

***Influence of Rosemary and Garlic on some Haematological parameters and Immunological Functions on Cirrhotic Liver Rats***

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***Abstract***

The study was conducted to investigate the effect of rosemary and garlic supplementation on some haematological parameters and immune functions in cirrhotic liver rats. Fifty two male albino rats weighing  $180 \pm 5$  g were used. The first main group (n=6) was fed on the basal diet during the experimental period and kept as a negative control group. The second main group (n =46) was intraperitoneally injected with CCl<sub>4</sub> at a dose of 0.5ml/100 g. body weight for the first time and followed by a dose of 0.3 ml/100 g b.w. twice a week over a period of six weeks in order to induce liver cirrhosis. Four injected rats were randomly chosen for pathological examination to ensure that livers become cirrhotic. The other rats (n=42) were divided into 7 subgroups (6 rats each) as follows: Subgroup (1) was fed on basal diet as a positive control group. Subgroups (2 and 3) were fed on basal diet supplemented with 5% and 10 % rosemary respectively. Subgroups (4 and 5) were fed on basal diet supplemented with 5% and 10% garlic respectively. Subgroups (6 and 7) were fed on basal diet supplemented with 5% and 10 % of (rosemary and garlic) at a ratio of (1:1) respectively. Rats were fed the experimental diet for a period of 8 weeks. Results indicated that the highest (IgM) and (IgG) levels in serum were observed in the group fed combination of rosemary and garlic compared to positive control. Diets supplemented with rosemary or garlic or their combination at different levels significantly increased ( $P<0.05$ ) the mean levels of serum total protein, albumin and globulin respectively, compared to the control positive group. The highest increase in protein parameters concentrations were observed at the groups fed on basal diet supplemented with a mixture of rosemary and garlic. Supplementation with combination of rosemary and garlic showed significant decrease ( $P<0.05$ ) in the mean serum levels of (AST), (ALT) and (ALP) respectively compared to the control positive group. On the other hand, the supplementation with rosemary, garlic and their combination significantly ( $P<0.05$ ) increased the mean level of RBCs, WBCs, PCV and Hemoglobin compared to the positive control group. The highest increase in the tested parameters was observed at the groups that fed on a combination of rosemary and garlic.

**Key Words:** rosemary-garlic-antioxidant-antiinflammatory-Liver cirrhosis- immune system-hematological parameters.

***Introduction***

The immune system is the network of cells, tissues, and organs that work together to defend the body against attacks by "foreign" invaders. These are primarily infection-causing organisms such as

bacteria, viruses, parasites and fungi. Because the human body provides an ideal environment for many microbes, the immune system's job is destroying them (*Delves and Roitt, 2000*).

Liver cirrhosis is a condition in which the liver slowly deteriorates and is unable to function normally due to chronic injury. Scar tissue replaces healthy liver tissue and partially blocks the flow of blood through the liver (*Kaidoet al., 2009*). As the liver fails, complications may include hypertension, edema, splenomegaly, metabolic bone diseases, insulin resistance and type 2 diabetes and liver cancer (*Wieland et al., 2010*). Cirrhosis can cause immune system dysfunction due to long-term malnutrition (*Bonnelet et al., 2011*). In the past, medicinal plants were considered as the only form of health care readily available to the majority of human populations, and plants or plant derived products were used for medicinal purposes for centuries. Moreover, it was estimated that about 80% of the world population used to rely on botanical preparations as medicines to meet their health needs (*Langner et al., 1998*). They were generally considered as safe and proved to be effective against certain ailments (*Ganguly et al., 2003*). The use of medicinal plants has shown a gradual increase, especially when the scientific investigations have overwhelmed us with their medicinal potentials. Two potentials are considered important in this regard; anti-mutagenic and immune modulators (*Kalpagam and Nirmala, 2003*).

Dietary polyphenols have been shown to inhibit LDL oxidation, scavenge superoxide and other reactive oxygen species (ROS), and increase antioxidant capacity (*Visioli et al., 2015*). Furthermore, some dietary phenolic compounds, mainly polyphenols, have been shown to affect human platelet function in (vitro and in vivo) (*Ostertag et al., 2010*).

Rosemary (*Rosmarinus officinalis*), due to its richness in essential oils and phenolic compounds together with other constituents, has attracted the investigators in the field of medicinal plants. In this regard, the crude extract of the plant and/or isolated compounds have shown immune modulator (*Al-Sereiti et al., 1999*) anti-mutagenic (*Alexandrov et al., 2006 and Peng et al., 2007*) and anti-oxidant effects (*Yesil-Celiktas et al., 2007 and Chang et al., 2008*).

