

## Protective Effect Of Rosmarinus Officinalis L. Against Hepatic Injury Stimulated By Ferric Chloride In Male Rats

Amel Taha Yaseen<sup>\*1</sup>; Mafaz Khalid Saeed<sup>\*2</sup>; Razan Y.Q. Al-Abachi<sup>\*3</sup>; Alaa Younis Mahdy Al-Hamadany<sup>\*4</sup>; Shihab A. Al-Bajari<sup>\*5</sup>

<sup>\*1,2</sup> Department of chemistry, College of science, Mosul university, Iraq

<sup>\*3,4,5</sup> Mosul Technical Institute, Northern Technical University, Mosul, Iraq

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### Abstract

Rosmarinus officinalis L., Lamiaceae, has been used in Iraq for decades to cure a variety of diseases and is considered as a desirable species owing to its medicinal benefits and bioactivity. The purpose of this research was to find out more about that the Rosmarinus officinalis L plant might significantly reduce the harmful effects of ferric chloride. Fifty male rats were divided into five main groups: control (no additions), FeCl<sub>3</sub> group, and the remainder of the groups were fed Rosmarinus officinalis L. components (flavonoid, glycoside, and alkaloids) to monitor the affected liver function. FeCl<sub>3</sub> raised aspartate transaminase, alanine transaminase, gamma-glutamyltransferase, and alkaline phosphatase levels, while Rosmarinus officinalis compounds reduced FeCl<sub>3</sub> toxicity. In contrast to the FeCl<sub>3</sub> group and FeCl<sub>3</sub> groups containing Rosmarinus officinalis L. plant components, there was a significant difference ( $P < 0.05$ ) in total lipid profile, total protein, albumin, and globulin levels in the control group. In compared to the control group, lipid peroxidation resulted in a significant amount of malondialdehyde in the FeCl<sub>3</sub> group; however, Rosmarinus officinalis components lowered the level of FeCl<sub>3</sub>, reducing oxidative damage. In compared to those fed just FeCl<sub>3</sub>, the levels of glutathione, catalase, and glutathione peroxidase rose in the groups fed Rosmarinus officinalis components. The levels of superoxide dismutase had significantly slightly differences amongst the groups that were examined.

**Key words:** Rosmarinus officinalis, lipid peroxidation, medical plants, ferric chloride

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### Introduction

Liver disorders are commonplace, and they are the leading cause of death worldwide. Hepatocellular malignancies go from steatosis to chronic hepatitis, fibrosis, cirrhosis, and hepatocellular carcinoma, with steatosis being the most common (Notaset al., 2009). Viral hepatitis, alcohol misuse, narcotics, metabolic disorders caused by an elements excess of iron or/and copper, autoimmune assault of hepatocytes, bile duct epithelium, or congenital bile duct epithelium anomalies can all cause chronic

damage that leads to fibrosis in the liver (**Michel et al., 2009**). In recent decades, herbal plants such as *Rosmarinus officinalis* L. and *Salvia officinalis* L. have piqued attention for the treatment and prevention of a variety of human injuries. *Rosmarinus officinalis* L., Lamiaceae (Rosemary), a perennial herb native to the Mediterranean, is a perennial herb. It has only lately gained worldwide recognition as an aromatic plant whose fresh and dried leaves are extensively used to flavor and spice foods. moreover, its liver-protective properties have been known for some years (**Hegazy et al. 2017**). *R. officinalis* contains antioxidants including rosmarinic acid, diterpenoids such as carnosic acid, carnosol, rosmanol, and epirosmanol, as well as tocopherols and carotenoids (**Amin & Hamza, 2005**). The therapeutic potential of *R. officinalis* extract has been related to phenolic diterpenes, which scavenge singlet oxygen, hydroxyl radicals, and lipid peroxyl radicals (**Gallego et al., 2013**). In a model of azathioprine-induced toxicity in rats, as well as acetaminophen-induced liver damage, *R. officinalis* extracts have been shown to have hepatoprotective activity (**Fadlalla & Galal, 2020**). Hepatic damage caused by hypothermic ischemia in rats, gentamicin-treated rats, and alcoholic liver disease (**Bahriet et al., 2020**), is ascribed to the essential oil's constitution as well as its polyphenol content (**Fadlalla & Galal, 2020**). The most prominent of which is rosmarinic acid (**Lucarini et al., 2014**). However, this biological activity is less well established, and to the best of our knowledge, there is no definitive data on the chemicals that cause it. Furthermore, there is no proof of the underlying mechanism of action, which is thought to be related to a decrease in oxygen-reactive species (ROS) (**Rakovicet et al., 2014**). On the other hand, the *in vitro* antioxidant activity of *R. officinalis* has been extensively investigated, with many vegetal compounds of the species having been reported: floral extracts, fresh aerial portions (**Karadaet et al., 2019**) or essential oil (**Borges et al., 2019**). Polyphenols are the compounds responsible for this action (**Karadaet et al., 2019**), but also from the essential oil's terpenes (**Borges et al., 2019**). As a reason, The goal of this research was to research further into antioxidant enzymes, lipid profile, and hepatoprotective properties of an aqueous extract of Iraqi rosemary against liver injury in more detail.

