Journal of Current Medical Research and Opinion

CMRO 09 (05) 4798-4806 (2026)
DOI: https://doi.org/10.52845/CMRO/2026/9-5-2
www.cmro.in

CMRO
ISSN (O) 2589-8779
(P) 2589-8760

Original Research

An Aqueous Extract of *Rosmarinus officinalis* Induced Hepatotoxicity in Male Albino Rats

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Abstract

Background: Because it induces oxidative stress and the loss of cellular glutathione, acetaminophen (paracetamol) is still a leading cause of drug-induced liver injury (DILI) worldwide. Further investigations are required to prove the unique hepatoprotective effect of the aqueous extract of the medicinal plant Rosmarinus officinalis (Rosemary) on Paracetamol toxicity. Rosemary is well-known for its antioxidant properties.

Objective: To find out if an aqueous extract of Rosmarinus officinalis can protect male albino rats from hepatotoxicity.

Methods: One hundred twenty-four rats aged 13-14 weeks and weighing from 225-250 grams were used for the research. There were 6 rats per each of 4 groups. G1, the control group that did not receive any treatment, was the first one. The second group G2 was the control group who were given 25 mg/kg of paracetamol. The third group (G3) was treated with aqueous rosemary extract (200 mg/kg). The oral dose of Paracetamol (25 mg/kg) was given to each group every 2 hours after giving an aqueous extract (200 mg/kg) in Group 4 (control group). All oral administration was made through the gavage tube in the 60 day trial.

Results: When paracetamol was dosed, there was a significant reduction ($P \leq 0.05$) in average GSH and CAT levels and significant rise ($P \leq 0.05$) in average MDA, ALT, and ALP levels as well as significant increase in the liver impairment score, indicating that this group was better than the control group. In the control group, no significant difference was found between the levels of AST, ALT and ALP at 200 mg/kg ROAE. With 200 mg/kg ROAE+25 mg

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Received: 21.02.2026 | **Revised:** 09.05.2026 | **Accepted:** 16.05.2026 | **Published:** 23-05.2026

Paracetamol group, however, there was a significant decrease in AST, ALT and ALP when compared with Paracetamol group. This indicates antioxidant and enzyme activity has been restored to normal level after pre-treatment with the aqueous rosemary extract. The significant increase in GSH and CAT levels and the significant decrease in MDA levels in the ROAE + paracetamol group when compared with the control group indicated that there was a remarkable improvement in the activity of these antioxidant enzymes. Histological examination showed that significant cell infiltration and central tubule degradation were present in the group receiving paracetamol. On the other hand, the groups that were given rosemary showed significant improvement. Preserved hepatocyte morphology, decreased necrotic areas and minimal inflammatory response indicated a strong regenerative response.

Conclusion: Based on its bioactive component content, which helped stabilize metabolism and neutralize hepatocyte membrane, the study found that the aqueous rosemary extract had a hepatoprotective effect against paracetamol injury.

