Protective Effect of Thyme Leaves on Hepatotoxicity Compared to Silymarin in Experimental Rats

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Research Journal Specific Education

Faculty of Specific Education

Mansoura University

ISSUE NO. 83 MAY , 2024

PROTECTIVE EFFECT OF THYME LEAVES ON HEPATOTOXICITY COMPARED TO SILYMARIN IN EXPERIMENTAL RATS

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Abstract:

Thyme (Thymus vulgaris L.) is aromatic native herb in Mediterranean region which used extensively to add a distinctive aroma and flavor to food. Thyme possesses various beneficial effects because of pharmacological properties. The present study aimed to inspect the protective effect of thyme leaves on carbon tetrachloride (CCl_4) and ethanol induced liver injury in experimental rats. Twenty rats were classified into negative control group, the second group kept as positive control fed on basal diet only, silymarin group fed on basal diet contained silymarin (7.6 mg/200 g b.w) daily and thyme group fed on basal diet contained 3% thyme leaves powder instead of cellulose in diet all over the period of the experiment. After four weeks second, third and fourth groups were administered CCL4 diluted in olive oil (1:2) at dose1.5 ml/kg b.w tube and 1 ml of ethanol mixture 0.4% for three weeks by stomach then these groups consumed also 5% lard (saturated fat) at the eighth week. Carbohydrate, fiber, protein, ash, moisture and fat were found in thyme leaves. The phenolic compounds of thyme leaves in descending manner were pyrogallic, caffein, catechein, caffeic, synergic, cinnamic, chlorogenic, chrisin. protocathoic, vanillic, ferrulic, coumarin and catechol acids. There was a significant increase for silymarin and thyme groups in nutritional parameters, albumin and A/G ratio and antioxidant parameters while, significant decrease in liver, renal function, lipid parameters in comparing with positive group. It can be recommended that the necessity of consuming thyme leaves as medicinal plant to protect the liver and prevent diseases.

Key words: Thyme leaves, CCL4, ethanol, liver injury, experimental animals

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INTRODUCTION

In recent times, focus on plant research has increased all over the world and a large body of evidence has collected to show immense potential of medicinal plants used in various traditional systems. Nutraceutical substance considered as food or a part of it that offers health or medical benefits, including prevention and treatment of diseases. The therapeutic effect of these plants for the treatment of various diseases is based on the chemical constituents present in them **(Hardy, 2000 and Dragland *et al.*, 2003)**. Thyme *(Thymus vulgaris L.)* is aromatic native herbs in the Mediterranean region and is used extensively to add a distinctive aroma and flavor to food. Thyme also possesses various beneficial effects because of its several biological and pharmacological properties. The leaves can be used fresh or dried for use as a spice. Essential oil extracted from fresh leaves and flowers can be used as aroma additives in food, pharmaceuticals and cosmetics. Thyme also possesses various beneficial effects such as antiseptic, carminative, antimicrobial and antioxidative properties **(Nieto, 2020; Ana *et al.*, 2021 and Hammoudi *et al.*, 2022)**. A lot of scientific investigations have been performed to discover possible functional properties of thyme, which could be efficient in preventing diseases like atherosclerosis, hyperglycemia, brain dysfunctions and cancer. Moreover, thyme has long been known for its antiviral, antibacterial, antifungal, and antiseptic activities, in addition to remarkable disruption of microbial biofilms **(Hassan *et al.*, 2020 and Hammoudi *et al.*, 2022)**.

Carbon tetrachloride (CCl4) has been widely used in animal models to investigate chemical toxin-induced liver injury. The most remarkable pathological characteristics of CCl4-induced hepatotoxicity are steatosis, fatty liver, cirrhosis and necrosis **(Recknagel *et al.*, 1989** and **Abd El-Ghany, 2006)**.Chronic ethanol-induced liver injury is linked with oxidative stress. In animals, chronic alcohol feeding causes fat accumulation, which leads to formation of steatosis in the liver. Accumulation of fat in alcohol-fed animals may result from increased hepatic triglyceride (TG) content, inhibition of fatty acid oxidation and excessive oxidative stress and may lead to fibrosis **(Sorensen *et al.*, 1984)**. Also, a combined effects of high-fat diet and ethanol induce oxidative stress in rat liver is well documented by **Demori *et al.*, (2006) and Khanal *et al.*, (2009)**.

