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Effect of *Rosmarinus officinalis* Ethanolic Extract on Liver Function in Wistar Rats: Biochemical and Histological Study

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ABSTRACT

Traditional medicine is widespread and the number of studies in the search for new molecules capable of preventing or even delaying the onset of complications related to liver dysfunction remains very limited. In Morocco, *Rosmarinus officinalis* (RO) is widely used for treating various liver disorders. The study aimed to assess the hepatoprotective effect of RO in Wistar female rats. Thirty Wistar female rats were divided into 5 groups; the control group received orally distilled water, while the other 4 groups received the ethanolic extract of RO at doses of 200, 300, 500, and 1000mg/kg/day administered for 28 days. At the end of the treatment, the rat blood samples were taken for biochemical analysis, including cholesterol, alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT), glucose, total cholesterol, and triglycerides (TG). Oxidative stress biomarkers, nitric oxide (NO), and the antioxidant enzyme catalase (CAT) were assessed in the liver. Samples of liver from each treatment group were histopathologically examined. The results show no notable hemolytic changes in erythrocytes, hematocrit, granulocytes, and leukocytes ($P>0.05$). No significant differences in biochemical and histological parameters; however, the 200, 300, and 500mg/kg of RO ethanolic extract exhibited antioxidant activity in the liver, as indicated by increased CAT levels and decreased NO levels. While 1000mg/kg exerts opposite effects, including an increase in the level of NO and a decrease of CAT compared to the control group ($P>0.05$). This study concluded that low doses of RO ethanolic extract showed appreciable protective effects on the liver by decreasing oxidative stress markers, suggesting using RO as a dietary supplement for patients with liver disorders.

Key words: *Rosmarinus officinalis*, Liver, Oxidant activity, Histology, Wistar rats.

INTRODUCTION

Liver dysfunction in humans and animals represents a significant health concern (Nevzorova et al. 2020). The liver plays a central role in the synthesis of body proteins (Vašková et al. 2023), storage of nutrients (Ozougwu 2017), recycling of larger molecules (Nanayakkara et al. 2023), detoxification of waste material (Kim et al. 2021), and body metabolism (Dutta et al. 2021). Various factors, including infectious agents (bacteria, viruses, and parasites), exposure to mycotoxins, chronic inflammation, and metabolic disorders, are the main causes of liver dysfunction (Bajaj et al. 2012; Awuchi et al. 2022; Qing et al. 2022).

Clinical signs of hepatic dysfunction include jaundice, weakness, anorexia, weight loss, abdominal pain and distension, and in severe cases, hepatic encephalopathy (Sivakrishnan and Pharm 2019). Laboratory findings typically revealed elevated liver enzymes including catalase, alanine transferase, and alkaline phosphatase, changes in bile concentration, and impaired coagulation profiles (Kalas et al. 2021). Histopathological changes may include hepatocellular degeneration, necrosis, fibrosis, and fatty infiltration (Ali et al. 2021).

In Morocco, traditional medicine is widespread. However, knowing that hepatopathies are a real scourge in Morocco, the number of studies in the search for new molecules capable of preventing or even delaying the onset of complications related to liver dysfunction remains very limited (Guariglia et al. 2023). For several years, development has been seen in the case of drug-induced nephrotoxicity. The liver is an organ that is particularly vulnerable to the toxicity of drugs present in the body (Karie et al. 2010). Drug-induced hepatotoxicity can be a sign of intoxication (dose-dependent phenomenon) or of an immunoallergic or vasomotor process with a normal dosage. The mechanisms are generally intertwined, but one of them predominates (Iluz-Freundlich et al. 2020).

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Medicinal plants have become one of the most accessible sources for improving human health due to their richness in bioactive compounds, also known as phytochemicals (Chakit et al. 2022a; Brikat et al. 2024; Babou et al. 2025b). These compounds serve as precursors for the synthesis of natural and synthetic drugs, as well as cosmetics and dietary supplements (Getachew et al. 2022; Kherrab et al. 2024a). Plant-derived drugs are readily available, less expensive, effective, and rarely exhibit side effects. Plants, which have been used for medicinal purposes for thousands of years, are the most obvious choice for current research aimed at discovering new therapeutically effective drugs (Getachew et al. 2022).

