



EFFECT OF ROSEMARY EXTRACTS ON DIABETIC AND LIVER MALFUNCTIONAL RATS

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ABSTRACT

In this study, alcoholic and aqueous extracts of rosemary and its oil were used to investigate their effects on diabetic and liver malfunctional rats by administrating them with STZ or CCl₄ for three weeks. The therapeutic effects of rosemary extracts (alcoholic and aqueous) and its oil against acute hyperglycemia induced by STZ in rats showed a decrease in the levels of fasting and postprandial blood glucose of rats treated with alcoholic extract of rosemary. In addition, results showed a restoration of these levels of the rats treated with aqueous extract or oil to their normal ranges after 3 weeks. There is a significant difference in lipid profile between rats treated with alcoholic or aqueous extracts and the negative control, where, the HDL in group administrated rosemary oil is significantly higher than both positive and negative control.

The protective effects of rosemary extracts (alcoholic and aqueous) and its oil against liver toxicity induced by CCl₄ in rats showed a significant difference between both (negative and positive control) and the activity of AST and ALT in the rats treated by alcoholic and aqueous extracts of rosemary. The protective effects of the plant extracts against liver toxicant occurred by decreasing the activities of these enzymes. Oil rosemary had restored effect more than both ethanolic and aqueous extracts, but not significant. This protective effect occurred by decreasing the activities of AST, ALT and Alp enzymes. No significant ($p \leq 0.05$) differences were noticed among the levels of serum total protein for all treatments compared

to the negative and positive controls. The levels of total cholesterol and triglycerides recorded high values for the positive controls compared to the negative control. The protective effects of rosemary extracts against liver toxicant occurred by restoring the levels of these parameters to their normal values.

INTRODUCTION

There are several factors lead to liver toxicity and diabetes namely the toxins such as aflatoxins, drugs and chemicals such as heavy metals and organic solvents especially CCl₄, streptozotocin (STZ) and alcohols.

There are many herbals which are used in therapeutic or protective effect against diseases in experimental animals. These herbals include rosemary and others.

Diabetes mellitus (DM) is a serious health problem being the third greatest cause of death all over the world, and if not treated, it is responsible for many complications affecting various organs in the body. Diabetes mellitus results in hyperglycemia and is characterized as type 1 in absolute insulin deficiency or type 2 in insulin resistance due to receptor insensitivity to endogenous insulin (El-Hilaly et al 2007).

The main active constituent of Rosemary plant is the volatile oil which includes camphene, camphor, cineol, boroneol, resin, bitters matter, rosemary acid and flavonoids (Begum et al 2013).

The main constituents of rosemary oil are: camphor, 1,8-cineol, α -pinene, boroneol, camphene, β -pinene, limonene, verbenone, caryophyllene and myrcene. The 1,8-cineol, boroneol and camphor compounds are presented with low quantity (Domokos et al 1997 and Salido et al 2003).

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Rosemary contains caffeic acid and rosmarinic acid, both of which are potent antioxidants as well as anti-inflammatory agents. Rosemary is also a good source of antioxidant vitamin E (alpha tocopherol) and other important antioxidants. In addition, rosemary contains 19 different chemical components with antibacterial action and volatile oil which reduce the airway constriction induced by histamine. The volatile oils in rosemary also reduce inflammation that contributes to liver and heart diseases. Herbalists think that rosemary may also help ease breast pain by acting as a natural drying agent to fluid filled cysts (Aruoma et al 1992 and Bakirel et al 2008).

Water-soluble extract from rosemary leaves is rich in rosmarinic acid (1.3%) and flavonoids (3%). A wide range of oxygenated monoterpenes are identified: oxides, such as 1,8-cineole, and alcohols, such as borneol and α -terpineol. Chemical composition of rosemary essential oil can vary between regions and it depends mostly on climate, soil composition, plant organ, age and stage of vegetative cycle (Faixova and Faix, 2008).