Because of these promising benefits of thyme, the present study was administrated to inspect the protective effect of thyme Leaves on carbon tetrachloride (CCl4) induced liver injury in experimental rats.

MATERIALS AND METHODS

Materials:

Fresh thyme *(Thymus Vulgaris)* leaves were purchased from a local market in Mansoura city, Egypt. Carbon tetrachloride and ethanol were obtained from El-Gomhoria Company for chemicals, Egypt. Silymarin drug is brown color capsules each one contains 140 mg of silymarin and it was obtained from Chemical Industries Development Company. The dose of silymarin drug was calculated according to **Paget and Barnes (1964)**. The standard diet was performed according to **NRC (1995)**. CCL4 and ethanol obtained from El-Gomhorea Company. CCL_4diluted in olive oil (1:2) at dose1.5 ml/kg b.w tube and 1 ml of ethanol mixture 0.4%.Twenty healthy albino rats Sprague –Dawley strain were purchased from Agricultural Research Center, Giza, Egypt. The average weights were 138 ± 5g.

Methods:

Thyme leaves were distributed uniformly as a thin layer on the trays and dried in an oven at 60 C for 12 h then crushed to powder. The powder was added as 3 % in diet**.** Moisture, crude protein, crude fat, ash and fiber were carried out in thyme leaves while total carbohydrates were calculated by difference according to the method of **A.O.A.C. (2000)**. Phenolic compounds were fractionated and identified in Central Laboratory of Food Tech. Res. Inst., Agric. Res. Center, Giza, Egypt by HPLC system according to **Goupy *et al.*, (1999).**

Rats were housed as four groups in wire cages and fed on basal diet for five days as adaptation period. Feed and water were *ad libitum*. The first group kept as negative control fed basal diet only, the second group kept as positive control fed on basal diet only, third group (silymarin group) fed on basal diet contained silymarin (7.6 mg/200 g b.w) daily and fourth group (thyme group) fed on basal diet contained 3% thyme leaves powder instead of cellulose in diet all over the period of the experiment.

After four weeks second , third and fourth groups were administered CCL4 and ethanol mixture for three weeks by stomach (**Demori *et al.*, 2006, Orhan *et al.*, 2007, , Khanal *et al.*, 2009 and Abd El-Ghany *et al.*, 2012)**. Then these groups consumed also 5% lard (saturated fat) instead of corn oil in diet at the eighth week **(Sorensen *et al.*, 1984)** to insure hepatotoxicity. Daily food intake and weekly body weight gain were recorded. Feed efficiency ratio was determined according to the method of **Chapman *et al.*, (1950)**.

A day post the end of eight week, the rats were sacrificed to obtain blood and liver samples. Serum amino transferase (AST & ALT) and alkaline phosphatase (ALP) enzymes; total protein, albumin, globulin, creatinine, uric acid total bilirubin were estimated. Liver triglycerides (T.G), cholesterol (CHO), total lipid content, total antioxidant capacity (TAC), catalase (CAT), superoxide dismutase (SOD), and malondialdehyde (MDA) were determined according to **Henry (2001)**. Histopathological examinations of livers from each group were applied in Pathological Department of Veterinary Medicine, Cairo University according to **Bancroft *et al.*, (1996**).

Statistical Analysis:

All the obtained data were statistically analyzed by SPSS computer software. The calculated occurred by analysis of variance ANOVA and follow up test LSD by SPSS ver.11 according to **Abo-Allam (2003)**.

RESULTS AND DISCUSSION

Table 1 showed that thyme leaves composition in descending manner were carbohydrate, fiber, protein, ash, moisture and fat. The value of total phenolic compounds in thyme leaves was 5.26 mg/g.

Table (1): Chemical composition and Total Phenolic compounds of Thyme leaves

Variables Samples	Moisture %	Ash (g/100g)	Fat (g/100g)	Protein (g/100g)	Carbohydrates (g/100g)	Fibers (g/100g)	T. Phenolic compounds mg/g
Thyme	11.11± 0.13	14.15± 0.34	3.54± 0.49	14.46± 0.23	67.85± 0.40	19.69± 0.03	5.26

Each value is the mean ± SD

The phenolic compounds of thyme leaves in descending manner were pyrogallic, caffein, catechein, cinnamic, caffeic, chrisin, synergic, chlorogenic. Protocathoic, vanillic, ferrulic, coumarin and catechol acids did not detected as found in table 2