The study of the hepatoprotective activity is dictated to us by the reputation that this plant has, within the Moroccan population, in the treatment of various liver illnesses and in the treatment of different forms of cysts. According to several herbalists, this plant is also recommended in the treatment of kidney stones; part of this work consists of verifying this activity on urinary lithiasis induced in laboratory animals (Chakit et al. 2022b).

In our study, we focused on rosemary (*Rosmarinus officinalis*), a member of the Lamiaceae family, chosen for its antioxidant and antimicrobial properties. It is widely used in pharmaceutical products and traditional medicine. Rosemary, considered to have both condiment and dietary uses, is commonly found in Mediterranean cuisine; it is also a popular honey plant. Rosemary is also included in List A ("medicinal plants traditionally used in allopathy and, for some of them, in homeopathy") of medicinal plants listed in the French Pharmacopoeia (11th edition, 2012). A medicinal plant or herbal drug is defined by the French and European Pharmacopoeias as a plant or part of a plant, used as is, most often in dried form or fresh, at least a part of which has medicinal properties. It is an aromatic herb appreciated for its aromatic, antioxidant, anticancer, antifungal, and antibacterial properties, and is widely used in traditional medicine. It is the subject of recent research in the culinary, cosmetic, and agri-food fields. This plant contains a large number of substances that are used in industry, food, cosmetology, and pharmacy. Among these compounds are alkaloids, tannins, terpenes, and flavonoids. The study aimed to assess the hepatoprotective effect of RO in Wistar female rats.

MATERIALS AND METHODS

Plant preparation

RO was harvested in July 2023 in the Ras Lma region of Taza, Northern Morocco. The leaves were dried at room temperature (25 ± 3°C), in the open air and protected from light to preserve the integrity of the molecules as much as possible, then finely ground using an electric grinder; grinding followed directly by sieving (250-500µm), resulting in a fine powder and a higher extraction yield. 100g of RO leaf powder was extracted with 500mL of 95% ethanol in a Soxhlet extractor. After extraction, the solvent was removed from the extract using a rotary evaporator operating at 60°C for 30 minutes at a speed of 2,000rpm, resulting in a solid, sticky residue.

Healthy female rats were divided according to their weight homogeneity into 5 groups (n = 6/group). One group of these animals (control group) received orally distilled water, while the other 4 groups received the ethanolic extract of RO at doses of 200, 300, 500, and 1000mg/kg/day administered for 28 consecutive days. At the end of the treatment, the rats spent the night fasting but with free access to water. Blood samples were taken for hematological and biochemical study, for the dosage of biomarkers of oxidative stress including nitric oxide (NO), and the antioxidant enzyme catalase (CAT) in the liver, as well as the study of histological sections of this target organ.

Biochemical analysis

Biochemistry analysis was performed using a spectrophotometer (J.P. SELECTA, s.a. Autovia, Abrera, Spain) for the following parameters: cholesterol, alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT), glucose, total cholesterol (CHO), and triglycerides (TG).

Determination of oxidative stress markers

NO determination

The method used to assess NO production from organ homogenates (liver and kidneys) is based on determining the concentrations of the end products of NO synthesis, namely nitrates and nitrites, as described by Bryan and Grisham in 2007 (Kherrab et al. 2024b). This determination was carried out using the Griess reagent, consisting of solution A (0.1% naphthylethylene diamine dichlorohydrate) and solution B (1% sulfanilamide). The experimental procedure involved mixing 100µL of Griess reagent with 100µL of the nitrite-containing sample in a spectrophotometer cuvette. The mixture is incubated for 30 minutes at room temperature, followed by measurement of optical density at 548nm, following the method of Chao et al. 1992. NO levels were expressed in µmol/g of tissue (Bryan and Grisham 2007; Elkaoui et al. 2025).