Effects of rosemary are attributed to the different chemical constituents including monoterpenes, diterpenes and the phenolic derivatives -mainly caffeic acid known as rosmarinic or carnosic acid and gallic acid. Among these bioactive constituents, the pharmacologic properties have been suggested to be highly attributed to rosmarinic acid, the predominant secondary metabolite in rosemary. Biochemically, rosmarinic acid is an ester of caffeic acid, hydroxydihydrocaffeic acid, chlorogenic acid and their hydrolyzed metabolites (El-Deeb, 1993; Rababah et al 2004; Wang et al 2004 and Herrero et al 2010). Rosemarinic acid has been suggested to have anti-atherogenic activity by preventing the oxidation (Hadafi et al 1998; Shetty, 2007; Dubois et al 2008; Park et al 2008; Vanithadevi and Anuradha 2008; Furtado et al 2010 and Moon et al 2010).

Rosemary contains a mixture of natural antioxidants. Generally, antioxidants dramatically change metabolism of glucose, lipid and proteins associated with cardiac diseases, (Fuhrman et al 2000). Antioxidants are found to perform several cardio protective properties including the ability to prevent LDL from oxidative modification by monitoring the levels of triglycerides, HDL and LDL. HDL (Nofer et al 2002) or accumulation of lipid peroxides on LDL (Mackness et al 1993). More or less, rosemary may exert antioxidant effects indirectly by increasing levels of antioxidants agents such as

glutathione reductase, vitamin C and β carotene (Labban et al 2014).

Free radicals/reactive oxygen species are associated with many biological phenomena, such as inflammation, aging, and carcinogenesis. The antioxidant activity of polar extracts of rosemary is related to the content of phenolic compounds (i.e. carnosol, carnosic acid). Constituents in rosemary have shown a variety of pharmacological activities for cancer chemoprevention and therapy in *in vitro* and *in vivo* models (Shabtay et al 2008).

This study aims to evaluate (i) the therapeutic effects of alcoholic, aqueous and oil extracts of rosemary on acute hyperglycemia induced by streptozotocin in male rats. (ii) The protective effects of the previous extracts of rosemary on the liver toxicity induced by CCl₄ in male rats. The protective and therapeutic effects of these plant extracts compared to the adverse effects of CCl₄ or STZ were determined through different blood biochemical assays.

MATERIALS AND METHODS

Materials

Plant material

Samples of dried Rosemary (*Rosmarinus officinalis* L.) and rosemary oil were obtained from Agricultural Seeds, Spices and Medicinal Plants Co., Al-Azhar st., Cairo, Egypt.

Reagent kits

Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Total protein, Albumin, Glucose, Cholesterol, High density lipoprotein cholesterol (HDL-C), Triglycerides, Alkaline phosphatase (ALP) kits were obtained from Egyptian company for Biotechnology, Obour city Industrial area, block 20008 pieces 19A. Cairo, Egypt.

Methods of analysis

Preparation of plant extracts

Ethanollic extract

Dried rosemary (100 g) was extracted three times with (400, 300 and 200 ml) ethanol (95%) for 24 hours at room temperature (25°C), and the samples were shaken and filtered after each extraction. Solvent was removed from the combined

extracts with a vacuum rotary evaporator at 40°C to obtain crude plant extracts. The dried extracts were weighed and stored at -20°C. Final extract was a dark green powder.

Aqueous extract

Dried rosemary (100 g) was extracted three times with (800, 600 and 400 ml) distilled water for 24 hours at room temperature (25°C), and the samples were shaken and filtered after each extraction. Solvent was removed from the combined extracts with a freezing drying to obtain crude plant extract. The dried extract was weighed and stored at -20°C. Final extract was a dark brown powder

GC-MS analysis of rosemary oil

GC-MS analysis of rosemary oil was carried out in faculty of science, Ain Shams University, Cairo, Egypt.

Biological Experiment

Experimental animals

A total of 54 adult male albino rats (weighing 160-200g) were obtained from Agricultural Research Center in Giza, Egypt. The animals were housed in acrylic cages in standard conditions of temperature prior to the experiments for one week in order to adapt to the laboratory condition, and they were also fed with commercial diet and water (Hanan, 2012).

