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Evaluation of Rheum Aqueous Extract Effects on Inflammatory Biomarkers in Valproic Acid-Induced Hepatotoxicity in Rat Model

Raed Mohammed Abdulrahman[1], Huda Ibraheem Al-Qadhi[1], Watban Abdullah Ahmed[2]

[1]Department of Pharmacology, College of Medicine, University of Baghdad, Baghdad, Iraq
[2]Department of Pharmacology & Toxicology, College of Pharmacy, Tikrit University, Tikrit, Iraq

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Corresponding author:
Raed Mohammed Abdulrahman
raed.abd2206m@comed.uobaghdad.edu.iq

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Abstract

Background: Valproic acid (VPA) is a widely used anticonvulsant and mood stabilizer, but its clinical utility is often limited by hepatotoxicity. Rheum species, commonly known as rhubarb, have demonstrated hepatoprotective and anti-inflammatory properties, but their effects on inflammatory biomarkers in VPA-induced hepatotoxicity remain unclear.

Objective: To evaluate the impact of Rheum aqueous extract on IL-6 and TNF-α levels in a rat model of VPA-induced hepatotoxicity, focusing on its potential hepatoprotective mechanisms.

Methods: Thirty male albino rats were divided into five groups: Control, VPA-induced hepatotoxicity, L-carnitine, Rheum aqueous extract, and a combination of L-carnitine and Rheum extract. VPA was administered orally at 250 mg/kg/day for 20 days to induce hepatotoxicity. Treatments with L-carnitine (250 mg/kg/day) and Rheum extract (80 mg/kg/day) were initiated post-induction for 15 days. IL-6 and TNF-α levels were measured to assess inflammatory responses.

Results: VPA treatment significantly increased IL-6 and TNF-α levels, indicating heightened inflammation. Both L-carnitine and Rheum extract independently reduced these inflammatory markers, with L-carnitine demonstrating a slightly stronger effect. However, combination therapy showed mixed outcomes: IL-6 levels were reduced, but TNF-α levels were unexpectedly elevated compared to the VPA group.

Conclusion: L-carnitine and Rheum extract exhibit significant anti-inflammatory effects individually, reducing IL-6 and TNF-α levels in VPA-induced hepatotoxicity. However, their combination revealed potential adverse interactions, emphasizing the need for further research into their combined therapeutic application. These findings contribute to the understanding of natural and synthetic interventions for mitigating drug-induced liver injury.

تقييم تأثير المستخلص المائي لنبات الراوند على المؤشرات الالتهابية في السمية الكبدية الناتجة عن حمض الفالبرويك في نموذج الجرذان

رائد محمد عبدالرحمن[1]، هدى إبراهيم القاضي[1]، وطبان عبد الله احمد[2]

[1]فرع الادوية، كلية الطب، جامعة بغداد، بغداد، العراق
[2] فرع الادوية والسموم، كلية الصيدلة، جامعة تكريت، تكريت، العراق