Determination of catalase activity (CAT)

Assessment of catalase activity in organ homogenates was performed following the method developed by Aebi (1984). This technique was based on the measurement of optical density changes resulting from H_2O_2 decomposition. For each sample, a quantity of 60µl (tissue extract or phosphate buffer per sample) was combined with 2340µl of phosphate buffer (0.05mM, pH 7.4) in a quartz cuvette. Initiation of the reaction was achieved by adding 600µl of H_2O_2 (1 M), and absorbance was recorded over 2 minutes (with readings every 30 seconds) at 240nm. Catalase activity is expressed in international units per minute per gram of tissue (IU/min/g tissue) or µmoles of H_2O_2 destroyed per minute per gram of tissue at 25°C (Aebi 1984; Babou et al. 2025a).

Histological assessment

Liver samples from each rat were examined for histopathological assessment. After fixation in 10% formalin, tissues were dehydrated and embedded in paraffin blocks. Sections 3 to 5µm thick were colored using hematoxylin and eosin (H&E). Samples were observed under a light microscope (Salokhe et al. 2020).

Statistical Analysis

The data obtained were analyzed using GraphPad software. All variables are presented as mean ± standard

error of the mean (SEM) and were compared using the one-way analysis of variance (ANOVA). Differences between control and treated groups were determined using the test of Tukey-Kramer; significance was considered at P<0.5%.

RESULTS

Glycemia

The effect of EERO on the biochemical parameter of glucose (glucose levels) was evaluated. The administration of EERO for 28 days caused a non-significant decrease in glucose levels in the groups of rats treated with the dose of 200mg/kg of EERO, while 300, 500, and 1000mg/kg of EERO induced a statistically non-significant increase in glucose levels compared to the control rats. The results for RO at glucose levels are shown in Fig. 1.

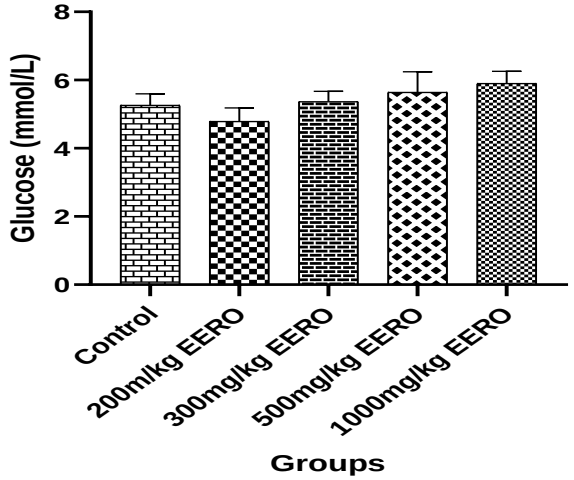

Fig. 1: Effect of *Rosmarinus officinalis* on glucose levels.

Cholesterol

The effect of EERO on the biochemical parameter of cholesterol (cholesterol levels) was evaluated. The administration of EERO at doses of 200, 300 and 500mg/kg of EERO for 28 days non-significantly decreased cholesterol levels, while the dose of 1000 mg/kg statistically non-significantly increased cholesterol levels in the treated rats compared to the control rats. The results for RO at cholesterol levels are shown in Fig. 2.

Triglyceride

Fig. 3 presents the effect of EERO on triglyceride levels. The administration of *R. officinalis* ethanolic extract for 28 days non-significantly decreased triglyceride levels in the groups of rats treated with 200 and 300mg/kg of EERO, while 500 and 1000mg/kg of EERO increased the triglyceride level in a statistically non-significant way compared to the control group.

ALAT and ASAT

Fig. 4 and 5 describe the effect of EERO on ALAT and ASAT levels. The administration of the ethanolic extract of *R. officinalis* for 28 days non-significantly decreased the levels of ALT and AST in the groups of rats treated with 200, 300, and 500mg/kg of EERO compared to the control group. The dose of 1000mg/kg of EERO non-significantly increased the level of ALAT and ASAT compared to the control group.