## Materials and Methods

### Chemicals and materials

SISCO Research PVT LTD's Laboratories provided the ferric chloride (Mumbai, India). Kits and other chemicals were obtained from BioLab Diagnostics (India Private Limited) for analytical grade. *Rosmarinus officinalis* L (Rosemary ) was collected from government of Nineveh. It was assigned a classification based on plant classification references for medicinal plants. A voucher specimen of the plant was also discovered and documented at the herbariums of the University of Mosul's College of Education.

### **Rosemary extraction**

The procedure described by AL-Saadon was used to prepare flavonoids, glycosides, and alkaloids extracts from the *Rosmarinus officinalis* plant (AL-Saadon, 2005).

### **Animals Experimentation**

Male albino rats weighing 190 to 240 grams were purchased from the University of Mosul's College of Veterinary Medicine. The animals were kept in conventional cages with unrestricted access to water and food (a typical laboratory pellet diet). The temperature in the animal home was kept between 24 to 29 °C, with a 12-hour light/dark cycle. The Institutional Animal Ethical Committee accepted the experimental protocol (IAEC).

### **Design Experimentation**

The protective role of *Rosmarinus officinalis* against  $\text{FeCl}_3$ -induced hepatotoxicity in rats was investigated using fifty animals.  $\text{FeCl}_3$  was combined 1to1 (w/w) with olive oil and tested for properties of hepatoprotective against  $\text{FeCl}_3$ -induced liver injury. The rats were split into four groups, each with ten rats, and were expected to give the following treatments:

**Group 1** (negative control): 4-week feeding of rats on a typical synthetic diet

**Group 2** (positive control): For 30 days, participants rats were given an equal combination of  $\text{FeCl}_3$  and 1 mL/kg olive oil orally (three times per week) through gastric gavage (bw).

**Group 3 to Group 5:** From day 15 to day 30, rats were given flavonoids, glycosides, and alkaloids, which are extracts of such *Rosmarinus officinalis* plant, at a dose of 100 mg/kg body weight. During the previous four weeks, oral gavage at a dosage of 1 mL/kg was supplied.

### **Biochemical analysis**

The biochemical parameters of liver enzymes, such as AST (aspartate transaminase), ALT (alanine transaminase), ALP (alkaline phosphatase), serum total protein, and albumin, as well as the lipid profile, which included TAG (triglycerides), TC (total cholesterol), HDL-c (high-density lipoprotein cholesterol), LDL-c (low-density lipoprotein cholesterol) were analyzed using an automat COBAS INTEGRA 800VR (Roche Diagnostics GmbH, Mannheim, Germany) following standard methods.

### **Estimating of antioxidant enzymes and Lipid peroxidation.**

Thiobarbituric acid reactions have been used to quantify the concentrations of malondialdehyde (MDA). The concentration of thiobarbituric acid (TBARS) in the tissue was measured using the Halliwell and Chirico (1993) method, and GSH was determined using the Mohammed and Kakey

method (2020). The activity of antioxidant enzymes (CAT, SOD, and GPx) were assessed using Wheeler et al. techniques's (1990).

### Statistical analysis

One-way ANOVA was used to analyze the data. The variance between treatment means was resolved using Duncan's novel multiple range test. The statistical program SPSS 26.0 has been used for all statistical analyses (SPSS Ltd., Surrey, UK).

