous extract preparation

Samples were extracted from the stems of the rhubarb plant using cold aqueous techniques. The stems are crushed using an electric mixer. The resulting powder is homogenized by mixing 10 grams of the plant with 100 ml of distilled water and incubating it in a shaking incubator for 24 hours. The mixture is then filtered through medical gauze to remove any remaining plant parts. The filtrate is centrifuged in a plain tube at 4400 rpm for 7 minutes and then filtered using Whitman No.1 filter paper to obtain a pure filtrate free of impurities and insoluble vegetable sediments. The extract was kept in glass containers at a temperature of -4 degrees until it was utilized (Roohparvar, 2023).

Experiment design

The study involved 30 albino male rats weighing 150-275 g (aged 5-8 weeks). Before the study began, the animals were acclimated for one week at the University of Baghdad, College of Medicine in Baghdad governorate, Iraq. The rats were maintained on a standardized pellet with unlimited access to water supplied by the facility.

The rats were allocated into five groups, The first one was received distilled water 1ml per rodent daily for 15 consecutive days. The second group received valproic acid VPA orally at a dose of 250 mg/kg/day for 20 consecutive days, third group received L-carnitine orally at a dose of 250 mg/kg/day for 15 consecutive days after induction by VPA, the fourth group received Rhubarb aqueous extract orally at a dose of 80mg/kg /day for 15 consecutive days after induction by VPA, and the fifth group received a mixture of L-carnitine and Rhubarb extract after induction by VPA.

On day 16 for the first group, 21 for the second group, and on day 36 for the rest groups, the rodents were euthanized using intraperitoneal (IP) anesthesia with Xylazine 2% (Xylazin Bio, Bioveta, Czech Republic), ketamine 10% (Vetased, Farmavet, Romania), Acepromazine 1% (Sedam, Farmavet, Romania) to study liver histopathology. The rats' abdominal cavities were opened, and the livers were promptly harvested, rinsed with cold normal saline, and immediately sent for histopathological analysis. (Coman et al., 2022).