Garlic (*Allium sativum*) has a long history of use as an immune system booster because of its antiseptic, anti-fungal and nutritive properties. It is a natural detoxicant protecting against bacterial and viral infections without any of the side-effects associated with antibiotics (*Ad'hiah et al., 2004*).

### **Aims of Study**

This study was conducted to investigate the effect of rosemary and garlic supplementation on some haematological parameters and immune functions in cirrhotic liver rats.

## **Materials and Methods**

### **Materials:--Chemicals:**

Casein, vitamins, minerals, cellulose and CCl<sub>4</sub> was obtained from El-Gomhoria Company, Cairo, Egypt. Kits for blood analysis were purchased from Gama Trade Company, Cairo, Egypt. **Plants:** Fresh rosemary (*Rosmarinus officinalis*) and garlic (*Allium sativum*) were obtained from Agriculture Research Center, Giza, Egypt. **Animals:** Adults male albino rats (n=52) of Sprague- Dawely Strain weighing (180 ± 5 g) were purchased from Helwan Farm for Experimental Animals, Helwan, Egypt .

### **Methods:**

**Preparation of dried rosemary and garlic:** fresh rosemary and garlic were cleaned, washed, dried by solar energy at the National Research Center, Dokki, Egypt and were well grinded to get the fine powder.

**Experimental Design:**

Fifty two rats were housed in well aerated cages under hygienic conditions and fed on basal diet for one week for adaptation. The diet was formulated according to **(Reeves et al., 1993)**. After this week rats were divided into two main groups as follows:-

**The first main group** (n=6) was fed on the basal diet during the whole experimental period and kept as a negative control group.

**The second main group** (n =46) was injected intraperitoneally with CCl<sub>4</sub> at a dose of 0.5ml/100 g. body weight for the first time and after one week at a dose of 0.3 ml/100 g b.w. twice a week over a period of six weeks in order to induce liver cirrhosis **(Zhao et al., 2014)**. Four rats from the injected rats were chosen randomly and slain and the liver were subjected to pathological examination to ensure that livers become cirrhotic. The other rats (n=42) were divided into 7 subgroups (6 rats each) as follows:

Subgroup (1) was fed on basal diet as a positive control group.

Subgroups (2 and 3) were fed on basal diet supplemented with 5% and 10 % rosemary respectively.

Subgroups (4 and 5) were fed on basal diet supplemented with 5% and 10% garlic respectively.

Subgroups (6 and 7) were fed on basal diet supplemented with 5% and 10 % of (rosemary and garlic) at a ratio of (1:1) respectively.

At the end of the experiment (8 weeks) the rats were fasted for 12 hour, and then sacrificed under ether anesthesia. Two blood samples were collected. The first sample was collected into a tube containing EDTA as anticoagulant and used for assessment of haematological indices. The second blood sample was collected into a centrifuge tube without any anticoagulant and was centrifuged for 15 minutes at 3000 r.p.m. to obtain serum which was stored at- 20°C until used for subsequent analysis.

**Biochemical analysis:**

Immunoglobulin M (IgM) and immunoglobulin G (IgG) were measured according to **(Ziva and Pannal, 1984)**. Total leucocytes count(WBC), red blood cell (RBC) count, haemoglobin concentrations were estimated, and packed cell volume (PCV) was determined using standard haematological technique as described by **(Ochei and Kolharktar 2008)**. Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined according to the method of **(Reitman and Frankel, 1957)** and alkaline phosphatase (ALP) according to **(Roy, 1970)**, while, Total protein, albumin and globulin were estimated according to **(Weissman et al., 1950)**.

Meanwhile, low density lipoprotein(LDL-C) and very low density lipoprotein(VLDL-C) were calculated according to **(fridewald et al.,1972)**. Serum total cholesterol (TC) **(Richmond,1973)** ,triglycerides (TG)**(Whalefeid,1974)** ,high density lipoprotein(HDL-C) **(Albers et al .,1983)** were determined.

**Statistical analysis:**

The obtained results were analyzed according to SPSS program, version (18). ANOVA test was used to compare results among groups and P<0.05 was considered to be significant **(Artimage and Berry,1987)**.