### Results

Table 1 showed the effect of FeCl<sub>3</sub> and Rosmarinus officinalis extraction compounds on lipid profile of rats males. Results revealed that Triglycerides, Cholesterol, LDL-c, VLDL-c values in the control group of male rats were lower (1.62±0.54, 5.08±1.02, 3.53±0.99, and 0.324±0.10) respectively than that of FeCl<sub>3</sub> group, (1.98±0.63, 5.96±1.12, 4.78±1.07, and 0.396±0.12) respectively, whilst the HDL-c results in the control group was showed slightly higher (1.25±0.12) than that of FeCl<sub>3</sub> group (1.14±0.12). See table 1.

The comparison between the control group and FeCl<sub>3</sub> groups with Rosmarinus officinalis L. extraction compounds ( Sodium valproate, flavonoid, glycoside and alkaloids), the results revealed that the level of Triglycerides, Cholesterol, LDL-c, VLDL-c in the control group were lower than the FeCl<sub>3</sub> group with Sodium valproate (1.83±0.52, 5.59±0.9, 4.41±0.86, and 0.366±0.10) respectively, FeCl<sub>3</sub> group with alkaloids (1.89±0.42, 5.60±0.94, 4.24±0.91, and 0.378±0.08) respectively, FeCl<sub>3</sub> group with flavonoid (1.87±0.37, 5.77±0.54, 4.21±0.50, and 0.374±0.07) respectively, and FeCl<sub>3</sub> group with glycoside (1.74±0.42, 5.79±0.89, 4.22±0.86, and 0.348±0.08) respectively. whilst, the HDL-c level in the control group (1.25±0.12) was higher the FeCl<sub>3</sub> groups with Sodium valproate, flavonoid, glycoside and alkaloids (1.17±0.08, 1.17±0.09, 1.18±0.11, and 1.15±0.09) respectively (Table1).

**Table 1: Effect of Ferric Chloride and Rosmarinus officinalis L. extraction compounds on lipid profile of male rats**

Groups Parameters	Control group (mean ± SD)	FeCl <sub>3</sub> group (mean ± SD)	FeCl <sub>3</sub> + Sodium valproate group (mean ± SD)	FeCl <sub>3</sub> + alkaloids group (mean ± SD)	FeCl <sub>3</sub> + flavonoid group (mean ± SD)	FeCl <sub>3</sub> + glycoside group (mean ± SD)
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<b>TG(mmol/l)</b>	1.62±0.54	1.98±0.63	1.83±0.52	1.89±0.42	1.87±0.37	1.74±0.42
<b>Cho (mmol/l)</b>	5.08±1.02	5.96±1.12	5.59±0.9	5.60±0.94	5.77±0.54	5.79±0.89
<b>HDL-c(mmol/l)</b>	1.25±0.12	1.14±0.12	1.17±0.08	1.17±0.09	1.18±0.11	1.15±0.09
<b>LDL-c(mmol/l)</b>	3.53±0.99	4.78±1.07	4.41±0.86	4.24±0.91	4.21±0.50	4.22±0.86
<b>VLDL-c(mmol/l)</b>	0.324±0.10	0.396±0.12	0.366±0.10	0.378±0.08	0.374±0.07	0.348±0.08

The results of effect of FeCl<sub>3</sub> and Rosmarinus officinalis extraction compounds on activated liver enzymes of rats males showed in table 2. Results revealed that ALT, AST, GGT, ALP, Globulin (g/dl) levels in the control group of male rats were lower (29.19±0.11, 36.96±1.5, 98±5.2, 28.79±1.59, 2.18±0.07) respectively than that of FeCl<sub>3</sub> group, (49.14±0.34, 41.87±1.92, 158±13.8, 55.26±1.37, 2.46±0.28) respectively, whilst the Albumin (g/dL) and Total Protein (g/dL), results in the control group was showed slightly higher (4.18±0.09, 6.38±0.32) than that of FeCl<sub>3</sub> group (3.27±0.23, 5.67±0.53,). See table 2.