Induction of Experimental Diabetes

A freshly prepared solution of Streptozotocin (STZ) (50 mg/kg body weight in 0.1 M citrate buffer, pH 4.5) was injected intraperitoneally for overnight fasted rats. STZ injected animals exhibited hyperglycemia within 48-36h (Singh et al 2007). The rats having fasting blood glucose (FBG) values of 250 mg/dl or above were considered for the study.

Protection of Experimental Hepatitis

A freshly prepared solution of carbon tetrachloride (CCl₄) (1ml/kg body weight dissolved in corn oil 50%v/v) was injected intraperitoneally twice a week for 21 days for all groups (Heibatollah et al 2008).

Experimental design

The experiment was carried out on (9) groups of six rats in each group to study the effect of rosemary extracts on diabetic and liver malfunctional rats as follows:

Group 1: Control rats received standard normal diet. The other groups received the different treatments besides being fed on the normal diet. **Group 2:** Diabetic control rats (rats + STZ). **Group 3,** STZ induced diabetic rats, was treated with alcoholic extract of Rosemary orally by stomach tube with a dose of (200mg/kg body weight) daily for 3 weeks. **Group 4,** STZ induced diabetic rats, was treated with aqueous extract of Rosemary orally by stomach tube with a dose of (200mg/kg body weight) daily for 3 weeks. **Group 5,** STZ induced diabetic rats, was treated with Rosemary essential oil orally by stomach tube with a dose of (200mg/kg body weight) daily for 3 weeks. **Group 6:** Hepatitis control rats (rats + CCl₄). **Group 7,** CCl₄ induced hepatotoxicity, was treated with alcohol extract of Rosemary orally by stomach tube with a dose of (500mg/kg body weight) day after day for 3 weeks. **Group 8,** CCl₄ induced hepatotoxicity, was treated with aqueous extract of Rosemary orally by stomach tube with a dose of (250mg/kg body weight) day after day for 3 weeks. **Group 9,** CCl₄ induced hepatotoxicity, was treated with Rosemary essential oil orally by stomach tube with a dose of (250mg/kg body weight) day after day for 3 weeks.

Statistical analysis

The data were presented as means ± SD from six replicates and subjected to one way ANOVA. The means of different treatments were compared using Duncan's multiple range tests at $p \leq 0.05$. Statistical analyses were performed using SPSS statistical software (IBM SPSS Statistics, version 20) (Snedecor and Cochran, 1980).

RESULTS AND DISCUSSION

1- GC-MS analysis of rosemary oil

GC-MS of rosemary oil is illustrated and calculated in **Table (1)**. The data refer that Rosemary oil contains mainly terpenes. Many of these compounds have antioxidant properties. The results agree with what obtained by (Wang et al 2008).

Table 1. GC-MS analyses of Rosemary oil

RT	Area Pct	Library/ID
8.81	3.01	p-Cymene
8.88	2.97	D-Limonene
8.94	50.96	1,8-Cineole
10.13	1.03	Linalool
10.87	9.32	(+)-2-Bornanone
11.23	2.41	endo-Borneol
11.60	1.74	alpha-Terpineol
14.84	2.32	caryophyllene
7.09	16.13	α -pinene
7.17	0.13	1-Methyl-3-piperidinemethanol
7.38	4.85	Camphene
7.92	1.50	beta-Pinene
8.20	1.36	beta-Myrcene
8.44	0.25	Bicyclo[3.1.0]hex-2-ene,2-methyl-5-(1-methylethyl)
8.66	0.56	cyclohexene,1-methyl-4-(1-methylethylidene)

2- Therapeutic effect of the extracts of rosemary (alcoholic, aqueous) and its oil on the acute hyperglycemia induced by streptozotocin in rats