Keywords: *Rosmarinus officinalis*, Aqueous extract, hepatotoxicity, Albino rats

Introduction

The liver has a great number of metabolic processes such as lipid, protein and glucose metabolism, and is an integral part of every mammalian organism, which is processing the substances that come in, detoxifying them, and maintaining biochemical balance. This is because the liver is especially exposed to environmental pollutants and pharmaceuticals due to its unique location within the body that receives blood directly from the digestive tract through the portal vein (Saleem et al., 2010). Drug-induced liver injury is closely associated with high morbidity and mortality rates worldwide, and is one of the most important clinical issues faced by contemporary medicine (Bosch et al., 2006). Similarly, Paracetamol (Acetaminophen) is one of the most widely used over-the-counter medicines in the world; it is used for fever and to relieve pain and is not prescription-only. It is used at therapeutic doses in the human body without any side effects, however, at higher doses can lead to severe liver problems with sudden liver failure and, in many cases, liver transplantation (Hasanein and Sharifi, 2017). Paracetamol is white, odourless, crystalline and dissolves in water. The chemical preparation of the compound involves three steps, in the first step, it gets reduced to amine group, with molecular weight 151.16 g/mol, melting point 169-170°C, pH at 25°C is 5.3-6.5, density is 1.293 g/cc and molecular formula is C8H9NO2. The next step is to mix para-aminophenol with acetic anhydride to create acetaminophen (Iloamaeke and Iwuozor, 2018). It was discovered by a chemist, Harmon Northrop Morcy, in the late 1800s, and first prepared in 1878. It had been used clinically as a fever reliever by Vaughn Mering in 1887, but didn't get used as a medicine for 15 years (Ogemdi, 2019). Rosemary is an herbaceous plant with a strong aroma and is a member of the mint family (Lamiaceae) and is usually shrubby, height of up to 1,5 m. It can be used from 20 to 35 degrees Celsius, but can withstand lower temperatures. It has thin, long simple opposite evergreen leaves with sharp tips that are curved. The length of the leaves is 2-4 cm and their width is 2-5 mm. On top, they have a glossy dark green colouration with golden yellow or silvery-white specks; underneath, they have a pale green hue. They have prominent midrib and are finely haired in white. An analysis using high-performance liquid chromatography–mass spectrometry (HPLC–MS/MS) showed that the water-based rosemary extract is abundant in the phenolic acids and diterpenes that give the plant its antioxidant properties (Mecarthy et al., 2001). The aqueous extract mostly contains rosmarinic acid, a phenolic chemical that has exceptional free radical scavenging and DNA protective properties. Two

chemicals in the plant, carnosic acid and carnosol, have the remarkable property of stabilizing and protecting the cell membranes from being damaged (Kola et al., 204). This study aimed to investigate the level of liver tissue and physiological parameters injuries caused due to paracetamol overdose in rats and to evaluate the protective effect of rosemary extract against these injuries.

Material and Methods

Animals' samples

Twenty-four (24) male rats Rattus rattus were used for this study, and were obtained from the laboratory of College of Education for Pure Sciences at the University of Karbala. The average age was 12-14 weeks and the average weight was 200-250 grams. These were retrieved from the 'Animal House' at the University of Karbala, College of Pharmacy. They were placed in plastic cages equipped with metal lids, which are ratproof. With great care, wood shavings were spread down on the floor of the cages, and they were changed periodically. The animals received water and animal feed ad libitum (available from the nearby markets) at a temperature of 25 °C with adequate ventilation. Each cycle of 12 hours of light and 12 hours of darkness was used. The animals were acclimatised for two weeks before the experiment was started (Kaman and Dutta, 2019).

Collecting and harvesting different plants

Rosemary (R. officinalis) was scientifically recognised by the University of Karbala, Iraq as purchased from local marketplaces of Karbala. The leaves were rinsed to wash away any debris, and left to dry in the shade, with constant mixing. Crushed in a blender to get a fine powder. The mixture of 20 grammes of powder with 400 millilitres of distilled water was allowed to sit at room temperature for a full day. Impurities were removed from this mixture by filtering it through multi-layered medical gauze. The next step was to spin-fry it for 10 minutes at 3000 rpm, wash it with filter paper, and then dry it in the oven until it reached 40°C. Last but not least, it was chilled before being given to animals in a 200 mg/kg solution after being dissolved in water (Hernandez et al., 1994).

Designing experiments

Twenty-four male white rats were used in the study (6 rats per group). For 2 months the following procedures were carried out: Negative Control Group (G1) No therapy was given to the animals. Aqueous Extract of Rosemary was administered orally to the animals at a dose of 200 mg/kg body weight for 2 months (G2) For the same period of time, aqueous extract of rosemary was orally administered at a dose of 200 mg/kg body weight, followed by oral administration of paracetamol to the animals (G3) Taking 25 milligrams per kilogram of body weight every day for 30 days. At the end of the experiment enough blood was removed from the heart by a heart puncture. This blood was then placed in the tubes which were free from the anticoagulant (Gel tube). It was allowed to coagulate for half an hour and then subjected to centrifugation in the centrifuge at 3000 r.p.m. for 15 mins, removing the serum. The serum was carefully divided into several sterile tubes and stored at -20 degrees of cold temperature prior to testing the liver enzymes (ALT, AST, ALP) and antioxidants (MDA, CAT, GSH). The liver was then extracted and stored in formalin for the preparation of histological sections.