الخلاصة

يُعد حمض الفالبرويك من مضادات الاختلاج ومثبّتات المزاج واسعة الاستخدام، ولكن فائدته السريرية غالبًا ما تحدّها السُميّة الكبدية. أظهرت أنواع نبات الراوند (Rheum) خصائص واقية للكبد ومضادة للالتهابات، ولكن تأثيرها على المؤشرات الالتهابية في السُميّة الكبدية الناتجة عن حمض الفالبرويك ما زال غير واضح. هدفت هذه الدراسة إلى تقييم تأثير المستخلص المائي لنبات الراوند على مستويات IL-6 و TNF-α في نموذج الفئران المصابة بالسُميّة الكبدية الناتجة عن حمض الفالبرويك، مع التركيز على آليات الحماية المحتملة للكبد .تم تقسيم ثلاثين فأرًا أبيضًا ذكرًا إلى خمس مجموعات: المجموعة الضابطة، مجموعة السُميّة الكبدية الناتجة عن حمض الفالبرويك، مجموعة الكارنيتين، مجموعة مستخلص الراوند، ومجموعة الجمع بين الكارنيتين ومستخلص الراوند. تم إعطاء حمض الفالبرويك عن طريق الفم بجرعة 250 ملغم/كغم/يوم لمدة 20 يومًا لتحفيز السُميّة الكبدية. بدأت العلاجات بالكارنيتين (250 ملغم/كغم/يوم) ومستخلص الراوند (80 ملغم/كغم/يوم) بعد التحفيز لمدة 15 يومًا. تم قياس مستويات IL-6 وTNF-α لتقييم الاستجابات الالتهابية. أدى علاج حمض الفالبرويك إلى زيادة كبيرة في مستويات IL-6 وTNF-α، مما يشير إلى زيادة الالتهاب. خفض كل من الكارنيتين ومستخلص الراوند هذه المؤشرات الالتهابية بشكل مستقل، مع إظهار الكارنيتين تأثيرًا أقوى قليلاً. ومع ذلك، أظهرت المعالجة المركبة نتائج مختلطة؛ حيث انخفضت مستويات IL-6 ، ولكن مستويات TNF-α ارتفعت بشكل غير متوقع مقارنة بمجموعة حمض الفالبرويك. أظهر كل من الكارنيتين ومستخلص الراوند تأثيرات مضادة للالتهابات بشكل ملحوظ عند استخدامهما بشكل فردي، مما أدى إلى خفض مستويات IL-6 و TNF-α في السُميّة الكبدية الناتجة عن حمض الفالبرويك. ومع ذلك، كشفت المعالجة المركبة عن تفاعلات سلبية محتملة، مما يؤكد الحاجة إلى مزيد من البحث في تطبيقهما العلاجي المشترك. تساهم هذه النتائج في فهم التدخلات الطبيعية والصناعية لتخفيف إصابة الكبد الناتجة عن الأدوية .

الكلمات المفتاحية: حمض الفالبرويك، السُميّة الكبدية، مستخلص الراوند، الكارنيتين، السيتوكينات.

Introduction:

Hepatotoxicity is a significant clinical concern associated with various pharmaceuticals, including anticonvulsants such as valproic acid (VPA) [(1)]. Valproic acid is a widely prescribed anticonvulsant and mood stabilizer, effective in managing epilepsy and bipolar disorder. However, its therapeutic use is often limited by hepatotoxicity, a serious adverse effect that can lead to liver failure. The mechanisms underlying VPA-induced hepatotoxicity involve mitochondrial dysfunction, oxidative stress, and activating inflammatory pathways. Notably, elevated levels of pro-inflammatory cytokines such as interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α) have been implicated in the pathogenesis of VPA-induced liver injury [(2)].

Natural products have garnered significant attention in the search for hepatoprotective agents, due to their therapeutic potential and favorable safety profiles. Among these, species of the Rheum genus, commonly known as rhubarb, have been traditionally utilized in various medicinal systems for their anti-inflammatory and hepatoprotective properties. Phytochemical analyses have identified bioactive compounds in Rheum species, including anthraquinones, stilbenes, and flavonoids, which exhibit antioxidative and anti-inflammatory effects [(3)].

Recent studies have explored the hepatoprotective efficacy of Rheum extracts in experimental models of liver injury. For instance, the aqueous extract of Rheum palmatum demonstrated significant protective effects against carbon tetrachloride-induced hepatic damage in rats, attributed to its ability to modulate oxidative stress and suppress inflammatory cytokine production [(4)]. However, the specific impact of Rheum extracts on IL-6 and TNF-α levels in the context of VPA-induced hepatotoxicity remains underexplored.

This study aims to evaluate the effects of Rheum aqueous extract on the inflammatory

biomarkers IL-6 and TNF-α in a rat model of VPA-induced hepatotoxicity. By elucidating the potential hepatoprotective mechanisms of Rheum, this research seeks to contribute to the development of effective strategies for mitigating drug-induced liver injury.

Methods:

Plant Material

Rheum stems were procured from the medicinal herbs section of the Baghdad Bureau in Iraq. The stems were prepared for research by air-drying at ambient temperature for two weeks to eliminate contaminants. Once dried, the stems were crushed using an electric grinder. The crushed material was then stored in opaque, sealed containers at -4°C until extraction.

