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Hepatoprotective Effect of Ethanolic Extract of *Rosemarinus officinalis* (Rosemary) Leaves Against CCL_4-induced Hepatic Damage in Albino Rats

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ABSTRACT

The aromatic herb *Rosmarinus officinalis* L. (Rosemary) is from the Lamiaceae family and has been traditionally used in culinary and folk medicine for various ailments, including liver disorders. In this study, we evaluated the hepatoprotective and nephroprotective effects of the ethanolic leaf extract of *Rosmarinus officinalis* against carbon tetrachloride (CCl_4)-induced toxicity in albino rats. Rats were administered 0.5 mL/kg b.w. of CCl_4 intraperitoneally and subsequently orally treated with either 200 or 400 mg/kg b.w. of rosemary extract for four weeks. The standard group received silymarin at 100 mg/kg body weight. The serum levels of biomarkers, including alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), electrolytes, urea, and creatinine, were then evaluated. Phytochemical screening results indicated the presence of alkaloids, flavonoids, tannins, steroids, phenols, and terpenes in the extract. Our results show that treatment with rosemary extract significantly ($p<0.05$) reduced the biomarkers' serum levels enzymes of ALT, AST, ALP, and kidney-related parameters compared to the treatment (CCl_4) group. While the antioxidant and hepatoprotective properties of Rosemary are well-documented in the literature, this study adds a minor novelty by assessing both liver and kidney protection in a dose-responsive model using ethanolic leaf extract against chemically induced injury. These findings further support the reported therapeutic potential of antioxidants from *Rosmarinus officinalis* in protecting vital organs from oxidative damage.

INTRODUCTION

The liver is a vital organ in the body that plays an important role in the metabolism and detoxification of xenobiotics and is at high risk of chemically induced toxicity. The major cause of liver disease nowadays in the Western world is drug-induced liver injury, which results in the majority of acute liver failure cases [1]. Although the pathophysiological mechanism of chemically induced hepatotoxicity is not fully understood, it is mostly associated with the conversion of xenobiotics to endogenous pro-oxidants, such as reactive oxygen species (ROS), which lead to oxidative stress and eventually result in cellular macromolecular damage[14]. Oxidative stress occurs as a result of an imbalance between the production of free radicals and pro-oxidants (ROS and RNS) and the neutralizing ability of the antioxidant defense system to counteract them. Recently, oxidative stress has been recognized as a crucial factor that plays a pivotal role in the pathophysiological changes observed in a wide range of liver diseases [1]. A better understanding of the role of oxidative stress in these liver disorders might help in the appropriate use of antioxidants as a therapeutic approach for liver diseases.

Carbon tetrachloride (CCl_4) is a chemical toxin used in experimental research to induce toxicity in experimental models, thereby mimicking oxidative stress in various pathophysiological conditions [2]. It can lead to severe centrifugal necrosis and steatosis with just a single exposure [15]. Carbon tetrachloride toxicity results from the activation of Cytochrome P450, leading to the formation of a trichloromethyl radical that subsequently attacks cellular structures and macromolecules, causing their oxidation and ultimately leading to hepatotoxic damage [3]. The use of natural antioxidant compounds emerges as a means to avoid the toxicity side effects of synthetic antioxidants [4]. Natural antioxidants help in endogenous antioxidant defense

against pro-oxidants (ROS & RNS), thereby neutralizing their effect [2]. *Rosmarinus officinalis* L. is an aromatic plant that originates from the Mediterranean region and belongs to the family of *Lamiaceae.* It is commonly known as Rosemary and is used in culinary applications as a condiment, flavoring agent in food, and also in the preservation of food due to its ability to prevent oxidation and microbial contamination in foods [5]. It is also used in the cosmetic industry as a raw material in the production of hair and cosmetic products [6]. The leaves are used in folk medicine to treat various ailments, including diabetes, stomachache, fatigue, peptic ulcer, gastrointestinal tract disturbances, biliary colic, renal colic, bronchial asthma, headache, and epilepsy [7]. They are empirically used as a choleretic and Hepatoprotective agent [1]. The leaves contain various constituents, including vitamin C, vitamin B, choline, and rosmarinic acid, which have been shown to provide defense against oxidative stress from oxidizing agents and free radicals. They are also used as a stimulant and mild analgesic [14] It is also used as an antispasmodic agent to reduce rheumatic pain in Mexico when macerated with ethanol as a topical agent [8]. The most important feature of rosemary antioxidant activity is an association between diterpenes and radical scavenging activity [9]. The present study is part of a therapeutic approach to evaluate the hepatoprotective effect of the ethanolic extract of Rosemarinus officinalis L. (Rosemary) leaves against CCl_4-induced hepatic damage in albino rats.