Markers were identified with the help of histopathology.

At the end of the experiment the animals were sacrificed. Subsequent, nasal biopsies were taken and stored in formalin 10%. Later these sections would be treated serially histologically to prepare for the study. The sections were stained with eosin & haematoxylin and studied at 10x under electron microscope (Suvarna et al., 2013).

Analysing Biochemical Processes

The serum biochemistry level of GSH, CAT, Hadwan and Kadhum (2018), MDA, ALT, AST and ALP were measured after the end of the

experiment by Muslih et al. (2001), Hadwan and Kadhum (2018), and Bergmeyer et al. (1986).

Analyze data using statistics.

The data was analyzed statistically using SPSS software. Duncan's test was used to compare the results based on the least significant difference (LSD) at a probability threshold of 0.05 (Moder, K. 2010).

Results

The effect of Paracetamol and ROAE on liver enzymes level.

The AST, ALT, and ALP levels were significantly lower in the Paracetamol groups when compared with the control groups (Table 1). AST, ALT and ALP levels were not significantly different between ROAE group (200mg/kg) and control group. In the ROAE + paracetamol group (200 mg/kg, 25 mg), there was however a significant reduction in AST, ALT and ALP when compared to the paracetamol group.

Table 1. We used SPSS to do the statistical analysis. Significance was determined by using LSD and Duncan's tests at the 0.05 level. A special comment is provided for tables that employ Duncan's test. Results with the same letter are not significantly different.

Treatment	Means± S.E		
	ALT (U/L)	AST (U/L)	ALP (U/L)
Control (G1)	35.93±1.17 c	60.79 ±1.95 c	181.45±1.30 c
Paracetamol (G2)	65.41±1.68 a	86.25±2.52 a	225.95 ±0.83 a
ROAE (G3)	38.18±0.67 c	63.79±1.14 c	180.33±0.85 c
Paracetamol + ROAE (G4)	43.20±0.79 b	70.93±0.56 b	183.03 ±0.96 c
LSD	3.453	5.152	3.229
P (VALUE)	0.05	0.05	0.05

The impact of paracetamol and ROAE on the levels of liver enzymes

The GSH and CAT levels were significantly lower while the level of MDA was significantly higher in the paracetamol group when compared to the control group (Table 2). The ROAE + paracetamol (200 mg/kg, 25 mg) group showed significant elevation of GSH and CAT level while there was significant decrease in MDA level compared to the control group, which was also seen in the ROAE group.

Table 2. We used SPSS to do the statistical analysis. The LSD and Duncan's tests were conducted at 0.05 level of significance. There is a special comment for tables which use Duncan's test. The results with common letters are not significantly different from each other.

Treatment	**Means± S.E**		
	GSH **µmol/L**	CAT **µmol/L**	MDA **µmol/L**
Control (G1)	77.95± 0. 38 b	31.46± 2 . 08 b	8.19± 0.05 b
Paracetamol (G2)	44.56± 0. 66 d	19.70± 0. 28 d	17.21 ± 0.45 a
ROAE (G3)	85.25± 0. 48 a	34.90 ± 0. 33 a	4.50 ± 0.08 d
Paracetamol + ROAE (G4)	73.21± 1.61 c	26.85± 0. 89 c	7.95 ± 0.33 c
LSD	2.42	3.29	0.71
P (VALUE)	0.05	0.05	0.05