Preparation of Aqueous Extract

The rhubarb stems were processed to produce an aqueous extract using a cold extraction method. The stems were crushed using an electric mixer to obtain a fine powder. For extraction, 10 grams of the rhubarb powder were mixed with 100 ml of distilled water and incubated in a shaking incubator for 24 hours. The mixture was filtered through medical gauze to remove large plant particles, followed by centrifugation at 4400 rpm for 7 minutes. The filtrate was further refined using Whatman No. 1 filter paper to remove impurities and insoluble residues. The resulting aqueous extract was stored in glass containers at -4°C until use [(5)].

Experimental Design

This study included 30 male albino rats aged 5-8 weeks and weighing between 150-275 g. The rats were acclimated for one week at the Medicine College of Baghdad University, where they were provided with a standardized pellet diet and free access to water. The rats were randomly selected and assigned to experimental groups using a stratified random sampling technique to ensure comparable weight and age distribution across groups.

The animals were divided into five groups as follows:

Control Group; Received 1 ml of distilled water per day via intraperitoneal (i.p.) injection for 15 consecutive days.

The Valproic Acid group administered valproic acid at a dose of 250 mg/kg/day, orally for 20 consecutive days to induce toxicity.

Third L-Carnitine Group; received L-carnitine at a dose of 250 mg/kg/day orally for 15 consecutive days following VPA induction.

Fourth Rhubarb Extract group; administered rhubarb aqueous extract at a dose of 80 mg/kg/day orally for 15 consecutive days following VPA induction [(6)].

The fifth combination group was treated with a combination of L-carnitine and rhubarb aqueous extract following VPA induction.

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

Statistical analysis

A one-way ANOVA test was used to compare the difference in mean between the groups; a p-value was considered significant if it was less than 0.05. All analyses were carried out using GraphPad Prism version 10.1.2 and Microsoft Excel 2019.

Results:

The 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 figure1 and table1.

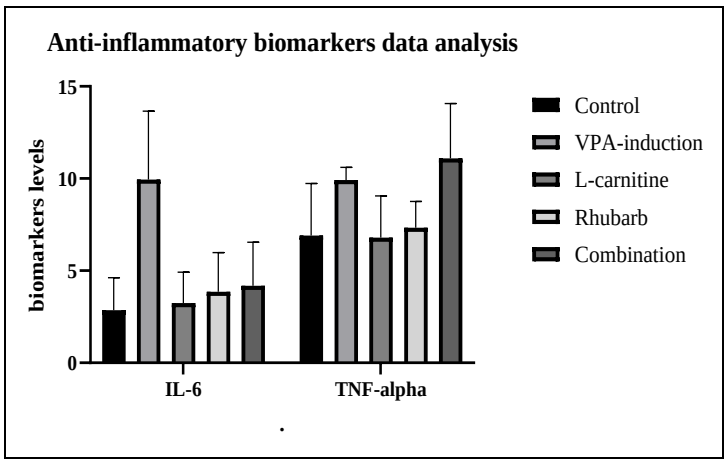

Figure1: Effect of VPA, L-carnitine, Rhubarb, and mixture of L-carnitine and Rhubarb on inflammatory biomarkers

Table 1: Effect of VPA, Rhubarb, and mixture of Rhubarb and L-carnitine on inflammatory biomarkers.

Group	IL-6 (M±SD)	TNF-α (M±SD)
Control group	2.86 ± 1.76	6.91 ± 2.82
Induction group	9.95 ± 3.71	9.92 ± 0.69
L-carnitine treatment	3.24 ± 1.68	6.79 ± 2.27
Rheum extract treatment	3.86 ± 2.12	7.33 ± 1.42
Combination treatment	4.18 ± 2.36	11.09 ± 2.98
P-Value	0.001151	0.01947

The results explained the anti-inflammatory potential of L-carnitine and Rheum extract in mitigating the effects of valproic acid (VPA)-induced inflammation. In the induction group, exposure to VPA led to a marked increase in both IL-6 and TNF-α levels, reflecting significant inflammatory activity. However, treatment with either L-carnitine or Rheum extract resulted in a noticeable reduction in these inflammatory markers, indicating their ability to counteract the pro-inflammatory effects of VPA.