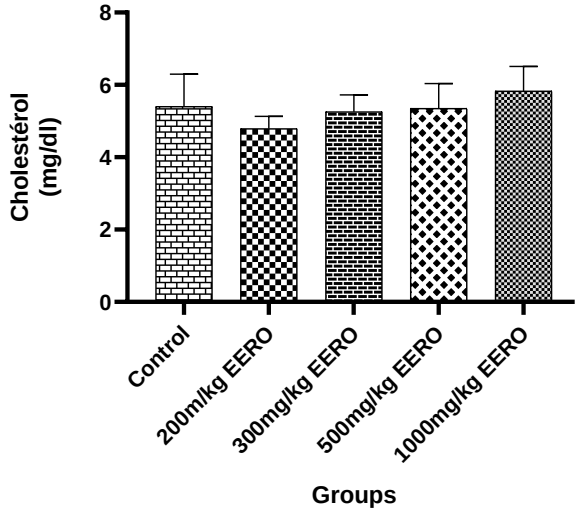

Fig. 2: Effect of *Rosmarinus officinalis* on cholesterol levels.

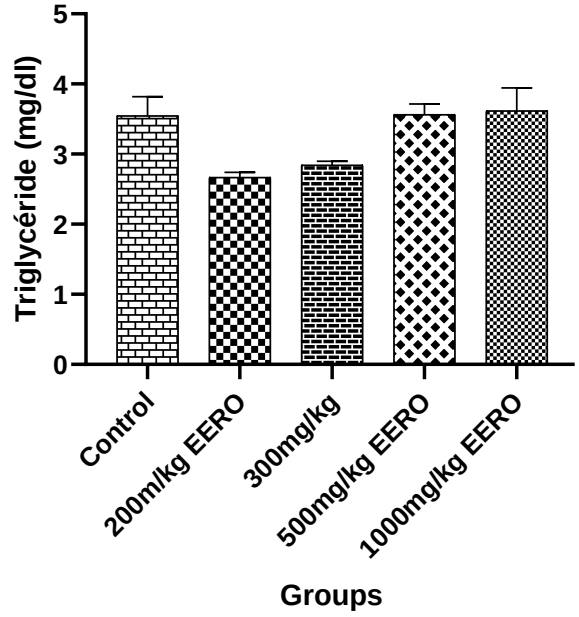

Fig. 3: Effect of the ethanolic extract of *Rosmarinus officinalis* on triglyceride levels (n=6).

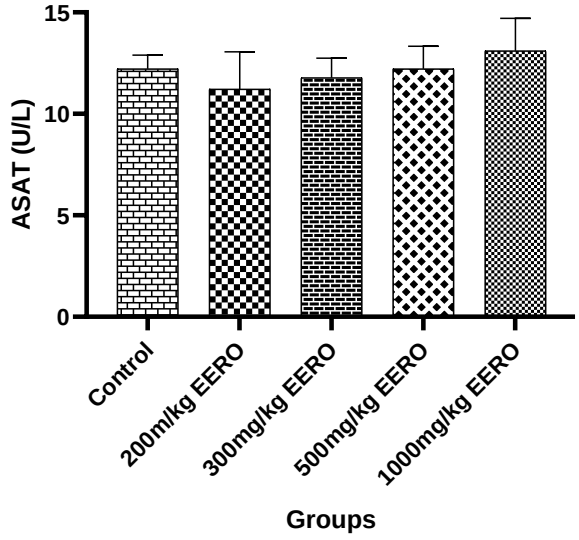

Fig. 4: Effect of ethanolic extract of *Rosmarinus officinalis* on the levels of ALAT (n=6).

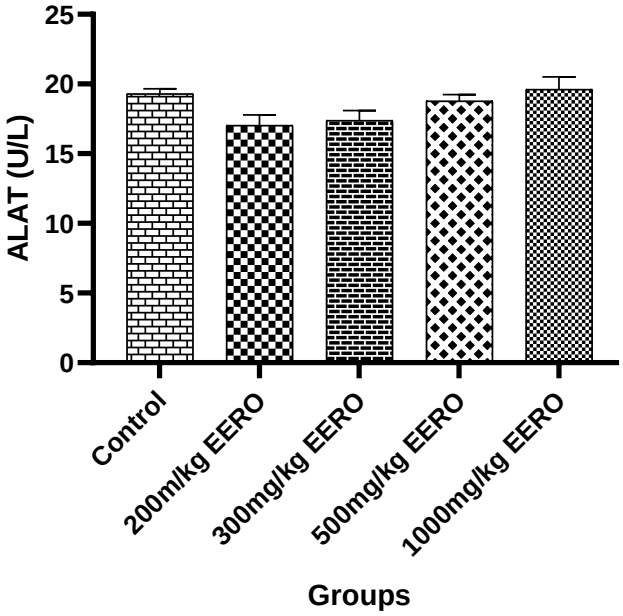

Fig. 5: Effect of the ethanolic extract of *Rosmarinus officinalis* on the levels of ASAT (n=6).