## Results

**Table (1):  
The crude chemical composition of rosemary and garlic .**

	Nutrients (/100 g)	Rosemary	Garlic
Macro nutrients	Calories (k.cal)	331	203
	Proteins (g)	4.9	8.6
	Fats (g)	15	0.7
	<u>omega-3</u> (g)	1.1	27.2
	<u>omega-6</u> (g)	1.2	311
	Carbohydrate (g)	64	45
	Total fiber (g)	43	2.7
Vitamins	Vitamin A (IU)	312	12.2
	Vitamin C (mg)	61	42.4
Minerals	zinc (mg)	3.2	1.6
	Iron (mg)	29	2.3
	Selenium	0.46	0.19

The chemical composition of rosemary and garlic as shown in Table (1) indicated that, rosemary is high in (calories, carbohydrates, total fat, total fiber, vitamin A, vitamin C and iron) but low in protein, zinc and selenium. While garlic is lesser in calories, carbohydrates and vitamin C but low in vitamin A, iron, zinc and selenium. However, it is high in total fat, omega-3 and omega-6 fatty acids.

**Table (2):  
Effect of rosemary and garlic on serum IgG and IgM in rats with induced liver cirrhosis.**

Groups	Parameters	IgG (mg/ml)	IgM (mg/ml)
Control (-ve)		5.35±0.22 <sup>a</sup>	3.93±0.03 <sup>a</sup>
Control (+ve)		1.31±0.05 <sup>g</sup>	0.49±0.08 <sup>f</sup>
Rosemary 5%		2.21±0.16 <sup>f</sup>	1.02±0.03 <sup>e</sup>
Rosemary 10%		2.95±0.13 <sup>e</sup>	1.21±0.05 <sup>e</sup>
Garlic 5%		3.69±0.05 <sup>d</sup>	1.91±0.04 <sup>d</sup>
Garlic 10%		3.82±0.23 <sup>d</sup>	1.99±0.06 <sup>d</sup>
Combination 5%		4.30±0.17 <sup>c</sup>	2.73±0.17 <sup>c</sup>
Combination 10%		4.83±0.12 <sup>b</sup>	3.38±0.19 <sup>b</sup>

Values were expressed as Means ± SE.

Values at the same column with different letters are significantly different at P<0.05.

Table (2) shows the effect of rosemary and garlic at different levels on serum immunoglobulins (IgM and IgG) of rats with induced liver cirrhosis. The injection with CCl<sub>4</sub> induced immune deficiency in rats. There are significant decrease (P<0.05) in the mean value of IgM and IgG compared to the negative control group. Diets supplemented with rosemary showed significant increase (P<0.05) in the mean levels of IgM and IgG, respectively compared to the positive control group. On adding garlic it showed significant difference (P<0.05) in the mean levels of IgM and IgG, compared to the control positive group. There was a significant difference (P<0.05) in the level of IgM and IgG among the groups that fed on the different levels of rosemary, garlic and combination of rosemary and garlic. The highest IgM and IgG level are observed in groups fed combination of rosemary and garlic.

**Table (3):**  
**Effect of rosemary and garlic on serum total protein, albumin and globulin in rats with induced liver cirrhosis.**

Parameters Groups	Total protein	Albumin	Globulin
	(g/dl)		
Control (-ve)	12.26±0.14 <sup>a</sup>	6.10±0.17 <sup>a</sup>	6.96±0.08 <sup>a</sup>
Control (+ve)	4.83±0.38 <sup>f</sup>	2.18±0.12 <sup>f</sup>	1.20±0.25 <sup>g</sup>
Rosemary 5%	6.73±0.37 <sup>e</sup>	2.99±0.12 <sup>e</sup>	2.63±0.31 <sup>f</sup>
Rosemary 10%	8.00±0.51 <sup>d</sup>	3.68±0.10 <sup>d</sup>	3.50±0.20 <sup>e</sup>
Garlic 5%	8.53±0.29 <sup>cd</sup>	3.86±0.18 <sup>d</sup>	4.33±0.28 <sup>d</sup>
Garlic 10%	9.13±0.07 <sup>c</sup>	4.03±0.08 <sup>d</sup>	5.43±0.08 <sup>c</sup>
Combination 5%	10.60±0.23 <sup>b</sup>	4.73±0.21 <sup>c</sup>	5.90±0.05 <sup>bc</sup>
Combination 10%	11.26±0.14 <sup>b</sup>	5.55±0.18 <sup>b</sup>	6.30±0.20 <sup>b</sup>

Values were expressed as Means ± SE.