Effects of the extracts of rosemary (alcoholic or aqueous) and its oil on the levels of fasting and postprandial blood glucose analyzed at the end of experiment period (21 days) in rats treated with streptozotocin are shown in (Tables 2 & 3). The rats treated with streptozotocin only (positive control) and diabetic rats received alcoholic, aqueous or oil extracts of rosemary recorded high levels of fasting blood glucose (309.9, 311.9, 306.5 and 359.8 mg/dl, respectively) compared to the negative control group (96.6 mg/dl) at zero time. Similarly the rats treated with streptozotocin (STZ) only (positive control) and diabetic rats received alcoholic, aqueous or oil extracts of rosemary recorded high levels of postprandial blood glucose (440.0, 428.2, 457.4 and 471.3 mg/dl, respectively) compared to the negative control group (126.4 mg/dl) at zero time. There were slight increase in the levels of fasting and postprandial blood glucose for the positive control, and gradual decrease for dia-

betic rats treated with alcoholic, aqueous or oil extracts of rosemary during three weeks. The levels of fasting blood glucose of rats induced by STZ and treated by alcoholic, aqueous or oil extracts after 21 days are (144.9, 119.7 and 99.6 mg/dl, respectively). As it is shown from the previous data that the alcoholic extract of rosemary was found to have therapeutic potential. It possesses hypoglycemic properties but the aqueous and oil extracts of rosemary were more efficient, (restoring the levels of fasting and postprandial blood glucose to their normal ranges after 21 days). These data are similar to these obtained by (Bakirel et al 2008).

Diabetic rats showed a significant increase of glucose level associated to a significant decrease of insulin level, in comparison to their respective values in control. STZ leads to diabetes by destroying the insulin secreting cells of the pancreas and correspondingly leads to hypoinsulinemia and hyperglycemia. It means that STZ leads to hyperglycemia by specific cytotoxic impact on pancreatic beta cells. One of the intracellular phenomena for its cytotoxicity is through the production of free radicals exhibited both *in-vivo* and *in-vitro* as stated by (Yadav et al 2002).

Table 2. Effect of alcoholic, aqueous or oil extracts of rosemary on the levels of fasting blood glucose (mg/dl) analyzed weekly in rats treated with (STZ).

Time	Zero	7 days	14 days	21 days
Control	96.6 ^b	101.3 ^c	91.7 ^d	103.1 ^c
	± 51.9	± 41.3	± 36.9	± 40.7
STZ only	309.9 ^a	325.6 ^a	334.3 ^a	347.5 ^a
	± 79.1	± 58.6	± 47.1	± 50.8
STZ + alcoholic	311.9 ^a	292.7 ^{ab}	234.9 ^b	144.9 ^b
	± 43.5	± 37.9	± 41.4	± 45.9
STZ +Aqueous	306.5 ^a	241.9 ^b	177.0 ^c	119.7 ^c
	± 44.3	± 39.9	± 42.3	± 44.5
STZ + Oil	359.8 ^a	288.1 ^{ab}	212.6 ^c	99.6 ^c
	± 41.3	± 41.2	± 45.9	± 57.2

The data represent the means ± SE calculated from six replicates. Different letters refer to significant differences at ($P \leq 0.05$).

Table 3. Effect of alcoholic, aqueous or oil extracts of rosemary on the levels of postprandial blood glucose (mg/dl) analyzed weekly in rats treated with (STZ).

Time	Zero	7 days	14 days	21 days
Control	126.4 ^d	131.6 ^d	119.6 ^d	135.8 ^c
	± 67.1	± 52.2	± 51.9	± 63.8
STZ only	440.0 ^{cb}	449.5 ^a	465.8 ^a	478.6 ^a
	± 61.2	± 50.7	± 54.3	± 66.6
STZ + alcoholic	428.2 ^c	376.2 ^b	282.1 ^b	160.9 ^b
	± 63.2	± 53.1	± 56.9	± 68.7
STZ +Aqueous	457.4 ^{ab}	366.5 ^b	274.4 ^b	140.9 ^c
	± 66.5	± 54.5	± 55.7	± 67.2
STZ + Oil	471.3 ^a	322.2 ^c	240.2 ^c	132.2 ^c
	± 69.2	± 60.9	± 63.8	± 75.5

The data represent the means ± SE calculated from six replicates. Different letters refer to significant differences at ($P \leq 0.05$).

The mechanism of the anti-diabetic activity indicates that rosemary extracts, especially rosemary oil works as a protection and stimulation of β -cells of pancreatic islets, since it increases β -cells and subsequent release of insulin. It also increases insulin receptor. Rosemary extract reduces blood sugar through regeneration β -cell. It could be said that this extract may have antidiabetic effect by preventing mucosal damage of β -cells, i.e. preventing of oxidative stress and regenerating of β -cells. This subsequent improves the increase secretion of insulin from β -cells of pancreas.