Oxidative stress assessment

Table 1 describes the effect of the ethanolic extract of *R. Officinalis* on nitric oxide (NO) and catalase (CAT) activity in rat liver tissues. The administration of 200mg/kg of ethanolic extract of *R. officinalis* produced a highly significant reduction in the levels of nitrite/nitrate (nitric oxide; NO) as compared to the control group P<0.01). A dose of 300mg/kg also caused a significant decrease (P<0.05), while 500mg/kg led to a smaller, non-significant reduction (P<0.05). In contrast, 1000mg/kg resulted in a non-significant increase in the level of NO (P> 0.05) as compared to the control group. Regarding CAT activity, the ethanolic extract of *R. officinalis* significantly increased (P<0.01) liver catalase at 200mg/kg, with an even greater significant increase (P<0.05) at 300mg/kg. Treatment with 500mg/kg EERO produced a non-significant increase, whereas treatment with 1000mg/kg caused a non-significant decrease in CAT levels as compared to the control group.

Histological analysis

It consists of an observation of whole livers from the experimental animals. It is qualitative and is limited to the external characteristics of the liver (color, volume and texture). The results of our study show that the livers of rats treated with plant extracts were good, with a normal architectural appearance of the liver cells in the form of normal nuclei and hepatocytes arranged around the central vein with normal capillaries (Fig. 6).

DISCUSSION

Medicinal plants are widely used by the Moroccan population for the prevention and treatment of several diseases, including hepatic illness. The study aimed to evaluate the hepatoprotective effects of *R. officinalis* in rats by biochemical and histological assessment. Phytotherapy is the basis of traditional medicine, which is a treatment technique based on the use of plant extracts and their active ingredients to overcome the causes and symptoms of various diseases (Aiboud et al. 2015; El Hasnaoui et al. 2015).

The effectiveness of any hepatoprotective substance (drug, plant extract, or food) depends primarily on its potential to correct negative effects that have been disrupted by known hepatotoxic agents.

Table 1: *Rosmarinus officinalis* ethanolic extract on nitric oxide and catalase activity in the liver of female rats after 28 days of treatment

	Control	*Rosmarinus officinalis* ethanolic extract			
		200mg/kg	300mg/kg	500mg/kg	1000mg/kg
CAT (IU/min/g tissue)	2.20±0.06	2.52±0.05**	2.46±0.05*	2.42±0.02	2.18±0.04
NO (mmol/g tissue)	1036.96±32.63	782.93±37.47**	859.54±20.58*	942.17±25.30	1092.18±26.17

NO: nitric oxide; CAT: catalase; significance level : (*) P<0.05, (**) P<0.01, (***) P<0.001