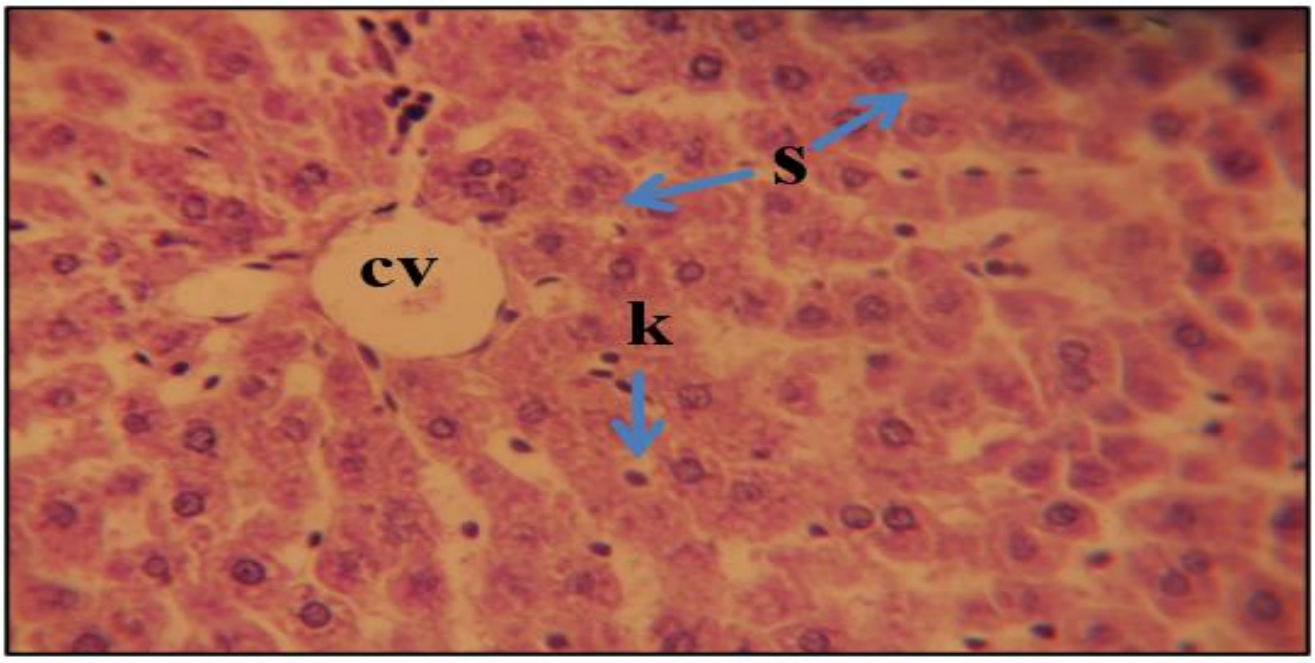

Image (1). transverse section of liver tissue of the control group, It shows hepatocytes (H), central vein (CV), Kupffer cells (K), and sinusoids (S).(400X) H&E.

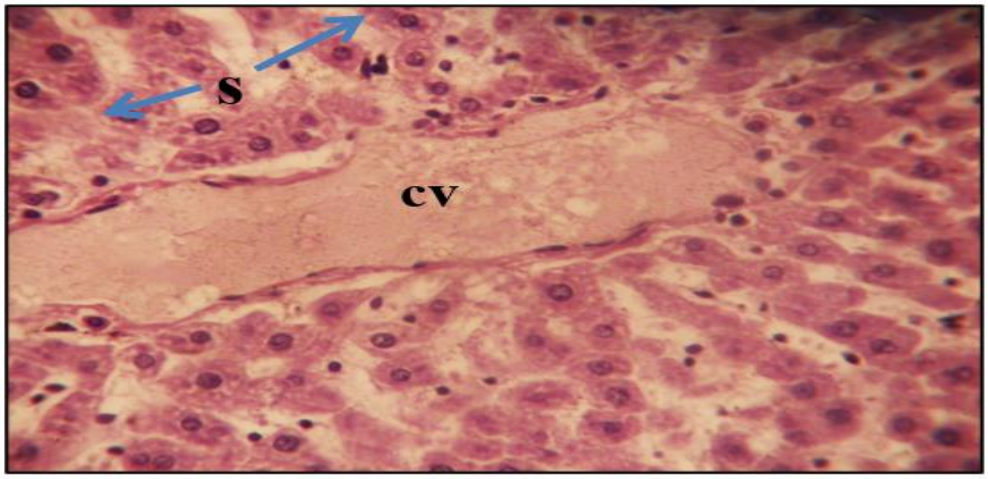

Image (2) transverse section of liver tissue of the paracetamol group, the central vein (CV) exhibits marked dilation and severe congestion, filled with an accumulation of eosinophilic proteinaceous material and cellular debris, accompanied by a noticeable disruption and focal denudation of its endothelial lining.(400X) H&E.