3- Therapeutic effect of alcoholic, aqueous or oil extracts of rosemary plant on Triglycerides (TG), total cholesterol (TC), (HDL) and (LDL)

Low Density Lipoprotein (LDL) is considered as a bad lipoprotein because it transfers cholesterol from liver to tissues, but High Density Lipoprotein (HDL) is considered as a good lipoprotein because it transfers cholesterol from tissues to liver.

The serum lipid parameters, triglycerides, total cholesterol and LDL in the STZ diabetic rats increased, while HDL decreased in comparison with negative control group, since it amounted in rats administrated alcoholic extract or aqueous extract of rosemary (46.1 and 49.8 mg/dl respectively), i.e. there is a significant difference between these two groups and the control one as it is shown in **Table**

(4). However the HDL in group administrated rosemary oil is amounted (55.1 mg/dl) and this amount is significantly higher than both positive control (42.2 mg/dl) and negative control (51.3 mg/dl).

4- Protective effect of Rosemary extracts on the liver toxicity induced by CCl₄ in rats

a) Effect of alcoholic, aqueous and oil extracts of rosemary on the liver functions in rats treated with CCl₄

The adverse effects of liver toxicity was produced by intraperitoneally administration of CCl₄ twice a week for 21 days at a dose of (1ml/kg body weight dissolved in corn oil 50% v/v) for experimental rats. The protective effects of alcoholic, aqueous or oil extracts of rosemary which were administered orally by stomach tube day after day for 21 days at a dose of (500,250 and 250 mg/kg respectively) was noticed. This administration was done to avoid these adverse effects through antioxidant and/or anti-inflammatory activity of extracts of this plant. The protective effects were monitored through activity determination of different liver enzymes namely alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) in the serum. These results agree with those obtained by (Kaliora and Andrikopoulos, 2005) who mentioned that rosemary extracts are found to exhibit different effects as hepatoprotective and anticancer.

b) Serum alanine aminotransferase (ALT) activity

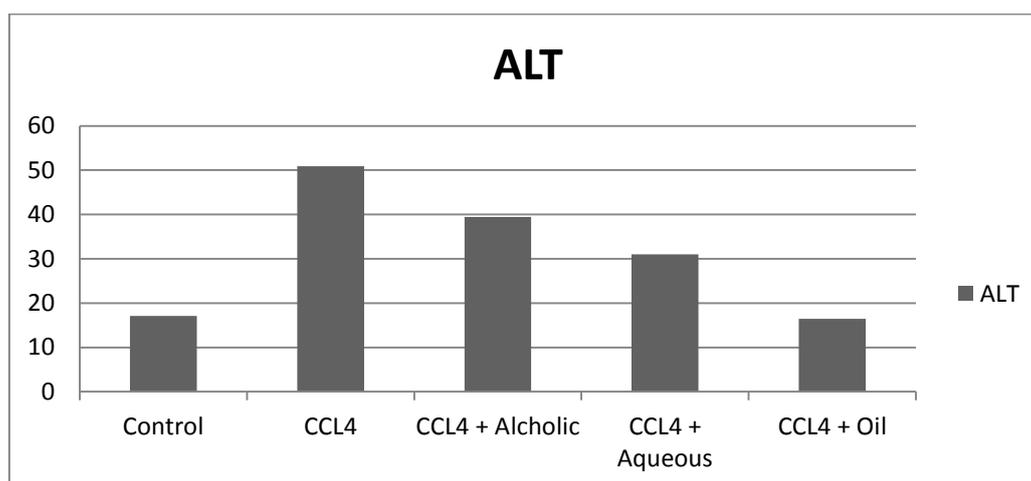
The enzymes ALT and AST are widely distributed with high concentration in liver and to a lesser extent in kidneys and heart. Although both serum ALT and AST become elevated whenever disease processes affect liver cell integrity, ALT is the more liver specific enzyme. The ALT and AST are enzymes involved in amino acid metabolism and are used as a marker in liver diseases.