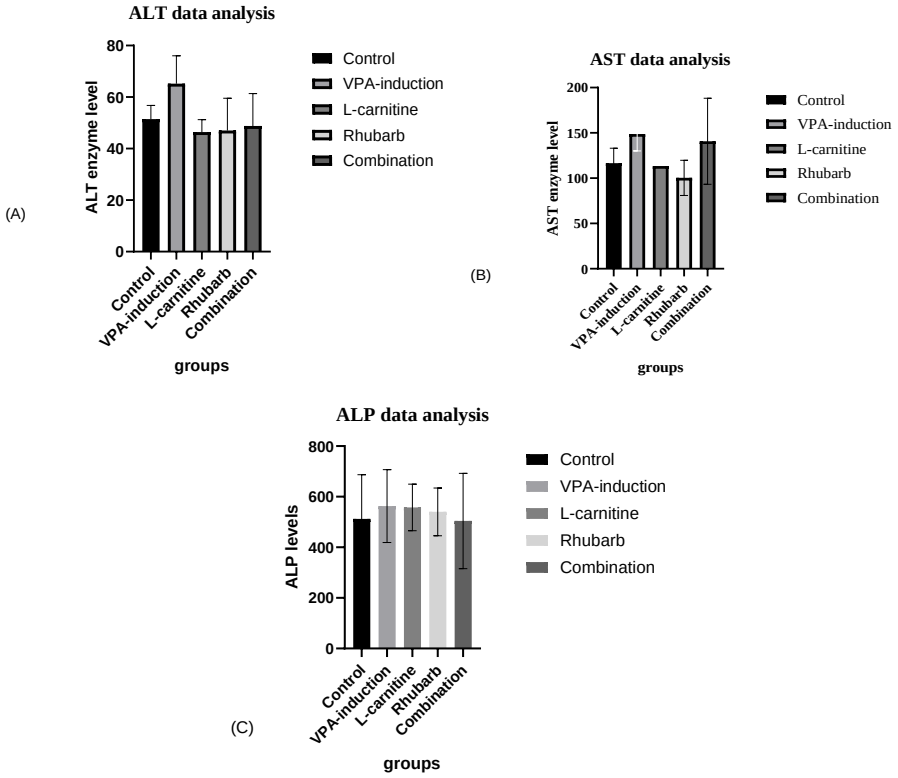
Statistical data analysis

A one-way ANOVA test was performed to compare the differences in the mean between groups, with a less than 0.05 p-value considered statistically significant. A GraphPad Prism (version 10.1.2) was used to perform all analysis and Microsoft Excel 2019.

Results

Identification of the effect of Rhubarb aqueous extract on hepatic function enzymes

Rhubarb aqueous extract and L-carnitine have a potential hepatoprotective effect against hepatotoxicity induced by valproic acid as shown in fig.1 and tab.1.



Figrure1: effect of VPA, L-carnitine, Rhubarb, and mixture of L-carnitine and Rhubarb on (A) ALT, (B) AST, and (C) ALP.

Table 1: Effect of VPA, Rhubarb, and mixture of Rhubarb and L-carnitine on hepatic function.

Groups	ALT(IU/L)	AST(IU/L)	ALP(IU/L)
Control	51.4±5.36	116.6±16.47	512.2±175.06
VPA	65.2±10.80	148.8±18.72	563.0±143.96
L-carnitine	46.4±4.87	113.4±14.51	557.6±92.2
Rhubarb	47.0±12.54	100.4±19.42	540±94.22
L-carnitine + Rhubarb	48.75±12.60	140.75±47.45	504±188.21
P-value	0.03671	0.03629	0.95

We investigated the effects of Rhubarb extract on liver injury induced by valproic acid and the levels of alanine transaminase ALT, aspartate transaminase AST, and alkaline phosphatase ALP. The liver was shown in this investigation to be affected by chronic use of valproic acid, but an aqueous extract of Rhubarb was shown to counteract the effect of VPA. We compared the effect of Rhubarb to L-carnitine which is a more famous medicine treating hepatotoxicity induced by VPA. We used a mixture of both L-carnitine and aqueous extract of Rhubarb to illustrate if there is a synergistic effect or not. Tab.1 reveals that VPA administration led to significant elevation of AST, ALT, and ALP levels. While using L-carnitine and Rhubarb resulted in decreasing levels of liver function enzyme. This finding was highly significant ($P < 0.05$) except in ALP which indicated a high p-value ($P>0.05$).

Histopathology review

A histopathological study of this investigation in (figure 2) detected a normal status for the central vein, hepatic cells, and liver sinusoids. And showed Kupffer cells in the control group. However, with the induction group by valproic acid, we detected severe congestion of the central vein, and this congestion started to disappear when L-carnitine was used as a hepatoprotective drug against hepatotoxicity induced by valproic acid. It is the same story with L-carnitine when we used Rheum aqueous extract against liver injury caused by VPA but with little disappearance for congestion of the central vein compared to the group using L-carnitine for the same duration of treatment (15 consecutive days). When we used a mixture of L-carnitine and an aqueous extract of Rhubarb we detected a synergistic effect for this mixture resulting in the full disappearance of central vein congestion.