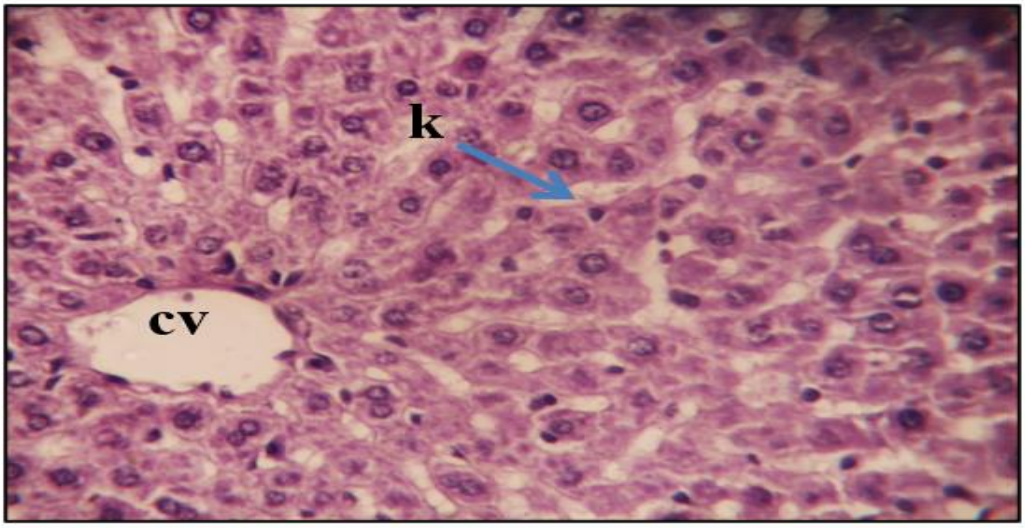

Image (3) transverse section of liver tissue of the aqueous extract of Rosmarinus officinalis group, It shows hepatocytes (H), central vein (CV), Kupffer cells (K), and sinusoids (S).(400X) H&E.

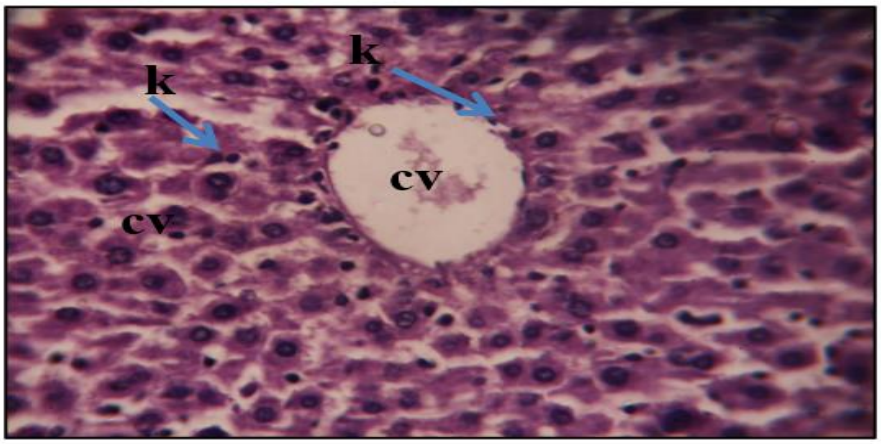

Image (4) transverse section of liver tissue of the aqueous extract of (Rosmarinus officinalis + paracetamol) group, it shows mild inflammation with normal liver tissue appearance, where hepatocytes (H), are show normal, central vein (CV), Kupffer cells (K), and sinusoids (S).(400X) H&E.

Discussion

The antioxidants glutathione (GSH) and catalase (CAT) were seen to be low in the paracetamol group while the malondialdehyde (MDA) was seen to be high. This may be due to poor functioning of the body's own antioxidant mechanisms, which disrupts the normal balance in the body. When the free radical level increases and defence mechanisms of the body are impaired, the function of organs and tissues gets reduced (Hajam et al., 2022). The result confirms the use of malondialdehyde (MDA) as a good marker to reflect the level of oxidative stress in tissues, which is a byproduct of fatty acid oxidation in cell membranes, representing the level of damages to the cells in the treated group (Wang et al., 2017). However, we observed that antioxidant level has increased and MDA level has decreased after the aqueous rosemary extract treatment in our study. The presence of such antioxidants such as rosmarinic acid in this plant may indicate its antioxidant activity, which helps to lower the production of free radicals and suppress enzymes that facilitate lipid oxidation. Everything is consistent with the findings of (Malo et al., 2011) which was carried out.