Effect of oral administration of alcoholic, aqueous extracts of Rosemary or its oil on the levels of serum ALT activity analyzed at the end of experiment period in rats treated with CCl₄ are shown in **Fig. (1)**. The levels of ALT when alcoholic extract, aqueous extract or oil of rosemary was administered to rats are (39.42, 30.9 and 16.42 U/L respectively). The results revealed that the activity of serum ALT enzyme records high levels (50.9 U/L)

Table 4. Effect of Rosemary extracts (alcoholic, aqueous or oil) on the levels of serum triglycerides, total cholesterol, HDL and LDL in rats treated with STZ at the end of the experiment

	Control	STZ	STZ+alcoholic	STZ+aqueous	STZ+ oil
Triglycerides (mg/dl)	88.0 ^e ± 1.6	201.3 ^a ± 2.7	185.5 ^b ± 1.1	154.9 ^c ± 1.3	114.5 ^d ± 3.1
Total cholesterol (mg/dl)	93.1 ^e ± 1.4	189.2 ^a ± 1.2	151.5 ^b ± 1.3	122.8 ^c ± 0.5	107.4 ^d ± 0.8
HDL (mg/dl)	51.3 ^b ± 0.8	42.2 ^d ± 1.1	46.1 ^c ± 0.8	49.8 ^b ± 0.4	55.1 ^a ± 0.5
LDL (mg/dl)	24.1 ^e ± 0.3	106.7 ^a ± 0.4	68.3 ^b ± 1.6	42.0 ^c ± 0.3	29.3 ^d ± 0.8

The data represent the means ± SE calculated from six replicates. Different letters refer to significant differences at ($P \leq 0.05$).

**Fig. 1.** Effect of Rosemary extracts "alcoholic, aqueous or oil" on the levels of ALT in rats treated with CCl₄ after three weeks

for the positive control (The rats treated with CCl₄ only) compared to the negative control (17.1 U/L). There is a significant difference between both negative control (17.1 U/L) and positive control (50.9 U/L) and the values of ALT in the rats treated by alcoholic or aqueous extracts of rosemary. The protective effects of the plant extracts against liver toxicant occurred by decreasing the activities of this enzyme.

c) Serum aspartate aminotransferase (AST) activity

The activity of AST in rats administrated by CCl₄+ alcoholic extract, CCl₄ + aqueous extract

and CCl₄ + rosemary oil were (49.42, 41.43 and 26.1 U/L respectively) as it shown in **Fig. (2)**. The data indicated that the activity of serum AST enzyme reached high levels (59.5 U/L) for the positive control (in rats treated with CCl₄ only) compared to the negative control (29.1 U/L). The difference between both negative control (29.1 U/L) and positive control (59.5 U/L) and the activity of AST in the rats treated by alcoholic and aqueous extracts of rosemary is significant. The protective effects of the plant extracts against liver toxicant occurred by decreasing the activities of this enzyme.

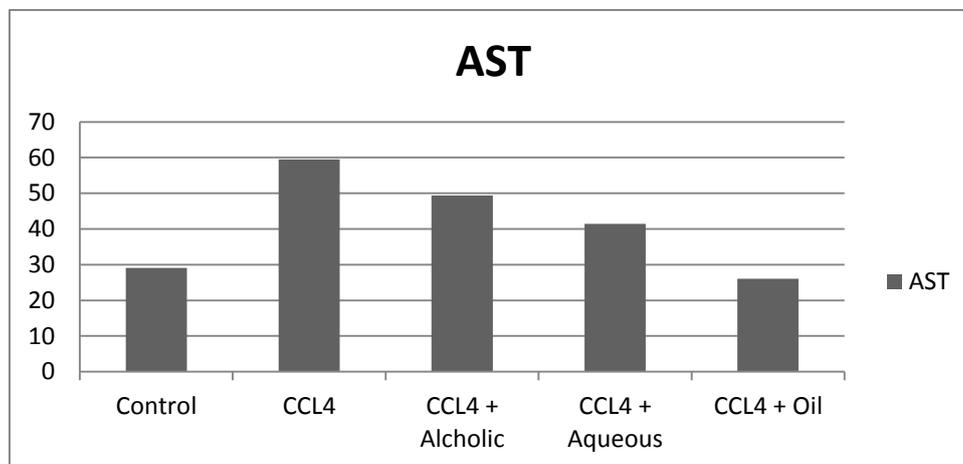


Fig. 2. Effect of Rosemary extracts "alcoholic, aqueous or oil" on the levels of AST in rats treated with CCl₄ after three weeks.