Values at the same column with different letters are significantly different at P<0.05.

Data in table (3) shows the effect of rosemary and garlic at different levels on serum protein parameters (total protein, albumin and globulin) in rats with induced liver cirrhosis. Diets supplemented with rosemary or garlic or their combination at different levels significantly increased (P<0.05) the mean levels of total protein, albumin and globulin respectively, compared to the positive control group. The highest increase in protein parameters concentrations were observed at the groups fed on basal diet supplemented with a mixture of rosemary and garlic.

**Table (4):**  
**Effect of rosemary and garlic on liver functions of rats with induced liver cirrhosis.**

Parameters Groups	AST	ALT	ALP
	(μ/L)		
Control (-ve)	71.60±3.59 <sup>g</sup>	25.06±2.03 <sup>e</sup>	154.43±3.05 <sup>f</sup>
Control (+ve)	144.40±3.12 <sup>a</sup>	58.23±1.68 <sup>a</sup>	285.50±1.60 <sup>a</sup>
Rosemary 5%	130.66±3.17 <sup>b</sup>	50.16±1.16 <sup>b</sup>	238.70±8.37 <sup>b</sup>
Rosemary 10%	123.26±2.45 <sup>bc</sup>	46.40±1.13 <sup>b</sup>	221.66±3.38 <sup>c</sup>
Garlic 5%	117.53±2.26 <sup>cd</sup>	40.60±2.22 <sup>c</sup>	226.60±5.70 <sup>bc</sup>
Garlic 10%	110.66±2.33 <sup>d</sup>	38.66±0.88 <sup>c</sup>	218.53±1.57 <sup>c</sup>
Combination 5%	98.53±1.07 <sup>e</sup>	33.34±0.87 <sup>d</sup>	186.00±3.52 <sup>d</sup>
Combination 10%	88.66±2.40 <sup>f</sup>	28.16±1.01 <sup>e</sup>	170.56±2.52 <sup>e</sup>

Values were expressed as Means ± SE.

Values at the same column with different letters are significantly different at P<0.05.

Results illustrated in table (4) revealed the effect of rosemary and garlic at different levels on liver functions of rats with induced liver cirrhosis. Rats injected with CCl<sub>4</sub> (positive control rats) had significant increase (P<0.05) in the mean value of serum AST, ALT and ALP compared to the negative control group. On the other hand, the supplementation with different levels of rosemary and garlic significantly decreased (P<0.05) the mean level of serum liver enzymes compared with the control positive group. There are significant differences (P<0.05) in the level of serum AST and ALT between the groups that fed on diet supplemented with rosemary and garlic compared to the positive control group. Supplementation with combination of rosemary and garlic showed significant decrease (P<0.05) in the mean levels of AST, ALT and ALP respectively compared to the positive control group.

**Table (5):  
Effect of rosemary and garlic on serum lipid profile in rats with induced liver cirrhosis.**

Parameters Groups	TC	TG	HDL-C	VLDL-C	LDL-C
	mg/dl				
Control (-ve)	79.10±2.99 <sup>d</sup>	45.33±2.18 <sup>e</sup>	60.16±2.24 <sup>a</sup>	9.06±0.43 <sup>e</sup>	11.43±0.68 <sup>e</sup>
Control (+ve)	116.16±3.63 <sup>a</sup>	88.00±4.04 <sup>a</sup>	34.33±2.40 <sup>e</sup>	17.60±0.80 <sup>a</sup>	64.23±1.18 <sup>a</sup>
Rosemary 5%	104.80±2.31 <sup>b</sup>	79.00±0.57 <sup>b</sup>	43.00±1.52 <sup>d</sup>	15.80±0.11 <sup>b</sup>	46.00±1.10 <sup>b</sup>
Rosemary 10%	97.33±1.76 <sup>c</sup>	68.67±0.88 <sup>c</sup>	46.00±2.08 <sup>cd</sup>	13.73±0.17 <sup>c</sup>	37.60±3.36 <sup>c</sup>
Garlic 5%	92.70±2.71 <sup>c</sup>	66.00±2.08 <sup>c</sup>	48.40±0.94 <sup>cd</sup>	13.20±0.41 <sup>c</sup>	31.10±1.72 <sup>d</sup>
Garlic 10%	90.10±1.81 <sup>c</sup>	63.83±1.16 <sup>c</sup>	51.66±1.20 <sup>bc</sup>	12.76±0.23 <sup>c</sup>	25.66±2.47 <sup>d</sup>
Combination 5%	76.66±1.76 <sup>d</sup>	57.00±1.52 <sup>d</sup>	56.33±3.17 <sup>ab</sup>	11.40±0.30 <sup>d</sup>	8.93±3.26 <sup>ef</sup>
Combination 10%	75.76±1.75 <sup>d</sup>	55.66±1.85 <sup>d</sup>	60.46±1.86 <sup>a</sup>	11.13±0.37 <sup>d</sup>	4.16±0.64 <sup>f</sup>

Values were expressed as Means ± SE.