Table (2): Phenolic compounds fractionation of Thyme leaves (ppm)

Content	Pyrogallic	Caffein	Catechein	Cinnamic	Caffeic	Chrisin	Synergic	Chlorogenic acid
(ppm)	676.28	175.63	56.71	42.76	27.15	15.89	14.08	8.23

Positive rat group showed significant decline in final weight, weight gain and weight gain percent in comparing with negative control group. The liver injured rat group which protected by thyme leaves showed significant increase in weight gain, weight gain percent and FER without significant differences in final weight and daily food intake in comparing with negative control group. There was a significant increase in final weight, weight gain and weight gain percent in liver injured rat groups protected by silymarin and thyme leaves in comparing with positive rat group as shown in table 3. The antioxidative effects of natural phenolic compounds in pure forms or in their extracts from different plant sources such as vegetables, fruits and medicinal plants led to speculation about the potential benefits of ingestion of plant phenolic **(Amarowicz *et al.*, 2008)**.

Table (3): Nutritional parameters of the experimental groups

Variables Groups	Initial Weight (g)	Final Weight (g)	Weight Gain (g)	Weight Gain %	Food Intake (g)	FER
Negative	a 138.20±7.82	a 214.80±18.93	b 76.60±11.14	b 55.42±11.95	a 18.52±0.87	b 0.07±0.002
Positive	a 138.00±7.09	c 190.60±20.92	d 52.60±11.78	c 38.06±9.43	ab 16.05±1.68	b 0.06±0.01
Silymarin	a 142.00±7.02	ab 206.00±20.48	c 64.00±8.54	b 45.18±11.88	a 17.47±1.87	b 0.07±0.01
Thyme	a 140.60+7.66	a 232.60+21.94	a 92.00+17.13	a 65.52+11.78	a 18.47+1.66	a 0.09+0.01

Each value is the mean ± SD.

Mean values in each column having different subscript (a, b, c, d) are significantly different at P< 0.05

In comparing with negative control group, positive rat group showed significant increase in AST, ALT, ALP enzymes activity and globulin but there were non-significant differences in total protein, albumin and A/G ratio values. Silymarin group showed significant decrease in ALP and significant increase in albumin and A/G ratio without significant differences in AST and ALT values compared to negative control while thyme group showed significant decrease in both ALP and globulin, and a significant increase in A/G ratio compared to negative control. On the other side, significant increase in A/G ratio and significant decrease in AST, ALT, ALP and globulin in silymarin and s, while albumin significantly increased in silymarin group and showed nonsignificant differences in thyme group in comparing with positive group as shown in table 4. There results were agreed with the results obtained from **Rana and Soni, (2008); Aristatile *et al.*, (2009) and Shati and Elsaid, (2009)**.

Table (4): Serum amino transferase (AST & ALT) and alkaline phosphatase (ALP) enzymes, total protein, albumin, globulin and albumin to globulin ratio (A/G) of the experimental groups

Variables Groups	AST U/L	ALT U/L	ALP U/L	T.P g/dl	Albumin g/dl	Globulin g/dl	A/G ratio
Negative	b 8.20+1.84	b 23.20+2.84	b 301.40+83.71	a 5.82+1.70	b 2.10+0.45	b 3.72+0.28	b 0.56+0.09
Positive	a 10.20+2.84	a 37.80+4.84	a 498.38+88.86	a 6.86+1.38	b 2.40+0.29	a 4.46+0.32	b 0.54+0.09
Silymarin	b 7.80+0.84	b 20.40+2.55	c 198.78+55.79	a 6.44+1.30	a 3.31+0.21	b 3.13+0.45	a 1.08+0.21
Thyme	b 8.80+0.84	b 18.60+3.55	c 197.94+67.89	a 5.00+1.20	ab 2.72+0.08	c 2.28+0.18	a 1.20+0.10

Each value is the mean ± SD.

Mean values in each column having different subscript (a, b, c, d,) are significantly different at $P< 0.05$

The positive rat group showed significant increase in creatinine, uric acid, and total bilirubin while silymarin and thyme groups showed significant increase in creatinine and uric acid and significant decrease in total bilirubin in comparing with negative control group. Silymarin and thyme groups had significant decrease in all above mentioned renal function parameters in comparing with positive rat group as shown in table 5. The obtained results were agreed with **Teissedre and Waterhouse, (2000) Carmiel *et al.*, (2003) and Shati and Elsaid, (2009)** who demonstrated a decrease of the antioxidant defense in a strain of genetically obese rats with histological signs of hepatic steatosis and elevated ALT serum levels.