d) Serum alkaline phosphatase (ALP) activity

ALP is an enzyme in the cells lining the biliary duct of the liver. ALP levels in plasma rise with large bile duct obstruction, intrahepatic cholestasis, or infiltrative diseases of the liver. Biliary tract disease produces relatively greater increases in ALP than increases in ALT, AST. ALP is associated with the plasma membrane of hepatocytes adjacent to the biliary canaliculus.

Effect of oral administration of alcoholic, aqueous and oil extracts of rosemary on the levels of serum ALP activity analyzed at the end of experiment period in rats treated with CCl₄ are shown in **Fig. (3)**. The activity of ALP were (131.2, 102.8 and 70.8 U/L respectively) in administrating rats with alcoholic, aqueous and oil extracts. The results revealed that the activity of serum ALP enzyme reached high levels (136.5 U/L) for the positive control (in rats treated with CCl₄ only) compared to the negative control (71.3 U/L).

The activity of serum ALT, AST and ALP are significantly increased due to the administration of CCl₄, which supports the finding that the liver is necrotized; therefore the increase of the liver enzymes activity in serum is mainly due to the leakage of these enzymes from the liver into blood stream, as mentioned by (**Mansour et al 2002**).

e) Serum proteins

The mean values of serum total proteins and albumin of CCl₄ induced rats are tabulated in **Table (5)**. The data did not show any significant dif-

ferences in the level of serum total proteins and albumin for rats treated with alcoholic extract, aqueous extract or oil of rosemary compared to the positive control group (CCl₄ only).

f) Serum triglycerides

Triglycerides are the main lipids present in the human plasma; the others are the cholesterol, phospholipids and nonesterified fatty acids. The measurements of serum triglycerides are important in the diagnosis of hyperlipoproteinemia, liver obstruction and other diseases involving lipid metabolism. In the human body, high levels of triglycerides in the blood stream have been linked to atherosclerosis and by extension, the risk of heart disease and stroke (**Yasim et al 2011**).

Effects of oral administration of alcoholic, aqueous or oil extracts of rosemary on the levels of serum triglycerides analyzed at the end of experiment period in rats treated with CCl₄ are (144.3, 123.3 and 90.8 mg/dl respectively) as it is shown in **Table (6)**. The results revealed that the levels of serum triglycerides reached high levels (338.6 mg/dl) for the positive control (in rats treated with CCl₄ only) compared to the negative control (110.1 mg/dl). There were no significant ($p \leq 0.05$) differences in the levels of serum triglycerides for the rats received the plant extracts compared to the negative control. The protective effects of the plant extracts (alcoholic, aqueous or oil) against the liver toxicant (CCl₄) occurred through appearance of this parameter levels in their normal ranges.

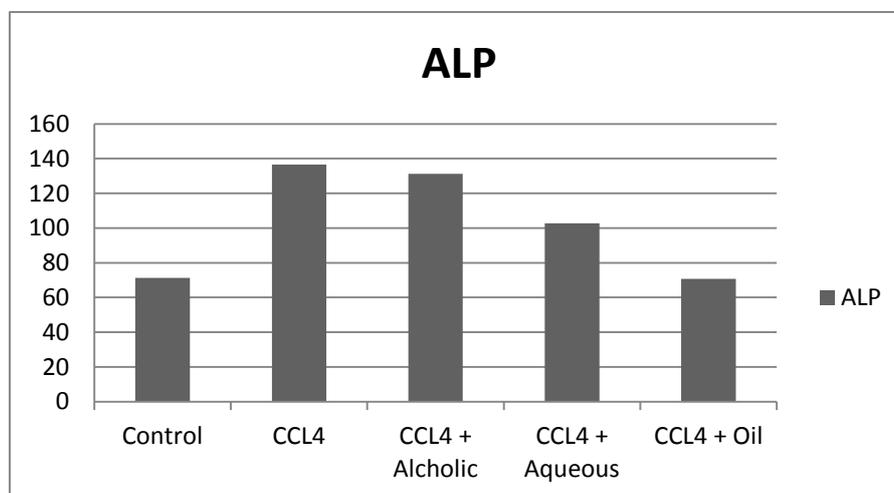


Fig. 3. Effect of Rosemary extracts "alcoholic, aqueous or oil" on the levels of ALP in rats treated with CCl₄ after three weeks