Values at the same column with different letters are significantly different at P<0.05.

TC:-Total cholesterol

TG:-Triglycerides

HDL:-High-density lipoprotein

LDL-C:-Low-density lipoproteins

VLDL-C:-Very low-density lipoproteins

Data in table (5) shows the effect of rosemary and garlic at different levels on TC, TG, HDL, VLDL and LDL in rats with induced liver cirrhosis. Diets supplemented with rosemary or garlic at different levels showed significant decrease (P<0.05) in the mean levels of TC, TG, VLDL and LDL, compared to the control positive group, while serum HLD-c was significantly increased (P<0.05). Also diets supplemented with garlic and combination of rosemary and garlic showed significant decrease (P<0.05) in the mean levels of TC, TG, VLDL and LDL, compared to the +ve group and increase in the level of HDL-c. It was observed that the more improvement of lipid profile was recorded at the groups fed on a mixture of rosemary and garlic

**Table (6)  
Effect of rosemary and garlic supplementation on blood cell parameters of rats with induced liver cirrhosis.**

Parameters Groups	Hemoglobin %	RBCs (million/c.mm)	WBCs(thousands /c.mm)	PCV%
	Control (-ve)	15.70±0.10 <sup>a</sup>	6.71±0.24 <sup>a</sup>	3.65±0.15 <sup>c</sup>
Control (+ve)	7.90±0.40 <sup>f</sup>	2.42±0.42 <sup>d</sup>	11.50±0.90 <sup>a</sup>	26.00±5.00 <sup>d</sup>
Rosemary 5%	11.20±0.20 <sup>e</sup>	4.47±0.02 <sup>c</sup>	6.22±0.67 <sup>b</sup>	38.52±1.50 <sup>b</sup>
Rosemary 10%	12.65±0.25 <sup>d</sup>	4.58±0.08 <sup>c</sup>	6.35±0.25 <sup>b</sup>	43.00±2.00 <sup>b</sup>
Garlic 5%	12.97±0.12 <sup>cd</sup>	4.44±0.06 <sup>c</sup>	6.40±0.40 <sup>b</sup>	37.44±0.50 <sup>b</sup>
Garlic 10%	13.72±0.22 <sup>bc</sup>	4.75±0.25 <sup>c</sup>	7.10±0.01 <sup>b</sup>	41.00±2.00 <sup>b</sup>
Combination 5%	14.10±0.10 <sup>b</sup>	5.72±0.15 <sup>b</sup>	4.70±0.16 <sup>c</sup>	51.48±1.50 <sup>b</sup>
Combination 10%	14.10±0.40 <sup>b</sup>	6.02±0.12 <sup>b</sup>	4.09±0.11 <sup>c</sup>	58.00±2.00 <sup>ab</sup>

Values were expressed as Means ± SE.

Values at the same column with different letters are significantly different at P<0.05.

Table (6) shows the effect of rosemary and garlic at different levels on blood cell parameters and Hemoglobin of rats with induced liver cirrhosis. The positive control group had significant decrease

( $P < 0.05$ ) in the mean value of Hb, RBCs, PCV and WBCs compared to the control negative group. On the other hand, the supplementation with rosemary, garlic and their combination significantly ( $P < 0.05$ ) increased the mean level of RBCs, WBCs, PCV and Hemoglobin, compared to the positive control group. The highest increase in the tested parameters was observed at the groups that fed on a combination of rosemary and garlic.

## ***Discussion***

Chronic liver damage is a wide spread pathology characterized by a progressive evolution from steatosis, fibrosis and cirrhosis. The production of reactive oxygen species (ROS) is considered to be a major factor in oxidative cell injury. The antioxidant activity or the inhibition of free radicals generation is important in providing protection against such hepatic damage (*Vitaglione et al., 2004*).