MATERIALS AND METHODS

Sample Collection and identification

Fresh leaves of *Rosmarinus officinalis (Rosemary) were collected from a commercial market in Jos,* Nigeria. The sample was transported to the Department of Biochemistry of Gombe State University, where the research was conducted. The plant sample was taken to the herbarium unit of the Botany Department at Gombe State University for identification and authentication. A voucher number of (GSU-H 284) was given to it.

Preparation of plant extract.

The fresh leaves of *Rosmarinus officinalis (Rosemary) were washed and shade*-dried. The dried sample was therefore pulverized with mortar and pestle into fine powder. A 200g powdered form of the sample was macerated in a 70% ethanol solution, and the solution was allowed to stand at room temperature for two weeks, with daily shaking. The mixture was filtered using Whatman no 1 filter paper. The filtrate was evaporated using a rotary evaporator at 40°C for 5 hours. Then, the extract was collected and used during the treatment exercise, according to the method described by Rodrigo et al. (2014), with minor modifications.

Experimental animals

Thirty (30) samples of albino rats, comprising both males and females, were obtained from the animal farm of the National Veterinary Research Institute in Vom LGA, Plateau State, Nigeria. During the experiment, the rats were housed in steel cages under controlled constant conditions. They were allowed free access to food and water during the experimental period..

Experimental design

A total of thirty albino rats (n=30), were randomly divided into five groups (n=6), and the following treatments was completed in four (4) consecutive weeks.

Grouping

Group 1: Normal controls were administered physiological saline (1.5 mL) orally three times a week and olive oil intraperitoneally twice weekly. Group 2: Toxic control received an IP injection of a CCL4 and olive oil (1:1 v/v) mixture at a dose of 0.5 mL/kg b.w. Twice weekly. Group 3: Standard control (Silymarin Si + CCL_4) was administered with 100 mg/kg b.w Silymarin for 5 days/week orally for 4 successive weeks, and an IP injection of CCL_4 and olive oil (1:1 v/v) mixture at a dose of 0.5ml/kg b.w, twice weekly. Group 4: (1^{st} treatment group) The plant extract was administered orally (200 mg/kg b.w.) daily. After 1 hour, a CCL4 and olive oil (1:1 v/v) mixture was induced intraperitoneally (0.5 ml/kg b.w.) twice a week. Group 5: (2^{nd} treatment group) Plant extract was administered orally (400 mg/kg b.w.) daily, followed by an intraperitoneal (IP) injection of CCL4 and olive oil (1:1 v/v) mixture (0.5 ml/kg b.w.) twice weekly.

Blood collection

The blood samples were collected by human decapitation, part of it put into an EDTA tube and the other in non-heparinized tubes. The samples were left at room temperature for 15 minutes; then, the tubes were centrifuged for 15 minutes at 3000 rpm to separate the serum, which was then kept frozen until use.

Preliminary (qualitative) test for phytochemicals in *Rosemarinus officinalis* L. crude extract

The presence of alkaloids, flavonoids, saponins, tannins, anthraquinones, and steroids were determined

Biochemical Parameters

The biochemical parameters were assessed for alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, total protein, and albumin. The Randox kit was used to check the levels of alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase in serum. The Agappe reagent kit was used to check the levels of total protein and albumin in the serum.

Statistical Analysis

One-way analysis of variance (ANOVA) was used to test for significant differences between the groups at $P \leq 0.05$, using the Statistical Package for the Social Sciences (SPSS, Version 16.0). Post-hoc analysis was carried out using Tukey's test, wherein values with $p \leq 0.05$ were considered to be statistically significant. Values were expressed as mean ± standard error of the mean (SEM).

RESULT AND DISCUSSION

Phytochemical Analysis of *Rosemarinus officinalis* Leaves Extract.