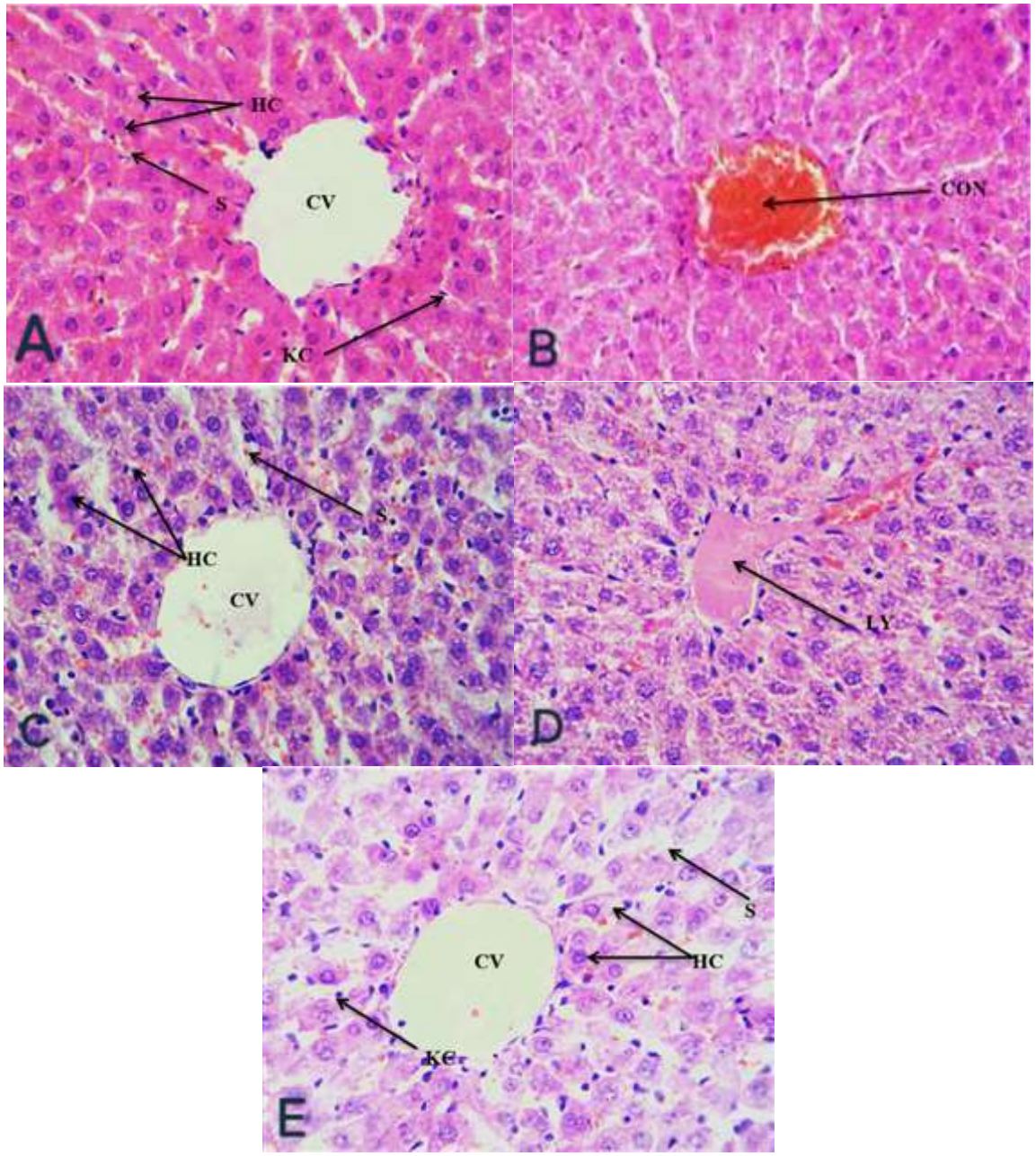


Figure 2: liver tissue section (H&E 400X) of (A) control group; (B) VPA-treated rats; (C) L-carnitine-treated rats after induction by VPA; (D) Rheum-treated rats after induction by VPA; (E) L-carnitine and Rheum-treated rats after induction by VPA.

Discussion:

Drug-induced liver injury (DILI) is a relatively uncommon hepatic condition caused by using medications, illicit drugs, herbal remedies, or dietary supplements (Garcia-Cortes et al., 2020). Although valproic acid is an anticonvulsant medicine used for treating epilepsy and many seizure disorders, it can cause severe liver injury with chronic use (Najafi et al., 2017).