Cirrhosis produces hepatocellular dysfunction and increased intrahepatic resistance to blood flow (*Hung et al., 2006*). The hepatic fibrosis developed due to increased accumulation of malondialdehyde (MDA), the stable end product of lipid peroxidation. MDA in this oxidative stress causes various diseases (*Lee et al., 2004*).

Medicinal plants were considered as the only form of health care readily available to the majority of human populations, and plants or plant derived products were used for medicinal purposes for centuries. Moreover, it is estimated that about 80% of the world population relies on botanical preparations as medicines to meet their health needs (*Langner et al., 1998*). This is reasoned by the fact that they are generally considered as safe and proved to be effective against certain ailments (*Ganguly et al., 2003*).

Rosemary contained the highest concentration of phenolic substances obtained from the leaves of the evergreen shrub rosemary (*Okamura et al., 1994*). The phenolic compounds responsible for rosemary antioxidant activity are mainly phenolic diterpenes such as carnosol, carnosic acid, rosmanol, epirosmanol and isorosmanol (*Cuvelier et al., 1994 and Del Bano et al., 2003*). Rosmanol is a potent antioxidant having inhibition effect of superoxide anion production, as well as lipid peroxidation and free radical scavenging activities (*Haraguchi et al., 1995 and Escuder et al., 2002*). The polyphenol, rosmarinic acid has 2 aromatic rings, each with 2 -OH groups that are capable of donating H. and chelating metals. (*Hra et al., 2000*) reported that rosemary extract exhibited antioxidant activity superior to  $\alpha$ -tocopherol. Rosemary oleoresin has been reported to contain several components such as rosmanol, rosmariquinone, rosmaridiphenol, and carnosol that may be up to four times as effective as BHA and equal to BHT as an antioxidant (*Houlihan et al., 1984 and Chang, 1984*).

Rosmarinic acid exhibits antioxidant and antiinflammatory effects (*Halliwell, 1996*). Rosemary extracts are able to donate electrons to reactive radicals, converting them to more stable and on reactive species, therefore preventing them from reaching biomolecules, such as lipoproteins, polyunsaturated fatty acids, DNA, amino acids, proteins and sugars, in susceptible biological systems. Also, it was concluded that rosemary extracts have a high scavenging capacity of different types of reactive oxygen and nitrogen species, mostly free radicals, is thought to be one of the main mechanisms of the antioxidant action exhibited by phenolic phytochemicals (*Moreno et al., 2006*).

Dietary supplement because of its powerful antioxidant activity (*Dorman et al., 2003*) may function as an immunoenhancing antioxidant that could help alleviate the oxidative stress condition and will probably be effective in some stressed condition such as protein or antioxidant deficiency (*Babuet et al.,*

1998). Immunomodulation is one of the main targets for synthetic drugs and chemicals. However, its high cost, anticipated toxicity, and adverse event effects render it undesirable for the patients. In contrast, the use of herbal plants as health promoters is gaining increasing attention in both consumers and scientific circles. In the literature, several plants have been listed that exhibit immunomodulatory actions, like modulation of cytokine secretion; phagocytosis promotion and macrophage activation; immunoglobulin production; allergic reactions and lymphocyte proliferation. Recently, garlic has been suggested as a promising candidate for maintaining the homeostasis of the immune system. Several studies have been carried out in animal models to examine the effect of different garlic components and formulations on immunomodulatory activities (*Mahima et al., 2012*).

The obtained results revealed that rosemary and garlic supplementation increased the immune functions, are in agreement with (*Ad'hiah et al., 2004*) who mentioned that the garlic has a long history of use as an immune system booster because of its antiseptic, anti-fungal and nutritive properties. It is a natural detoxicant protecting against bacterial and viral infections without any of the side-effects associated with antibiotics. In the current study, the protective effects of rosemary powder against CCl<sub>4</sub>-induced hepatotoxicity in adult male rats were investigated. In this respect, the effect of the extract on the liver functions and oxidative stress markers (hepatic glutathione reductase activity and malondialdehyde level) as well as histopathological changes of the liver were evaluated (*Wang et al. 2008*).

The current results are in agreement with the finding by (*Liu et al., 2007*) who reported that administration of rats with rosemary extract alleviated the deleterious effect of CCl<sub>4</sub> on liver. Rosemary may act as a co-factor in the synthesis of biological endogenous antioxidant material (*Reddy and Rao, 2006*). In the current study, high dose (as well as continuous administration) induced high degree of protection and improvement. This could be attributed to the high percentage of antioxidants in the high dose, which resulted in strongest inhibition of oxidative radicals from reaching the biological molecules. By the same manner, the continuous administration of rosemary extract besides CCl<sub>4</sub> built a continuous resistant-barrier with more protection battery against oxidative radicals (*Mata et al., 2007*).