The comparison between the control group and FeCl<sub>3</sub> groups with Rosmarinus officinalis L. extraction compounds ( Sodium valproate, flavonoid, glycoside and alkaloids), the results revealed that the level of ALT, AST, GGT, ALP, Globulin (g/dl) in the control group were lower than the FeCl<sub>3</sub> group with Sodium valproate (39.37±1.39, 37.46±1.15, 121±3.1, 48.27±1.3, 2.70±0.12) respectively, in FeCl<sub>3</sub> group with alkaloids, the results revealed that ALT (U/L), AST (U/L), Total Protein (g/dL), and Albumin (g/dL) were higher in controls group than that in FeCl<sub>3</sub> group with alkaloids (27.82±1.28, 34.53±1.57, 6.24±0.48, and 3.94±0.38 ) respectively, whilst the results of GGT (U/L), ALP (U/L), and Globulin (g/dL) showed lower levels in controls group than that in FeCl<sub>3</sub> group with alkaloids (156±3.4, 34.39±1.22 , and 2.86±0.16)respectively. in FeCl<sub>3</sub> group with flavonoid, AST, GGT, ALP, Total Protein (g/dl),and Globulin (g/dl) were lower in controls group than that in FeCl<sub>3</sub> groupwith flavonoid (41.19±1.72, 133±4.5, 34.52±1.33, and 6.39±0.44) respectively, whilst the results of ALT, and Albumin (g/dl) showed higher levels in controls group than that in FeCl<sub>3</sub> group with flavonoid (25.79±1.51,3.59±0.12) respectively. Finally, FeCl<sub>3</sub> group with glycoside the results showed higher levels of ALT, AST, Total Protein (g/dl), and Albumin (g/dl) in controls group than that in FeCl<sub>3</sub> group with glycoside (28.99±0.34, 34.16±1.5, 6.18±0.19, and 3.48±0.09) respectively. whilst, the results of GGT, ALP, and Globulin (g/dl) level were lower in the control group than in the FeCl<sub>3</sub> groups with, glycoside (118±5.2,39.79±1.59, and 2.68±0.07) respectively (Table2).

**Table 2: Effect of Ferric Chloride and Rosmarinus officinalis L. extraction compounds on activated liver enzymes of male rats**

Groups  Parameters	Control group (mean $\pm$ SD)	FeCl <sub>3</sub> group (mean $\pm$ SD)	FeCl <sub>3</sub> + Sodium valproate group (mean $\pm$ SD)	FeCl <sub>3</sub> + alkaloids group (mean $\pm$ SD)	FeCl <sub>3</sub> + flavonoid group (mean $\pm$ SD)	FeCl <sub>3</sub> + glycoside group (mean $\pm$ SD)
ALT (U/L)	29.19 $\pm$ 0.11	49.14 $\pm$ 0.34	39.37 $\pm$ 1.39	27.82 $\pm$ 1.28	25.79 $\pm$ 1.51	28.99 $\pm$ 0.34
AST (U/L)	36.96 $\pm$ 1.5	41.87 $\pm$ 1.92	37.46 $\pm$ 1.15	34.53 $\pm$ 1.57	41.19 $\pm$ 1.72	34.16 $\pm$ 1.5
GGT (U/L)	98 $\pm$ 5.2	158 $\pm$ 13.8	121 $\pm$ 3.1	156 $\pm$ 3.4*	133 $\pm$ 4.5	118 $\pm$ 5.2
ALP (U/L)	28.79 $\pm$ 1.59	55.26 $\pm$ 1.37	48.27 $\pm$ 1.3	34.39 $\pm$ 1.22	34.52 $\pm$ 1.33	39.79 $\pm$ 1.59
Total Protein (g/dL)	6.38 $\pm$ 0.32	5.67 $\pm$ 0.53	5.89 $\pm$ 0.38	6.24 $\pm$ 0.48	6.39 $\pm$ 0.44	6.18 $\pm$ 0.19
Albumin (g/dL)	4.18 $\pm$ 0.09	3.27 $\pm$ 0.23	3.19 $\pm$ 0.38	3.94 $\pm$ 0.38	3.59 $\pm$ 0.12	3.48 $\pm$ 0.09
Globulin (g/dL)	2.18 $\pm$ 0.07	2.46 $\pm$ 0.28	2.70 $\pm$ 0.12	2.86 $\pm$ 0.16	3.01 $\pm$ 0.15	2.68 $\pm$ 0.07