In the present study, we used an aqueous extract of Rheum (Rhubarb) to counteract the effect of hepatotoxicity induced by VPA, we compared its effect with L-carnitine supplementation to evaluate its effect. In the current study, we used VPA for 20 days with a dose (250mg/kg/day i.p.) that shows an elevated in enzymes of liver function like AST, ALT, and ALP. A study by Ezhilarasan shows a substantial increase in AST, ALT, and ALP with chronic use of valproic acid (Ezhilarasan & Mani, 2022). Oxidative stress, glutathione depletion, microvesicular hepatic steatosis, lipid peroxidation, apoptosis, and hyperammonemia are caused by VPA. ROS-

antioxidant imbalances generate physiological and metabolic dysfunctions (Koroglu et al., 2021). In the investigation conducted by Shaaban AA, increased levels of all enzymes (AST, ALT, and ALP) were detected in rats that were treated with only 300 mg/kg VPA (Shaaban & El-Agamy, 2017).

In a compatible study, administration of Valproic acid significantly increased serum levels of AST, ALT, and ALP (Abdelkader et al., 2020). In this study, L-carnitine has a notable reduction in AST, ALT, and ALP compared to their levels with the induction group by VPA. A study by Askarpour M detected that L-carnitine supplementation reduced liver ALT, AST, and GGT (Askarpour et al., 2020). The systematic review and meta-analysis by Abbasnezhad revealed that L-carnitine supplementation significantly reduced blood levels of ammonia, bilirubin, ALT, AST, BUN, and Cr in hepatic encephalopathy patients (Abbasnezhad et al., 2019).

Furthermore, this current investigation shows that Rhubarb aqueous extract declined liver function enzyme levels compared to the induction group by VPA and this was enhanced by Xing which evaluated Rhubarb's effect on liver injury induced by CCL4 (Xing et al., 2011). Another research investigation by Sun X-J evaluating the Protective effect of rhubarb against intestinal mucosal barrier injury in rats with obstructive jaundice revealed that Fourteen days after rats were intragastrical given Rhubarb granular solution, ALT and AST levels were similar in the control and treatment groups (Sun et al., 2018).

However, in the group treated with L-carnitine and the group treated with Rhubarb extract, there was no noticeable decrease in ALP levels within 2 weeks of treatment. ALP is a marker enzyme for the plasma membrane and endoplasmic reticulum, it is, therefore, an ectoenzyme of the plasma membrane (Adeyemi et al., 2015). However, these enzymes are recommended as indicators of hepatocellular (ALT activity) and hepatobiliary (ALP and GGT activities) injury in preclinical studies, Endogenous phospholipases mediate ALP release in cholestatic disease, which is associated with elevated serum bile acid concentrations (Ennulat et al., 2010). An investigation by Chin Y revealed ALP levels remain high in animals, especially with severe cholestasis damage caused by VPA (the significance of treatment of p-value always $p>0.05$), and need a long time for healing maybe months to get back to normal status. Histopathological examination also showed an evident improvement in the hepatic tissues of hepatotoxic rats (Chen et al., 2019).

The results of this study show a synergistic effect when using a mixture of both L-carnitine and an aqueous extract of Rheum (Rhubarb) indicating declined levels of ALP, AST, and ALT in treating liver injury caused by VPA.

Conclusion:

This study explained the hepatoprotective properties of Rhubarb aqueous extract and L-carnitine against valproic acid (VPA)-induced liver injury. Both treatments significantly reduced AST, ALT, and ALP levels, with histopathological improvements, including the alleviation of central vein congestion. The combination of L-carnitine and Rhubarb demonstrated a synergistic effect, providing enhanced protection and full tissue recovery. These findings suggest that Rhubarb, alone or in combination with L-carnitine, could be a promising approach for managing VPA-induced hepatotoxicity. Further research is recommended to explore their long-term benefits and mechanisms.

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