In this study, paracetamol treatment led to a significant increase in liver enzymes ALT, AST and ALP. The liver is one of the most important organs as it contains many enzymes that are important in the metabolism of proteins, carbs and fats, but it can also be subjected to a wide range of pollutions including chemicals present in medicines (Kowsalya et al., 2015). Paracetamol is hepatotoxic, which affects the liver cells (hepatocytes) leading to increased cytoplasmic volume that converts hepatotoxins to free radicals, which affects liver enzymes. When the free radicals penetrate the cell membranes, lipid peroxides can be generated as a result of damage to unsaturated fatty acids (Bomgning et al., 2021). The results of this study support the results of (Rajesh and Latha,2004) that changes in the mitochondrial functional unit of liver cells cause liver damage. Liver cells release cellular enzymes, such as ALT, AST, and ALP, when they are damaged. As a result, following treatment high blood serum levels of these enzymes trigger acute cholestasis and viral hepatitis. Kanakamani et al. (2018) said that liver injury due to paracetamol and reduced liver secretion are two mechanisms which may contribute to a small to moderate rise in transaminases which are secreted by the cells of the hepatic duct or the hepatocytes. Treatment with an aqueous extract of rosemary brought the levels of the liver enzymes back to normal. The enzymes were significantly lower in the paracetamol group compared to the protective group which received rosemary aqueous extract along with paracetamol. The results of our study are similar to those obtained by Fadlalla and Galal (2020). They found that the aqueous extract could protect subjects from the acute elevation of ALT, AST and ALP levels as a result of the administration of 1000 mg/kg of paracetamol after 30 days. This may be owing to the presence of active bioactive compounds in the extract, such as phenolic acids, flavonoids such as luteolin, apigenin, hesperidin and rutin, which can help to stabilize the membranes of liver cells.

The paracetamol group had pathological changes in the liver tissue as noticed in the histological examination by Hasanein and Sharifi (2017). These changes ranged from widespread necrosis in the central lobular area to dilation and congestion. These changes may be attributed to the metabolic pathway of paracetamol, which by itself is mainly metabolised by glucuronidation and sulfation at therapeutic doses in the liver. The final product consists of a group of chemicals which are harmless and excreted in the urine. These pathways are used when the liver is overloaded, however, during an overdose it must use an alternate pathway with the help of the cytochrome P450 enzyme, CYP2E1. The second route produces a very reactive intermediate called N-acetyl-p-benzoquinoneimine (NAPQI) according to Wahid et al. (2016). Under typical circumstances, the most important endogenous antioxidant in the liver, reduced glutathione, is able to neutralise the hazardous molecule NAPQI. If GSH becomes depleted, however, free NAPQI

may interact with mitochondrial/cellular proteins, resulting in mitochondrial dysfunction and programmed cell death or necrosis in liver toxicity. Raised liver enzymes clinically imply this chain reaction has triggered lipid peroxidation (Shao et al., 2007) in cell membranes and enhanced the production of ROS. In the rats given aqueous Rosemary extract, the normal arrangement of hepatic cords around the central vein was maintained, which showed good protection. The microscopic pictures also reveal that the inflammation and necrosis of the hepatocytes were reduced or completely disappeared and the size of the nucleus and the cytoplasm came back to its normal size. This proves that rosemary can protect organs and tissues from chemical toxins, such as those found in Elmetwally et al. (2024) and Carnosol, carnosic acid, and rosemanol, which are potent compounds in neutralising reactive oxygen species (Guimarães et al., 2023).

Conclusion

The results demonstrate that rosemary extract is very effective in decreasing Paracetamol-induced liver injury. The strong antioxidant activity found in this natural extract is mainly responsible for its hepatoprotective effects.

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