Furthermore, Table 3 showed the results of antioxidant enzymes such as MDA (lipid peroxidation), GSH, CAT, GPx as well as SOD to evaluate the impact on the function of liver of rat males. Results revealed that MDA level in the control group (0.79 $\pm$ 0.04) was lower than that in FeCl<sub>3</sub> group, FeCl<sub>3</sub> group with Sodium valproate, and FeCl<sub>3</sub> group with alkaloids, FeCl<sub>3</sub> group with flavonoid group, and FeCl<sub>3</sub> group with glycoside (1.76 $\pm$ 0.02, 1.06 $\pm$ 0.02, 1.56 $\pm$ 0.02, 1.41 $\pm$ 0.02, and 1.26 $\pm$ 0.02) respectively, the GSH level in the control group was higher (77 $\pm$ 3.13) than that in FeCl<sub>3</sub> group, FeCl<sub>3</sub> group with Sodium valproate, and FeCl<sub>3</sub> group with alkaloids, FeCl<sub>3</sub> group with flavonoid group, and (55 $\pm$ 2.9, 66 $\pm$ 2.9, 59 $\pm$ 2.9, 52 $\pm$ 2.9) respectively, whilst GSH level in FeCl<sub>3</sub> group with glycoside group (85 $\pm$ 2.9) was higher than in that controls group (Table 3).

Also, the results revealed that CAT (mmol/L) level in the control group (0.18 $\pm$ 0.02) was higher than that in FeCl<sub>3</sub> group, FeCl<sub>3</sub> group with alkaloids, FeCl<sub>3</sub> group with flavonoid group, and FeCl<sub>3</sub> group with glycoside (0.08 $\pm$ 0.01, 0.12 $\pm$ 0.02, 0.14 $\pm$ 0.01, and 0.16 $\pm$ 0.02) respectively, whilst CAT (mmol/L) level in FeCl<sub>3</sub> with Sodium valproate group (0.18 $\pm$ 0.01) was same in controls group. With GpX (U/mg protein), the results showed that GpX (U/mg protein) level in the control group (5.98 $\pm$ 0.47) was higher than that in FeCl<sub>3</sub> group, FeCl<sub>3</sub> group with Sodium valproate, and FeCl<sub>3</sub> group with alkaloids, FeCl<sub>3</sub> group with flavonoid group, and FeCl<sub>3</sub> group with glycoside (3.75 $\pm$ 0.31, 4.75 $\pm$ 0.41, 3.95 $\pm$ 0.17, 5.75 $\pm$ 0.30, and 5.15 $\pm$ 0.11) respectively.

Finally, the results revealed that SOD level in the control group ( $2.62 \pm 0.3$ ) was higher than that in  $\text{FeCl}_3$  group, valproate, and  $\text{FeCl}_3$  group with alkaloids,  $\text{FeCl}_3$  group with flavonoid group, and  $\text{FeCl}_3$  group with glycoside ( $2.21 \pm 0.2$ ,  $2.22 \pm 0.2$ ,  $2.42 \pm 0.2$ , and  $2.44 \pm 0.2$ ) respectively, whilst SOD level in  $\text{FeCl}_3$  with Sodium valproate group ( $2.8 \pm 0.1$ ) was higher than that in controls group (Table 3).

**Table 3: Effect of Ferric Chloride and Rosmarinus officinalis L. extraction compounds on antioxidants enzymes of male rats**

Groups Parameters	Control group (mean $\pm$ SD)	$\text{FeCl}_3$ group (mean $\pm$ SD)	$\text{FeCl}_3$ + Sodium valproate group (mean $\pm$ SD)	$\text{FeCl}_3$ + alkaloids group (mean $\pm$ SD)	$\text{FeCl}_3$ + flavonoid group (mean $\pm$ SD)	$\text{FeCl}_3$ + glycoside group (mean $\pm$ SD)
MDA (mM/100g tissue)	$0.79 \pm 0.04$	$1.76 \pm 0.02$	$1.06 \pm 0.02$	$1.56 \pm 0.02$	$1.41 \pm 0.02$	$1.26 \pm 0.02$
GSH (mM/100g tissue)	$77 \pm 3.13$	$55 \pm 2.9$	$66 \pm 2.9$	$59 \pm 2.9$	$52 \pm 2.9$	$85 \pm 2.9$
CAT (mmol/L)	$0.18 \pm 0.02$	$0.08 \pm 0.01$	$0.18 \pm 0.01$	$0.12 \pm 0.02$	$0.14 \pm 0.01$	$0.16 \pm 0.02$
GpX (U/mg protein)	$5.98 \pm 0.47$	$3.75 \pm 0.31$	$4.75 \pm 0.41$	$3.95 \pm 0.17$	$5.75 \pm 0.30$	$5.15 \pm 0.11$
SOD	$2.62 \pm 0.3$	$2.21 \pm 0.2$	$2.8 \pm 0.1$	$2.22 \pm 0.2$	$2.42 \pm 0.2$	$2.44 \pm 0.2$