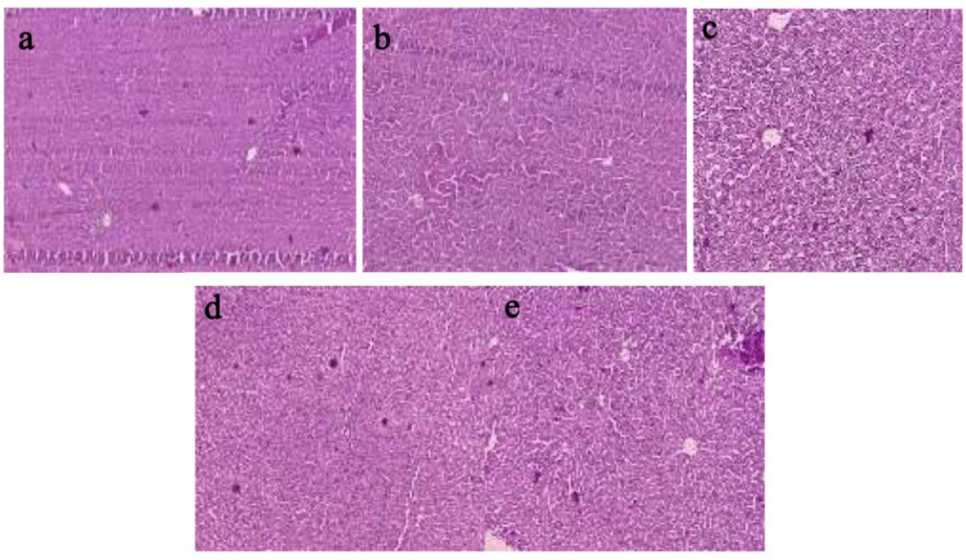

Fig. 6: Histological sections of the liver were observed under an optical microscope. a: Control; b: 200mg/kg; c: 300mg/kg; d: 500mg/kg; e: 1000mg/kg.

Administration of EERO for 28 days causes a non-significant decrease in glucose levels in the groups of rats treated with 200mg/kg of EERO, while 300, 500, and 1000mg/kg of EERO induced a statistically non-significant increase in glucose levels compared to the control rats, which suggests that the ethanolic extract of *R. officinalis* did not produce any hepatic toxicity when administered orally for 28 days, and the dose of 200mg/kg of EERO improved the state of liver function. These results are in line with other studies, which have demonstrated that administration of 200mg/kg of ethanolic extract of *R. officinalis* reduces blood glucose levels (Betul et al. 2017). Another study has also shown that administration of *Rosmarinus* aqueous extract at a dose of 200mg/kg decreases glucose levels (El-Desouky et al. 2020).

The decrease in glucose levels as the duration of food deprivation increases clearly indicates how the body mobilizes its own tissues as an energy source, resulting in the destruction of visceral organs. Glucose release into the blood occurs when food intake is reduced, resulting in a workload for the liver and muscles to release glucose (Didou et al. 2025).

EERO administration at 200, 300 and 500mg/kg during 28 days non-significantly decreased cholesterol levels, while 1000mg/kg statistically non-significantly increased cholesterol levels in the treated groups of rats compared to the control rats, which suggests that *R. officinalis* extract improves hepatic function when administered orally for 28 days. These results are in line with other studies that demonstrated that the administration of EERO at 100, 200, and 400 mg/kg significantly reduces the cholesterol level (Wang et al. 2017). In addition, another study showed that administration of EERO at 10mg/kg by nasogastric gavage for 8 weeks reduces the cholesterol level (Fareed et al. 2023). These results are in line with other studies that have demonstrated that the administration of the hydroalcoholic extract of *R. officinalis* for 28 days in the different groups of rats treated with 200, 500 and 1000mg/kg does not cause any significant difference in cholesterol levels and no alteration of these parameters compared to the control rats (Salokhe et al. 2020).

Administration of EERO for 28 days non-significantly decreased triglyceride levels in the groups of rats treated with 200 and 300 mg/kg of EERO, while 500 and 1000mg/kg of EERO increased the triglyceride level in a statistically non-significant way compared to the control rats, which suggests that *R. officinalis* extract improves hepatic status at doses of 200 and 300 mg/kg of EERO. Likewise, it did not produce any hepatotoxicity when administered EERO at doses of 500 and 1000 mg/kg for 28 days. These results are in line with other studies that demonstrated that the ethanolic extract of rosemary at 100, 200, and 400 mg/kg significantly reduced triglyceride and cholesterol levels. Another study showed that administration of EERO at 10 mg/kg by nasogastric gavage for 8 weeks reduced the level of triglyceride (Fareed et al. 2023). These results are in line with other studies that have demonstrated that the administration of the hydroalcoholic extract of *R. officinalis* for 28 days in the different groups of rats treated with 300, 500, and 1000mg/kg does not cause any significant difference in triglyceride levels and no alteration of these parameters compared to the control groups (Salokhe et al. 2020).

Amino transaminases are enzyme molecules of the cytoplasm and mitochondria. Normal plasma contains low levels of alanine and aspartate transaminase activity. Adaptive changes resulting from cell injury are primarily cytoplasmic and related to increased or impaired metabolic function. ALT activity is more liver-specific than AST. Increased activity reflects cellular injury, particularly in the liver, heart, kidneys, or muscle (Babou et al. 2025c). Liver injury is caused by hepatitis and toxic lesions, which can accompany many liver insults, including drug overdose (Marshall et al. 2005).