Rosemary inhibited and reduced the CCl<sub>4</sub>-induced hepatotoxicity in rats possibly by scavenging or blocking the formation of free radicals generated during CCl<sub>4</sub> metabolism. These improving effects of rosemary could be attributed to the bioactive constituents that alleviated the deleterious effect of CCl<sub>4</sub> either by the well-known scavenging action or the antioxidant properties that inhibited lipid peroxidation, stabilized the reactive radicals, preserve the cellular integrity and restrain the severity of CCl<sub>4</sub> (*Sotelo-Félix et al., 2002*).

Garlic is one of the earliest documented examples of plants employed for treatment of disease and maintenance of health. Garlic also contains a high concentration of selenium, which is responsible, in part, for garlic's antioxidant and cancer preventive effects. Hence, some growers add selenium to the soil to increase garlic's selenium content (*Amagase, 2006*).

Oral administration of rosemary leaf extract caused significant declines in the blood levels of triglycerides, total cholesterol, LDL-cholesterol, but increased HDL cholesterol. Moreover, it seemed that rosemary leaf extract had a hypolipidemic potential. This may be an indication of progressive metabolic control of rosemary leaf extract on mechanisms involved in elimination of the lipids from the body, this hypolipidemic properties have been confirmed in many plant species and plant products in medicinal use (*Kono et al., 1992*).

The most important constituents of rosemary are caffeic acid and its derivatives such as rosmarinic acid. These compounds have antioxidant effect (*Al-Sereiti et al., 1999*). A variety of phenolic compounds, in addition to flavonoids, are found in fruit, vegetables and many herbs. The phenolic compounds (such as caffeic, ellagic, and ferulic acids, sesamol, and vanillin) inhibit atherosclerosis (*Decker, 1995*). In addition to a well documented role in reverse cholesterol transport, HDL-cholesterol has recently been recognized to have several other important cardio protective properties including the ability to protect LDL from oxidative modification (*Nofer et al., 2002*), the polyphenols glabridin (derived from licorice), rosmarinic acid or carnosic acid (derived from rosemary), as well as garlic (which contains a mixture on natural antioxidants) inhibited LDL oxidation in a dose-dependent manner (*Fuhrman et al., 2000*). Moreover, several studies showed that plant extracts lowered LDL oxidation. However, the present data demonstrated that consumption of rosemary can lead to reduction in the risk of hyperlipidemic symptoms and heart diseases. It can be concluded from presented results that rosemary have improved in the lipid profile. (*Naidu and Thippeswamy, 2002*).

Several studies have been carried out in animal models to examine the effect of different garlic components and formulations on immunomodulatory activities. Different studies have shown that garlic compounds are able to perform antiapoptotic (*Chen et al., 2014*), antiparasitic (*Gharavi et al., 2011*), proapoptotic, anticancerigenic (*Xu et al., 2014*), and immunomodulatory (*Bauer et al., 2014*) effects on different cells. The presence of garlic compounds varies with respect to mode of garlic preparation and extraction as follows: (1) fresh bulbs main compounds are S-allyl-L-cysteine sulfoxide (alliin) and  $\gamma$ -glutamyl cysteine derivatives; (2) in steam distilled oils, sulfide family compounds are the main compounds; (3) powder from crushed and dried garlic contains alliin and diallyl disulfide (DADS); (4) macerates (ground garlic) are enriched extractions with sulfide family compounds, dithiines, and (E–Z)-ajoene compounds, and (5) AGE (soaked, sliced, aged garlic extract in ethanol solution) contains S-allyl-L-cysteine (SAC) and S-allylmercaptocysteine (SAMC) (*Trio et al., 2014*).

Garlic compounds can be divided to several groups or families of compounds. Among these families,  $\gamma$ -glutamyl cysteine derivatives, the primary precursor components of the alliin and allyl methyl cysteine (methiin) compound families (*Amagase et al., 2001*), that produced, by enzymatic action of alliinase (alliinlyase, EC: 4.4.1.4), the diallylthiosulfinate (Allicin) and allyl methyl thiosulfinate (AM) compound families (*Augusti et al., 2012*), which are precursors of several organosulfur compound families (i.e., the ajoene and dithiin families) (Lawson et al., 2005). Additionally, garlic preparations contain nonorgan sulfured compounds, such as tetrahydro-beta-carbolines (*Ichikawa et al., 2006*), fructans, and glucose-linked  $\beta$ -D-fructofuranosyl (*Chandrashekar et al., 2011*), identified in AGE preparations (*Kim et al., 2013*).