Table 5. Effect of Rosemary extracts "alcoholic, aqueous or oil" on the levels of total proteins, albumin in rats treated with CCl₄ at the end of the experiment.

	Control	CCl ₄	CCl ₄ + alcoholic	CCl ₄ + aqueous	CCl ₄ + oil
Total proteins (g/dl)	6.53 ^a ± 0.75	7.41 ^a ± 0.28	6.37 ^a ± 0.68	6.83 ^a ± 0.08	7.20 ^a ± 0.56
Albumin (g/dl)	4.53 ^a ± 0.78	4.95 ^a ± 0.27	4.90 ^a ± 0.20	5.10 ^a ± 0.03	5.06 ^a ± 0.42

The data are presented as means ± SD from six replicates. Different letters refer to significant differences at ($P \leq 0.05$).

Table 6. Effect of Rosemary extracts (alcoholic, aqueous or oil) on the level of serum triglycerides, total cholesterol, HDL and LDL in rats treated with CCl₄ at the end of the experiment

	Control	CCl ₄	CCl ₄ + alcoholic	CCl ₄ + aqueous	CCl ₄ + oil
Triglycerides (mg/dl)	110.1 ^{bc} ± 3.6	338.6 ^a ± 25.7	144.3 ^b ± 2.9	123.3 ^{bc} ± 2.6	90.8 ^c ± 3.7
Total cholesterol (mg/dl)	56.5 ^{cd} ± 1.6	151.5 ^a ± 1.3	65.6 ^b ± 1	60.4 ^c ± 1.6	53.5 ^d ± 1.1
HDL (mg/dl)	18.9 ^a ± 4.1	22.6 ^a ± 2.5	24.9 ^a ± 2.6	29 ^a ± 3.4	21.9 ^a ± 2.8
LDL (mg/dl)	15.5 ^b ± 0.5	61.2 ^a ± 4.7	11.8 ^b ± 0.4	16.0 ^b ± 0.3	13.5 ^b ± 1.2

The data are presented as means ± SD from six replicates. Different letters refer to significant differences at ($P \leq 0.05$).

g) Serum total cholesterol and cholesterol fractions

Effects of oral administration of alcoholic, aqueous or oil extracts of rosemary on the levels of serum total cholesterol analyzed at the end of experiment period in rats treated with CCl₄ are shown in **Table (6)**. The results revealed that the levels of serum total cholesterol recorded high levels (151.5 mg/dl) for the positive controls (in rats treated with CCl₄ only) compared to the negative control (56.5 mg/dl). The protective effects of the plant extracts against liver toxicant occurred by decreasing the levels of serum total cholesterol and there were no significant ($p \leq 0.05$) differences in the levels of serum total cholesterol for the rats received the plant extracts (aqueous and oil) compared to the negative control.

Table (6) reveals the effect of alcoholic, aqueous extracts or oil of rosemary on the levels of serum HDL and LDL in rats treated with CCl₄. The amounts of HDL of rats treated by alcoholic, aqueous or oil extracts of rosemary are (24.9, 29 and 21.9 mg/dl respectively). There were no significant ($p \leq 0.05$) differences in the levels of serum HDL-cholesterol for all treatments compared to the negative and positive controls.

The data illustrated that the levels of serum LDL recorded high levels (61.2 mg/dl) for the positive controls compared to the negative control (15.5 mg/dl). There are no significant ($p \leq 0.05$) differences in the levels of serum LDL for the rats received the plant extracts (alcoholic, aqueous) or oil compared to the negative control. The protective effects of both plant extracts against liver toxicant occurred through appearance of this parameter levels in their normal values.

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