EERO administration for 28 days non-significantly decreased the levels of ALT and AST in the groups of rats treated with 200 mg/kg, 300 and 500 mg/kg of EERO compared to the control rats. While 1000 mg/kg of EERO non-significantly increased the level of ALAT and ASAT compared to the control group. This suggests that the extract of R. officinalis improves hepatic function. Similarly, the dose of 1000 mg/kg of EERO did not produce any signs of renal and hepatic toxicity when administered orally for 28 days. These results are in agreement with other studies showing that the administration of the hydroalcoholic extract of *R. officinalis* for 28 days in the different groups of rats treated with 300, 500 and 1000 mg/kg does not cause any significant difference in the levels of ALT and AST and no alteration of these parameters compared to the control groups (Salokhe et al. 2020). Similarly, another study showed that administration of a dose of 200 mg/kg EERO reduced the level of ALT, AST, and blood glucose (Betul et al. 2017).

Administration of the EERO for 28days resulted in a significant ($P<0.001$) improvement in transaminase levels compared to the intoxicated group of rats. The rats treated with the ethanolic extract had 64.117±1.757 U/L for ALAT and 200.483±9.533 U/L for ASAT, and for the other group treated with the butanolic extract, this level was around 56.45±1.145 U/L for ALAT and 175.783±3.291 U/L for ASAT, which is close to the values obtained in the negative control group. Indeed, the administration of EERO significantly prevented hepatocyte damage. The reduction in AST and ALT levels is an indication of hepatoprotection and other studies even describe it as a process of liver cell regeneration (Zhang et al. 2014). This hepatoprotective effect is associated with the correction of daily weight variation in rats treated with our plant extracts.

The observed hepatoprotective effect can only be interpreted by the composition of these extracts and their antioxidant activities. Indeed, the results of our phytochemical analysis suggest that the *R. officinalis* extracts studied are rich in secondary metabolites such as total polyphenols, flavonoids, and moderate amounts of condensed tannins. These metabolites have the ability to neutralize radicals and thus prevent potential tissue damage. Indeed, many plant extracts rich in flavonoids have shown hepatoprotective and antioxidant effects against hepatotoxin-induced liver injury (Mensor et al. 2001).

Studies on the effects of plant extracts with hepatoprotective properties have shown that extracts of *Silybum marianum* exert preventive effects and have no therapeutic effect in acute treatments. These results allow

R. officinalis to be classified in this category of plants and confirm the results obtained with silymarin. Similarly, a comparative study carried out in intoxicated mice showed significant hepatoprotective activity of extracts of *R. officinalis*, *Peumus boldus*, and *Eupatorium cannabinum*, ranked in order of increasing effect with silymarin. Flavonoids present in these plants are recognized for their hepatoprotective activities (Anila and Vijayalakshmi 2003; Kumar and Pandey 2013).

Conclusion

Medicinal plants, especially *R. officinalis*, have become one of the most accessible sources for improving human health due to their richness in bioactive compounds, also known as phytochemicals. Our findings show that low doses of *R. officinalis* have a protective effect by causing an increase in the activity of antioxidant enzymes, and a decrease in signs of liver damage may therefore be due to the presence of flavonoids. These effects are likely related to the powerful anti-inflammatory and antioxidant properties of rosemary. However, higher doses of this plant exert non-significant opposite effects on liver function. These results suggest the use of *R. officinalis* with specific doses as a dietary supplement for patients with liver disorders, as well as a medication for the treatment of these diseases. Further trials are needed to confirm its therapeutic effects.

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Conflict of Interest: No competing interests.

Data Availability: ??

Ethics Statement: The experimental procedures were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and approved by the Local Institutional Research Committee of Animal Ethics.

Author's Contribution: Said Babou performed the experiments, analyzed the data, and wrote the paper. Miloud Chakit reviewed and provided comments on the content and interpretation of the manuscript. Youssef Sqalli-Houssaini supervised the work, revised and approved the manuscript.

Generative AI Statement: The authors declare that no Gen AI/DeepSeek was used in the writing/creation of this manuscript.

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