## Discussion

This study investigated the effects of rosemary (*Rosmarinus officinalis*) aqueous extract on  $\text{FeCl}_3$ -induced hepatotoxicity in adult male rats. Also, this issue looked examined the effect of the extract on liver functioning and oxidative stress biomarkers (hepatic glutathione reductase activity and malondialdehyde levels). Extracts, bioactive pollutants, and oils from oilseeds, fruits, vegetables, and medicinal plants had potent antioxidant activity that might protect the liver from injury. *Rosmarinus officinalis* is a herbal medicine with a potentially beneficial impact due to its polyphenolic component source (Borrás-Linares et al., 2014). Several studies have showed that these chemicals are good natural antioxidant sources, demonstrating their direct impact on human health. Several studies have demonstrated the benefits of enhancing a healthy diet by incorporating specific foods rather than specific nutrients that protect us from liver and heart disorders (Eilat-Adar et al., 2013).

The results of the present study revealed that increase in the activity of serum ALT, AST, GGT and ALP as a result of FeCl<sub>3</sub> intoxication which harmony with other studies such as Wang et al. that documented elevation of activated liver function parameters as a results of toxication (**Wang et al., 2008**). In addition, Abdel-Wahhabet al. found that Rosmarinus officinalis substantially improved the changed activities of serum ALT, AST, GGT, and ALP caused by CCl<sub>4</sub> intoxication. Consumption of oxygen radical scavengers (phytochemicals and antioxidants) has been proposed as a potentially helpful hepatoprotective protective mechanism. (**Abdel-Wahhabet al., 2011**). Another study by Yi et al. was reported that pre-treatment with plant extracts as antioxidants may dramatically lower blood levels of ALT, AST, total cholesterol, and triglycerides in mice treated with alcohol (**Yi et al., 2014**).

Significant changes in plasma lipoprotein levels were seen in the FeCl<sub>3</sub>-treated group in this study. serum lipoprotein levels, both high and low, have a direct relationship with normal liver function. Both low density lipoproteins (LDL) and high density lipoproteins (HDL) are required for lipoprotein passage (HDL). FeCl<sub>3</sub> caused liver injury by raising cholesterol, triglycerides, and LDL while decreasing HDL levels. Also, the results showed the effect of compounds extracted from Rosmarinus officinalis (Sodium valproate, flavonoids, glycosides, and alkaloids) on lipidemic in groups under study. according to many epidemiological studies, A high consumption of natural antioxidant-rich foods boosts the body's antioxidant capacity and lowers the risk of a variety of illnesses. Rosemary herbal treatments or dietary supplements are becoming more popular as a way to prevent or cure hyperlipidemia, particularly in people who have cholesterol levels that are on the borderline. (**Nyangonoet al., 2012**). It's been suggested that this might be related to its capacity to combat oxidative stress by quenching free radicals produced in the body as a result of the HFD. When compared to dried leaves or extracts, the total polyphenols, flavonoids, and rosmarinic acids content of the R. officinalis studied tincture may be correlated with antioxidant activities, as they exhibited substantially relevant quantities (**Girdet al., 2017**). According to Olorunnisolaet al., administration of phytochemicals in combined effect such as flavonoids reduced liver damage and improved liver function test results (**Olorunnisolaet al., 2012**). Furthermore, Saleh et al. documented that the lowering in the serum levels of transaminase enzymes might be produced by the presence of flavonoid substances, phenolics, and sesquiterpenes, which may protect the liver from harm (**Saleh et al., 2013**). The components of Rosmarinus officinalis functioning as free radical scavengers intercepting those free radicals might be one mechanisms for protection against liver damage by Rosmarinus officinalis (**Nassar et al., 2007**).