L-carnitine demonstrated a strong anti-inflammatory effect, significantly lowering both IL-6 and TNF-α levels compared to the VPA group. Similarly, Rheum extract treatment also reduced these markers, though the effect was slightly less pronounced than that of L-carnitine. This suggests that both agents have distinct but effective mechanisms in modulating the inflammatory response.

Interestingly, the combination treatment of L-carnitine and Rheum extract showed mixed results. While it effectively reduced IL-6 levels, it unexpectedly elevated TNF-α levels above those observed in the VPA group. This outcome suggests a potential interaction between the two treatments that might enhance pro-inflammatory pathways, warranting further investigation into their combined use. Overall, these findings underscore the therapeutic potential of L-carnitine and Rheum extract as individual treatments for managing VPA-induced inflammation. However, their combined application requires careful evaluation due to the possibility of an adverse interaction.

Histopathological 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.

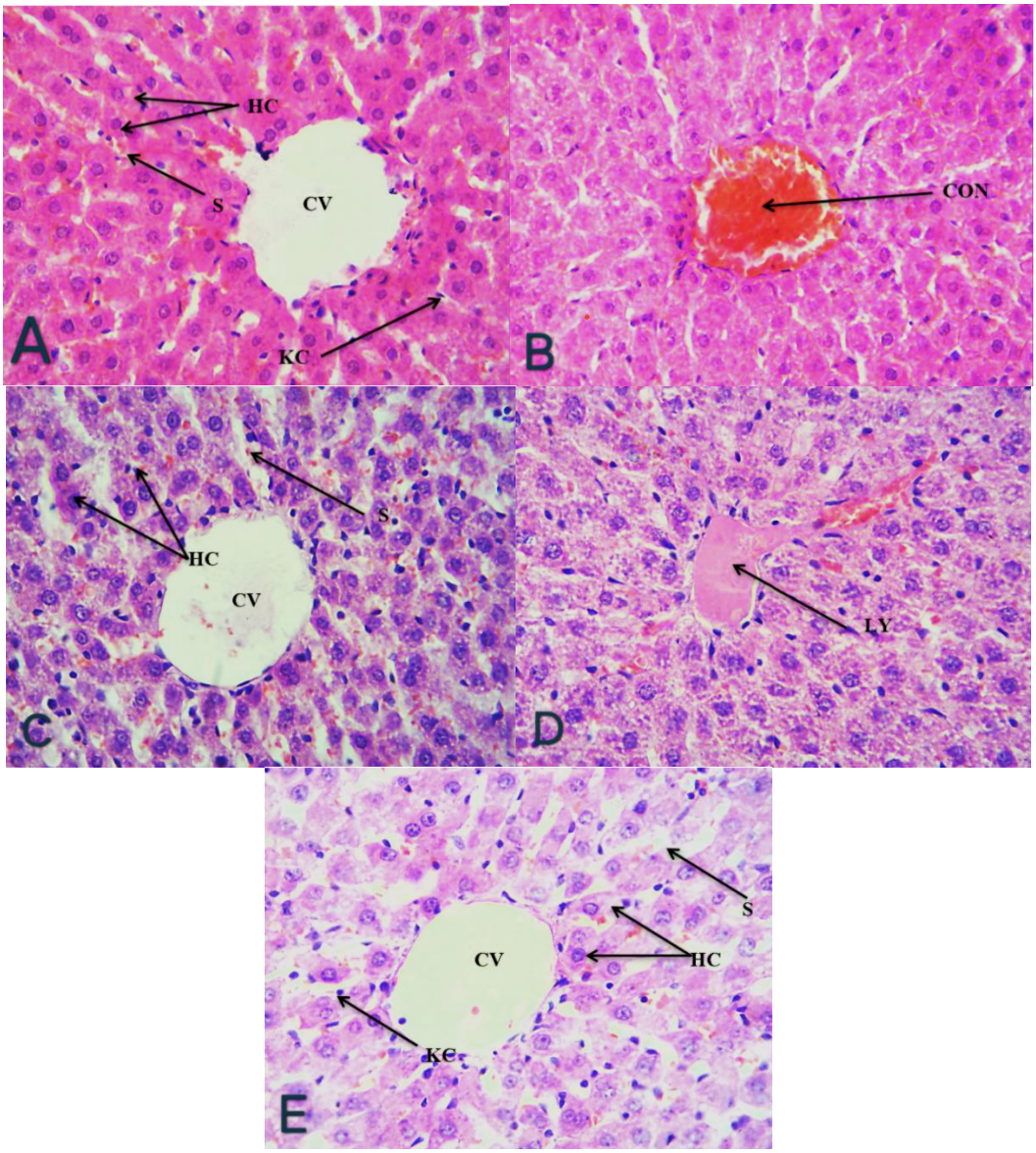

Figure 2: liver tissue section (H&E 400X) of (A) control group; (B) VPA-treated rats; (C) L-carnitine-treated rats after induction by VPA; (D) Rheum-treated rats after induction by VPA; (E) L-carnitine and Rheum-treated rats after induction by VPA.

Discussion

This study highlights the anti-inflammatory effects of L-carnitine and Rheum extract on valproic acid (VPA)-induced inflammation, as indicated by changes in IL-6 and TNF-α levels. VPA is known to induce oxidative stress and elevate pro-inflammatory cytokines, contributing to hepatic toxicity and other adverse effects [(7)]. The significant increase in IL-6 and TNF-α levels in the VPA-treated induction group aligns with existing literature on VPA-induced inflammatory responses.

L-carnitine demonstrated a notable anti-inflammatory effect by significantly reducing both IL-6 and TNF-α levels compared to the induction group. Recent studies have shown that L-carnitine mitigates inflammation by enhancing mitochondrial function and exerting antioxidant properties, which suppress oxidative stress and inhibit the activation of nuclear factor kappa B (NF-κB), a key regulator of pro-inflammatory cytokine production [(8)(9)]. For instance, L-carnitine has been reported to attenuate inflammatory responses in hepatic tissues by modulating the NF-κB signaling pathway, leading to decreased expression of IL-6 and TNF-α [(10)].