Table (5): Serum creatinine, uric acid and total bilirubin of the experimental groups

Variables Groups	Creatinine mg/dl	Uric Acid mg/dl	Total Bilirubin mg/dl
Negative	c 0.37 +0.02	c 2.35+0.18	b 0.51+0.02
Positive	a 0.68+0.03	a 4.88+0.86	a 0.99+0.02
Silymarin	b 0.46+0.06	b 3.13+0.47	d 0.37+0.05
Thyme	b 0.42+0.07	b 3.67+0.74	c 0.44+0.05

Each value is the mean ± SD.

Mean values in each column having different subscript (a, b, c, d) are significantly different at P< 0.05

The positive group showed significant increase in TG , CHO and total lipids in liver tissue however silymarin and thyme groups showed non-significant differences in TG, CHO and total lipids content of liver tissue in comparing with negative control group. There was a significant decrease in TG, CHO and total lipids content in liver tissue of silymarin and Thyme groups in comparing with positive group as shown in table 6. High fat diet is often associated with a metabolic syndrome characterized by obesity by alterations such as oxidative stress, insulin resistance and liver steatosis **(Angulo 2002 and Hassan *et al.,* 2020)**. Moreover**, Hayes and Strange (1995)** found a net decrease of all these enzyme activities was found in the livers of high fat diet-fed rats, indicating that high fat diet impaired antioxidant defense. According to **Pietta *et al.,* (1998),** increased generation of reactive oxygen species and oxidized lipoproteins play a major role in the development of atherosclerosis. The alterations of low density lipoprotein (LDL) mediated by elevated glucose levels may contribute to vascular disease in diabetes. Antioxidants in thyme scavenge reactive oxygen species may be of great value in preventing the onset and/or the propagation of oxidative diseases. Antioxidants are also compounds that inhibit or delay the oxidation of other molecules by inhibiting the initiation or propagation of oxidizing chain reactions Of late, more attention has been paid to the role of natural antioxidants mainly phenolic compounds, which may have more antioxidant activity than vitamins C, E, β-carotene and lycopene (**Gazzani *et al.*, 1998)**. Antioxidants can neutralize free radicals, may be of central importance in the prevention of carcinogenicity, cardiovascular and neurodegenerative changes associated with aging **(Halvorsen *et al.*, 2006 & Abd El-Ghany and Nanees (2010)**.

Table (6): Some lipid profile of liver tissue of the experimental groups

Variables Groups	T.G mg/g	CHO mg/g	Total lipids mg/g
Negative	b 67.94+8.82	b 36.68+5.98	b 151.64+22.79
Positive	a 111.56+21.88	a 56.96+6.99	a 211.04+33.90
Silymarin	b 77.58+15.88	b 34.58+4.64	b 178.16+25.90
Thyme	b 65.22+11.61	b 38.42+3.76	b 150.40+19.92

Each value is the mean ± SD.

Mean values in each column having different subscript (a, b, c, d) are significantly different at P< 0.05

In comparing with negative control group, the positive rat group showed significant increase in MDA and significant decrease in TAC, CAT and SOD. Silymarin group showed significant increase in TAC and MDA but the values of CAT and SOD were in non- significant difference. Silymarin and thyme groups showed significant increase in liver TAC, CAT and SOD and significant decrease in liver MDA as shown in table 7.

Imbalance of oxidative stress affects the transcription of numerous biochemical mediators (mainly cytokines) able to modulate the tissue and cellular events that characterize different types of liver diseases such as apoptosis, necrosis, fibrosis, cholestasis and regeneration. Many polyphenol-rich plants have been used for centuries in folk medicine for liver dysfunctions. A single or more compounds have positive health effect due to the presence of potential in vivo antioxidant effect of individual food polyphenols or extracts in cultured cells. Epidemiological studies show that the consumption of vegetables and fruits can protect humans against oxidative damage by inhibiting or quenching free radicals and reactive oxygen species **(Youdim *et al.,* 2000, Schroeter *et al.,* 2001 and Pataki *et al.,* 2002)**. There is growing interest in the antioxidant and anti-inflammatory capacities of natural herbs relative to prevention or treatment of chronic diseases that involve inflammation. Polyphenolic compounds from edible plants are the main antioxidants in the human diet **(Halvorsen *et al.,* 2006 and Jensen, 2006)**.