In the phytochemical analysis of the *Rosemarinus officinalis* leaves extract, saponin was found to be absent, while the phytochemicals tannins, steroids, flavonoids, phenols, alkaloids, and terpenes were found to be present, as shown in **Table 1**.

Table 1. Results of phytochemical analysis of Rosemary leaves extract.

Phytochemicals	Results
Saponin	-
Tannins	+
Steroids	+
Flavonoid	+
Phenols	+
Alkaloid	+
Terpenes	+

Effect of *Rosemarinus officinalis* leaves extract on serum AST, ALT, and ALP in CCl_4-induced Albino rats

Administration of 0.5ml CCL_4 in olive (1:1 v/v) intraperitoneally to the albino rats in CCl_4 group resulted in a significant increase ($p < 0.05$) in serum ALT activity compared to normal control group. However, groups treated with 100 mg/kg body weight (bwt) silymarin, 200 mg/kg, and 400 mg/kg bwt of the extract exhibited a marked reduction ($p < 0.05$) in the serum level of ALT compared to the CCl_4 group (**Table 2**). However, administration of 0.5 mL CCl4 in olive oil (1:1 v/v) twice weekly significantly increased ($p < 0.05$) serum AST activity in the CCl4-Treated Group compared to the normal control group. However, groups treated with 100 mg/kg bwt silymarin, 200 mg/kg, and 400 mg/kg bwt showed drastically reduced serum AST activity levels ($p < 0.05$) compared to the CCl_4 group (**Table 2**).

Moreover, administration of 0.5 mL CCL4 in olive oil (1:1 v/v) intraperitoneally to the albino rats in the CCl4 group resulted in a significant increase ($p < 0.05$) in serum ALP activity compared to the normal control group. However, groups treated with 100 mg/kg body weight (bwt) silymarin, 200 mg/kg, and 400 mg/kg bwt of the extract exhibited a marked reduction ($p < 0.05$) in the serum level of ALP compared to the CCl_4 group (**Table 2**).

Table 2. Effect of oral administration of *Rosemarinus officinalis* leaves extracts on liver parameters (ALT, AST & ALP) in CCl_4-induced Albino rats.

Groups	ALT U/L (a)	AST U/L (b)	ALP U/L (c)
Control	37.8 ± 1.715^a	24.6 ± 1.9798^a	57.50 ± 0.377^a
CCl_4	86.00 ± 2.846^b	82.00 ± 4.417^b	250.80 ± 1.320^b
Silymarin+CCL4	41.40 ± 1.979^c	24.00 ± 3.131^a	60.72 ± 2.180^a
CCl_4+200 mg/kg bwt RE	38.60 ± 2.561^a	40.00 ± 3.674^c	141.70 ± 3.961^c
CCl_4+400 mg/kg bwt RE	47.60 ± 4.244^d	29.00 ± 3.209^d	67.85 ± 4.1244^d

All values are expressed as mean(x) ± standard error of mean (SEM). Values with different superscripts are significantly different ($P<0.05$) down the column.

Effect of the ethanolic extract of *Rosmarinus officinalis* on serum chloride, potassium, and sodium in CCL4-induced albino rats.

Administration of 0.5ml CCL_4 in olive (1:1 v/v) intraperitoneally to the albino rats in CCl_4 group resulted in a significant increase ($p < 0.05$) in serum chloride compared to the normal control group. However, groups treated with 100 mg/kg body weight (bwt) silymarin, 200 mg/kg, and 400 mg/kg bwt of the extract exhibited a reduction ($p < 0.05$) in serum chloride compared to the CCl_4 group (**Table 3**). However, administration of 0.5 mL CCl4 in olive oil (1:1 v/v) twice weekly significantly increased ($p < 0.05$) serum potassium levels in the CCl4-Treated Group compared to the normal control group. However, groups treated with 100 mg/kg body weight (bwt) silymarin, 200 mg/kg, and 400 mg/kg bwt showed drastically reduced serum potassium levels ($p < 0.05$) compared to the CCl_4 group (**Table 3**). Moreover, administration of 0.5 mL CCL4 in olive oil (1:1 v/v) intraperitoneally to the albino rats in the CCl4 group resulted in a significant increase ($p < 0.05$) in serum sodium compared to the normal control group. However, groups treated with 100 mg/kg body weight (bwt) silymarin, 200 mg/kg bwt, and 400 mg/kg bwt of the extract exhibited a marked reduction ($p < 0.05$) in serum sodium compared to the CCl_4 group (**Table 3**).