## **Conclusions**

Rosemary inhibited and reduced the CCl<sub>4</sub>-induced hepatotoxicity in rats possibly by scavenging or blocking the formation of free radicals generated during CCl<sub>4</sub> metabolism. These improving effects of rosemary could be attributed to the bioactive constituents that alleviated the deleterious effect of CCl<sub>4</sub> either by the well-known scavenging action or the antioxidant properties that inhibited lipid peroxidation, stabilized the reactive radicals, preserve the cellular integrity and restrain the severity of CCl<sub>4</sub>. The medicinal importance of garlic preparations has already been substantiated by the availability of garlic pearls for human use. Present study has shown that garlic has promising anti-inflammatory activity.

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## تأثير الروزماري والثوم علي بعض قياسات الدم والوظائف المناعية علي التليف الكبدي للفئران

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### الملخص العربي

أجريت هذه الدراسة لمعرفة تأثير الروزماري والثوم على بعض قياسات الدم وبعض الوظائف المناعية في الفئران المصابة بالتليف الكبدي تم استخدام 52 من ذكور الجرذان من سلالة الالبيينو وزنهم  $180 \pm 5.5$ . تم تقسيم المجموعات كالتالي المجموعة الاولى الرئيسية وهي المجموعة الضابطة السالبة (6 فئران) وتتغذي علي الغذاء الاساسي فقط . المجموعة الثانية الرئيسية (46 فئران) تغذت علي الغذاء الاساسي وتم حقنها بجرعة من رابع كلوريد الكربون لاحداث التليف الكبدي للفئران. ثم بعد ذلك تم تقسيمهم الي 7مجاميع فرعية (6 فئران لكل مجموعة). المجموعة الفرعية (1) تغذت علي الغذاء الاساسي فقط وهي تمثل المجموعة الضابطة الموجبة . المجموعة الفرعية (2) تم تغذيتها علي الغذاء الاساسي مع اضافة الروزماري بنسبة 5% والمجموعة الفرعية (3) تم تغذيتها علي الغذاء الاساسي مع اضافة الروزماري بنسبه 10% من والمجموعة الفرعية (4) تم تغذيتها علي الغذاء الاساسي مع اضافة الثوم بنسبه 5% والمجموعة الفرعية (5) تم تغذيتها علي الغذاء الاساسي مع اضافة الثوم بنسبه 10% والمجموعة الفرعية (6) تم تغذيتها علي الغذاء الاساسي مع اضافة 5% خليط من الروزماري والثوم بنسبه 1:1 والمجموعة الفرعية (7) تم تغذيتها علي الغذاء الاساسي مع اضافة 10% خليط من الروزماري والثوم بنسبه 1:1 لمدة 8 أسابيع . أشارت النتائج الي ان المجموعة التي تغذت علي خليط من الروزماري والثوم ادي الي حدوث ارتفاع في المناعة ذو دلالة احصائية  $P < 0.05$  مقارنة بالمجموعة الضابطة الموجبة. وأن الغذاء المحتوي علي روزماري او الثوم او الخليط بينهم ادي الي حدوث في ارتفاع مستوياتالبروتينوالألبومينوالجلوبولينعلالتواليبيدرجة ذات دلالة احصائية  $P < 0.05$  وأن مقاييس الدم قد ارتفعت في المجموعة التي تغذت علي خليط بين الروزماري والثوم. واطهرت المجموعة التي تغذت علي خليط من الروزماري والثوم انخفاضاً كبيراً بدرجة دلالة احصائية  $P < 0.05$  في مستويات انزيمات الكبد (ALT,AST وALP) مقارنة بالمجموعة الضابطة الموجبة. ومن ناحية اخري اضافة الروزماري او الثوم او الخليط بينهم ادي الي حدوث في ارتفاع عدد كرات الدم الحمراء وكرات الدم البيضاء والهيموجلوبين ذات دلالة احصائية  $P < 0.05$  مقارنة بالمجموعة الضابطة الموجبة . وأظهرت النتائج أن أعلى معدلات في القياسات المختبرة في المجموعة التي تغذت على خليط من الروزماري والثوم .