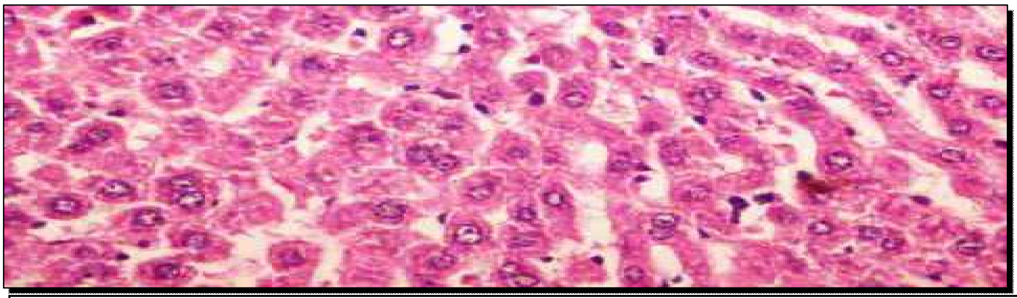
Table (7): Some antioxidant parameters of liver tissue of the experimental groups

Variables Groups	TAC mmol/g	CAT U/g	SOD U/g	MDA n mol/mg ptn
Negative	b 2.72+0.35	a 1.81+0.10	a 355.55+88.99	d 19.75+0.80
Positive	c 1.50+0.13	b 0.07+0.09	b 212.04+77.83	a 42.52+7.83
Silymarin	a 4.02+0.15	a 1.23+0.10	a 276.73+63.99	b 30.15+5.73
Thyme	b 2.82+0.37	a 1.04+0.17	a 321.94+93.95	b 29.86+4.32

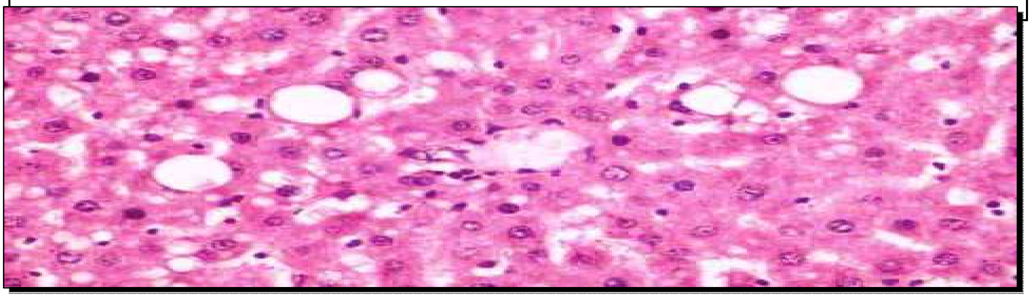
Each value is the mean ± SD

Mean values in each column having different subscript (a, b, c, d, ….) are significantly different at $P< 0.05$

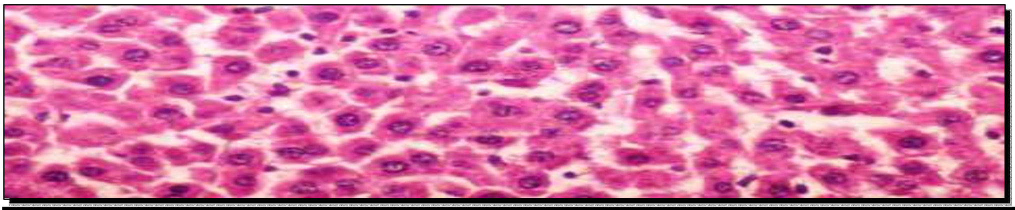
Microscopically, liver of rat from the negative group revealed the normal histological structure of hepatic lobule (Pict. 1). Examines sections from positive group showed vacuolar degeneration of hepatocytes and kupffer cells activation (Pict. 2). Liver from silymarin group showed no histopathological changes (Pict. 3). Liver of rats from thyme group revealed no histopathological changes (Picts. 4). The results proved the protective and treatment effect of thyme leaves, on liver tissue. Histopathological results were agreed with obtained biochemical results and agreed with results of **Zheng and Wang (2001)**, **Nadia and Nadia (2008)** and **Nieto (2020)**.