Similarly, Rheum extract exhibited significant anti-inflammatory effects, reducing IL-6 and TNF-α levels, though slightly less pronounced than L-carnitine. Rheum species contain bioactive compounds like anthraquinones and stilbenes, which have been shown to possess anti-inflammatory properties [(11)]. Recent research indicates that Rheum extract can inhibit pro-inflammatory mediators by modulating signaling pathways such as mitogen-activated protein kinase (MAPK) and NF-κB [(12)]. These mechanisms correspond with the observed decreases in inflammatory cytokines in the Rheum-treated group.

The combination treatment of L-carnitine and Rheum extract, however, presented unexpected results. While IL-6 levels were reduced, TNF-α levels were elevated compared to the induction group. This paradoxical effect suggests a potential interaction between L-carnitine and Rheum extract that may influence cytokine production differently when combined. Recent studies have reported similar findings where combinations of natural compounds resulted in antagonistic effects or unexpected modulation of specific cytokines [(13)]. This indicates that the combined use of certain bioactive agents may not always produce additive or synergistic anti-inflammatory effects and could potentially activate alternative inflammatory pathways.

These findings emphasize the importance of understanding the interactions between different therapeutic agents. While L-carnitine and Rheum extract individually show promise in attenuating VPA-induced inflammation, their combined use may require caution. Further research is needed to elucidate the underlying mechanisms of their interaction and to determine optimal dosing strategies that maximize therapeutic benefits while minimizing potential adverse effects.

Conclusion

This study explained the hepatoprotective and anti-inflammatory effects of L-carnitine and Rheum aqueous extract against valproic acid (VPA)-induced hepatotoxicity. Both treatments significantly reduced inflammatory biomarkers (IL-6 and TNF-α) and improved liver tissue structure, with L-carnitine showing slightly stronger effects. However, their combination unexpectedly increased TNF-α levels, suggesting a potential interaction requiring further investigation. These findings emphasize the therapeutic potential of L-carnitine and Rheum extract individually, while caution is needed when combining them.

Future studies should explore their interactions to optimize efficacy and safety.

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