Table 3. Effect of ethanolic extract of *Rosmarinus officinalis* on serum chloride, potassium, and sodium CCL_4-induced albino rats.

Grouping	Chloride ion (Cl^-) mmol/L (A)	Potassium ion (K^+) mmol/L (B)	Sodium ion (Na^+) mmol/L (C)
Control	99.4 ± 2.84^a	5.30 ± 0.18^a	134.2 ± 1.5^a
CCL_4	120.6 ± 1.8^b	7.62 ± 0.34^b	159.7 ± 2.7^b
Silymarin + CCL_4	101.7 ± 2.3^a	5.56 ± 0.15^a	136.1 ± 0.75^a
CCL_4 + 200 mg/kg bwt	109.9 ± 2.3^c	6.33 ± 0.24^c	145.1 ± 1.16^c
CCL_4 + 400 mg/kg bwt	105.4 ± 1.6^a	5.58 ± 0.38^a	137.5 ± 0.36^a

Note: All values are represented as mean ± standard error of the mean (SEM) of different replicates. Values with different superscripts down the group are statistically different from group I at ($P \leq 0.05$).

Effect of oral administration of ethanolic extracts of *Rosmarinus officinalis* on Serum Urea and Creatinine

Table 4 shows the results of kidney markers, which include serum urea and creatinine. The levels of serum urea and creatinine significantly increase ($p < 0.05$) in the CCl4 group compared with the normal control group. The levels of both serum urea and creatinine drastically reduced ($p < 0.05$) down the column in groups that were subsequently treated with 100 mg/kg bwt silymarin, 200 mg/kg, and 400 mg/kg bwt of the extract compared to the CCl_4 group.

Table 4. Effect of oral administration of ethanolic extracts of *Rosmarinus officinalis* on serum urea and creatinine.

Groups	UREA(mmol/l)	Creatinine(µmol/L)
Control	27.08 ± 1.20^a	0.28 ± 0.08^a
CCL_4	56.76 ± 0.70^b	0.97 ± 0.10^b
Silymarin + CCL_4	30.31 ± 0.65^a	0.37 ± 0.80^a
CCL_4 + 200 mg/kg bwt	46.48 ± 2.60^c	0.67 ± 0.04^c
CCL_4 + 400 mg/kg bwt	32.98 ± 1.70^a	0.39 ± 0.09^a

Note: All values are represented as mean ± standard error of the mean (SEM) of different replicates. Values with different superscripts down the group are statistically different from group I at ($P \leq 0.05$).

DISCUSSION

Oxidative stress and inflammation are proposed as possible mechanisms for the pathogenesis of hepatic encephalopathy. Frequent, unavoidable exposure to environmental pollutants, which are metabolized and detoxified in the liver, leads to a state of oxidative stress, resulting in hepatic, renal, and even neurotoxic effects [13]. In this study, the Hepatoprotective effect of *Rosemarinus officinalis* was investigated by measuring the levels of AST, ALT, and ALP in serum as biomarkers of liver injury. From our result, there was a significantly high level of AST, ALT, ALP level in CCl_4 treated group when compared with the normal control group, which shows that CCl_4 has induced damage to the hepatocytes leading to hepatocytolysis and subsequently leakage of these enzymes in to bloodstream which is similar to the findings of Abdel-Gawad *et al.,* 2014, Aouad *et al.,* 2021, and Essawy *et al.,* 2018 and elevation of ALP indicates interruption of bile flow from liver and also serves as evidence for liver injury [3].

The increase in serum liver enzymes can be attributed to hepatocytolysis, which disrupts the structural integrity of the liver by altering the extracellular and intracellular membranes of hepatocytes, reflecting cellular necrosis induced by the toxic metabolism of CCl4, leading to elevated plasma levels [8].