Also, Ramesh et al. reported that phenolic substances in the diet can prevent LDL oxidation, decreasing cardiovascular disease risk factors. Flavonoids are a type of polyphenolic substance that appears to have antioxidant effects by inhibiting the production of reactive oxygen and nitrogen



species and preserving the antioxidant defense system. Flavonoids may also enable liver cells eliminate LDL more efficiently from blood (**Ramesh et al., 2008**). Flavonoids accomplish this via increasing the density of LDL receptors in the liver and binding to apolipoprotein B. (**Pourghassem-Gargaret al., 2009**). This facts suggest that these reactions might be caused by phenolic chemicals including those contained in the fixed oil. As previously stated, the decrease in cholesterol micellar solubilization in the digestive tract, an increase in the flow of bile, concentration of bile acid and bile cholesterol, as well as an increase in fecal steroid excretion, this effects leading to cholesterol reduction (**Gorinstein et al., 2005**). This protection may be due to rosmarinic acid, which has been shown to reduce the generation of superoxide and 3-nitrotyrosine in Raw 264.7 macrophages by inhibiting the expression of inducible nitric oxide synthase (iNOS) (**Qiao et al., 2005**). The rosemary extract has the highest antioxidant activity against LDL oxidation and hyperlipidemia, according to our studies. The flavonoid and phenolic substances are examples of secondary metabolites that have a wide variety of biological characteristics, including antioxidant, anti-atherosclerosis, cardiovascular protection, and endothelial function improvement. It has been found that the antioxidant activity of phenolic compounds is mostly due to their redox properties, which allow them to act as reducing agents, by adsorbing and neutralizing reactive free radicals and chelating ferric ions, hydrogen donors play a critical role in lipid peroxidation catalysis, and have been proposed as a potential therapeutic therapy for free radical-related illnesses (**Nyangono et al., 2012**).

In our study, the levels of CAT, GSH, and SOD as well as GSH-Px, were remarkably decreased, whilst increased of MDA in FeCl<sub>3</sub> group compared to controls group. Comparison between FeCl<sub>3</sub> group and FeCl<sub>3</sub> with Rosmarinus officinalis extraction compounds (Sodium valproate, flavonoid, glycoside and alkaloids), the results showed increased of GSH, SOD, GSH-Px, and CAT levels whilst decreased of MDA compared to FeCl<sub>3</sub> group. This results agreement with other studies which reported polyphenols and flavonoids may be responsible for the reduced levels of MDA and increased levels of SOD activity. Superoxide anions are converted to hydrogen peroxide by both CAT and GPx, which is then converted to water by SOD (**Bhosale et al., 2012**). Singh et al. found that extracting plants rich in phenolic compounds reduces MDA and increases SOD activity, which is in accordance with the findings (**Singh et al., 2010**).

In separated study by Yi et al. who reported that GSH, SOD, GSH-Px, and CAT levels in the liver were significantly raised, whereas MDA levels and steatosis of microvesicular in the hepatic were significantly reduced through betulinic acid. It has been proposed that betulinic acid's hepatoprotective action is linked to an increase in antioxidant enzyme capacity, largely through strengthening of the tissue redox system and protection of the liver's antioxidant system (**Yi et al., 2014**). The remarkable decrease in plasma MDA and increase in antioxidant enzyme levels in hyperlipidemic rats, as well as their tendency to recover to near-normal levels following rosemary

extract treatment, showed their potent antioxidant activity. Antioxidants protect cells from oxidative stress by rupturing the antioxidant chain, which stops free radical processes from propagating. The ethanol diet was shown to produce direct decreases in enzymatic and non-enzymatic antioxidants, as well as increased lipid and protein alterations. Surprisingly, after taking herbals like green tea, enzyme activity and levels of non-enzymatic antioxidants, as well as lipid and protein oxidation products, were partially recovered (Li et al., 2015). Nutrient antioxidants, which are chain-breaking antioxidants that work with enzyme antioxidants to maintain reactive oxygen species (ROS) below physiological limitations, are among the dietary antioxidants (Singh et al., 2010).

### Conclusion

*Rosmarinus officinalis* L. was shown to be an essential role in the inhibition of  $\text{FeCl}_3$ 's detrimental effects in the current study. Treatment with *Rosmarinus officinalis* L. may enhance or reduce lipid peroxidation, as well as dramatically improve antioxidative enzymes. According to the current findings, *Rosmarinus officinalis* L. exhibits considerable hepatoprotective effect in addition to antioxidant activity. *Rosmarinus officinalis* L. plant active components might be utilized as one of the next generation of medicines to treat a variety of acute liver injuries.

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