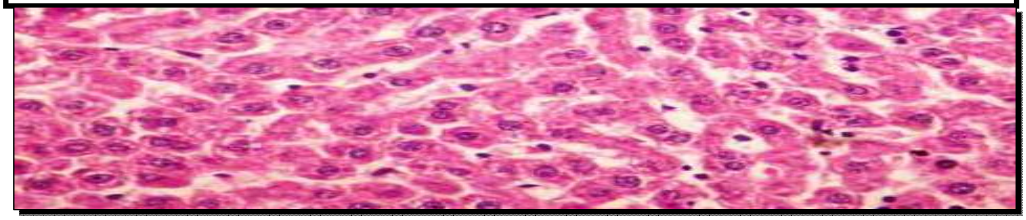
Pict (1): Liver of rat from the negative group showing the normal histological structure of hepatic lobule (H and E X 200)



Pict.(2): Liver of rat from positive group showing vacuolar degeneration of hepatocytes and kupffer cells activation. (H and E X 200).



Pict.(3): Liver of rat from silymarin group showing no histopathological changes. (H and E X 200).



Pict.(4): Liver of rat from the Thyme group showing no histopathological changes. (H and E X 200).

CONCLUSION

Thyme leaves could protect liver from injury because of high nutritional and nutraceutical values of and antioxidants. Further studies are needed to investigate the effects of thyme leaves at longer duration on various biological and biochemical parameters.

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التأثير الوقائي لأوراق الزعتر على السمية الكبدية مقارنه بالسيليمارين في فئران التجارب

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الملخص العربي:

الزعتر عشب من الاعشاب المحلية العطرية المتواجد في منطقة البحر الأبيض المتوسط ويستخدم على نطاق واسع لإضافة رائحة ونكهة مميزة للاطعمة و يمتلك الزعتر تأثيرات مفيدة مختلفة بسبب خصائصه الدوائية. ولقد أجريت هذه الدراسة لمعرفة التأثير الوقائي لأوراق الزعتر على الاصابة الكبدية برابع كلوريد الكربون (٤CCl) مع الإيثانول والوجبات عالية الدهون في فئران التجارب.

تم تقسيم عشرون من الفئران إلى المجموعة الاولي(السالبه) والثانيه (الموجبه) تغذت علي الوجبة القياسية فقط، ومجموعة السيليمارين تغذت على الوجبة القياسية المحتوية على سيليمارين (٧.٦ ملجم/٢٠٠ جرام وزن الجسم) يوميا ومجموعة الزعترالتي تغذت على الوجبة القياسية المحتوية على ٣ ٪ من مسحوق أوراق الزعتر بدلا من السليلوز في الوجبة القياسية ت طوال فترة التجربة. بعد أربعة أسابيع تم إعطاء المجموعات الثانية والثالثة والرابعة ٤CCL المخفف بزيت الزيتون (١:٢) بجرعة ١.٥ مل/كجم من وزن الجسم مع ١ مل من خليط الإيثانول ٠.٤٪ لمدة ثلاثة أسابيع بالأنبوبة المعدية وفي الاسبوع الثامن تم تناول المجوعات الثلاثة ٥٪.الدهون المشبعة.

واسفرت نتائج الدراسة علي ان اوراق الزعتر تحتوي على نسبه مرتفعه من الكربوهيدرات والألياف والبروتين والرماد والرطوبة والدهون كما تحتوي علي المركبات الفينولية كالبيروجاليك، الكافيين، الكاتشين، الكافيين، التآزري، السيناميك، الكلوروجينيك، الكريسين. أحماض البروتوكاثويك والفانيليك والفيروليك والكومارين والكاتيكول.

واثبتت التحاليل البيوكميائية ان هناك زيادة معنوية في كل القيم الغذائية لجميع المجوعات المعالجة كما أظهرت مجموعة السيليمارين والزعتر زيادة معنوية في نسبة الألبومين ونسبة A/G ومؤشرات مضادات الأكسدة وانخفاض معنوي في مؤشرات الكبد ووظائف الكلى والدهون مقارنة بالمجموعة الموجبة. وتوصي الدراسة بضرورة استهلاك أوراق الزعتر كنباتات طبية لحماية الكبد والوقاية من الامراض

الكلمات المفتاحية: أوراق الزعتر- رابع كلوريد الكربون – الإيثانول- الدهون- امراض الكبد- حيوانات التجارب

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