The level of AST, ALT and ALP was shown in this study to decrease in the group administrated with 200 mg/kg bwt and 400 mg/kg bwt rosemary ethanolic extract, which shows that Rosemary exerts beneficial effects on the treatment of CCl_4 hepatotoxicity by modulating the hepatic oxidative state through the trapping of harmful free radicals, but also through the activation and induction of the enzymatic mechanisms of physiological defense and immune stimulation which limits the extent of lipid peroxidation and thus the preservation of cell membranes and/or intracellular hepatocyte membranes in the liver tissue of animals, or may act directly on the process of transformation of ethanol into secondary metabolites in the animal organism by increasing its clearance, which may be another possible mechanism of action [8]. Rosemary reduced and inhibited the CCl_4 stimulate liver toxicity in rats by Breaking molecular bonds or preventing the free radical formation produced through CCl_4 metabolism. These enhanced effects of Rosemary can be attributed to the bioactive Ingredients that moderate the detrimental influence of CCl_4 by scavenging or the antioxidant features that prevent lipid peroxidation, stabilize reactive radicals, maintain cellular integrity, and restrict the riskiness of CCl_4 [9].

Chloride is used to replenish lost water and salt in the body due to certain conditions, such as hypernatremia or hyponatremia. Administration of 0.5ml CCL_4 in olive (1:1 v/v) intraperitoneally to the albino rats in CCl_4 group resulted in a significant increase ($p < 0.05$) in serum chloride compared to the normal control group. However, groups treated with 100 mg/kg body weight (bwt) silymarin, 200 mg/kg bwt, and 400 mg/kg bwt of the extract exhibited a reduction ($p < 0.05$) in serum chloride compared to the CCl_4 group (**Table 3**). However, administration of 0.5 mL CCl_4 in olive oil (1:1 v/v) twice weekly significantly increased ($p < 0.05$) serum potassium in the CCl_4-Treated Group compared to the normal control group. However, groups treated with 100 mg/kg body weight (bwt) silymarin, 200 mg/kg, and 400 mg/kg bwt showed drastically reduced serum potassium levels ($p < 0.05$) compared to the CCl_4 group (**Table 3**). Sodium helps regulate the amount of water that is in and around your cells [10]. Administration of 0.5 mL CCL_4 in olive oil (1:1 v/v) intraperitoneally to the albino rats in the CCl_4 group resulted in a significant increase ($p < 0.05$) in serum sodium compared to the normal control group. However, groups treated with 100 mg/kg body weight (bwt) silymarin, 200 mg/kg, and 400 mg/kg bwt of the extract exhibited a marked reduction ($p < 0.05$) in serum sodium compared to the CCl_4 group (**Table 3**).

Serum urea concentration reflects the balance between urea production in the liver and urea elimination by the kidneys in urine [11]. **Table 4** shows that a significant increase ($P > 0.05$) was observed in the group given a CCL_4 and olive oil (1:1 v/v) mixture at a dose of 0.5 ml/kg body weight of the extract, compared to the normal control group, indicating renal dysfunction. This is a result of an increase in the glomerular filtration rate of the kidney (renal tubule), leading to the excretion of more urea in the urine [12]. Serum creatinine, as shown in **Table 4**, indicates a significant increase ($P > 0.05$) in the group given CCL_4 and olive oil (1:1 v/v) mixture at a dose of 0.5 mL/kg body weight of the extract compared to the normal control group. Serum creatinine levels were drastically reduced ($p < 0.05$) down the column in groups that were subsequently treated with 100 mg/kg bwt silymarin, 200 mg/kg, and 400 mg/kg bwt of the extract compared to the CCl_4 group. It is known that elevated creatinine level is associated with abnormal renal function, especially glomerular filtration [10], and this extract does not show such an incremental effect on serum creatinine.

CONCLUSIONS

In this study, the ethanolic leaf extract of *Rosmarinus officinalis* was demonstrated to confer significant protection against CCl_4-induced hepatic and renal damage in albino rats. The observation that reductions in liver enzymes, such as serum electrolytes, urea, and creatinine, indicated a dual protective role on both hepatic and renal systems. These protective effects are likely be mediated via the antioxidant mechanisms, which are supported by the phytochemical constituents identified in this study. Although Rosemary's hepatoprotective properties are well known, this study further contributes to existing knowledge by highlighting its nephroprotective potential and dose-dependent efficacy in a chemical injury rat model. *Rosmarinus officinalis* presents a promising, natural, and accessible alternative in the context of rising interest in plant-based therapies to counteract oxidative organ damage. Our current studies include research aimed at elucidating the molecular pathways involved and validating these effects through histological and mechanistic investigations.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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