

Study the ability of rosemary leaf Ethanol extract to protect male rabbits' liver and kidney against poisoning cypermethrin

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Abstract: *Rosmarinus officinalis*, L. (Lamiaceae) offers medicinal potential against hepatotoxicity and nephrotoxicity Due to its antioxidant and anti-toxicity properties. The objective, of this research is to explore into the ethanol extract of *Rosmarinus officinalis* leaves and its efficacy against cypermethrin induce toxicity in The liver and The kidney of male rabbits. Forty male rabbits were employed in this experience, group one (without exposure); rabbits received corn oil (1 ml) group two; rabbits oral exposure to cypermethrin with dose (66.5 mg/kg/b.w (1/10 of LD50) dissolved in corn oil , group three(protective group) received rosemary extract with dose 100mg/kg plus cypermethrine with dose 66.5 mg/kg/b.w., group four received rosemary extract with dose 200mg/kg plus cypermethrine with dose 66.5 mg/kg/b.w. the above doses once per day for 6 weeks respectively. In accordance with the current study's findings, liver enzyme levels significantly increased. ALT, ALP and ALP), Lipid profile (TC, TG, LDL-c and VLDL_c) and kidney function tests (urea, creatinine, uric acid and blood urea, nitrogen concentrations) but significant decrease in (Total protein and HDL_c value) in the treated group with cypermethrin , whereas the animals received rosemary extract in both dose plus cypermethrin produced notable decrease in the levels of the vital parameters of the liver and kidneys are with the normal level. : the current study displayed that the *Rosmarinus officinalis* has antitoxicity activities as seen via decreasing liver enzymes, lipid profile and kidney function tests is accompanied by elevated the levels of total protein .

Keywords: cypermethrin, liver, rosemary

Introduction: Pesticides are employed extensively in agricultural and public health initiatives as posing serious health and environmental risks, especially in developing nations. (1) Since pyrethroid pesticides exposure in animals have shown physiological abnormalities and other clinical traits, their harmful effects on mammals have attracted a lot of attention recently. (2) Cypermethrin, a synthetic pyrethroids insecticide has been used in agriculture and buildings (3) Cypermethrin is reasonably toxic. material when intake through ingestion. or directly through dermal absorption (4) The most common effects of dermal exposure are irritability, itchiness in the skin and eyes, numbness, tingling, and burning sensations, loss of bladder control, lack of incoordination, convulsions, and occasionally, death. Intoxication with cypermethrin primarily affects the nervous and muscle systems. (5) Cypremethrin's harmful effects on several body organs and systems, including the liver, digestive, respiratory, nervous systems, and immune systems, have been detailed in a several studies. (6) Pyrethroid insecticides preferentially distribute into internal tissues rich in lipids, such as body

fat, skin, liver, kidney, ovaries, and the central and peripheral nervous systems. They are absorbed by the respiratory and digestive tracts. (7). Herbs are typically employed to defend against toxicities brought on by toxin material. Herbs are generally considered safe (8). Rosemary. (*Rosmarinus Officinalis*) is a herb used in food processing as a spice and flavoring enhancer. It is made up of dried flowers and leaves, making it a very fascinating source. a range of phenolic compounds, including carnosol, carnosic acid, rosmanol, 7-methyl-epirosemanol, isorosmanol, rosmadial, and caffeic acid, which are biologically active phytochemicals. (9,10).

Therefore, this plant has been known to have a therapeutic, potential in the prevention and/or treatment of several diseases such as bronchialasthma (11), peptic ulcers (12) hepatotoxicity (13), atherosclerosis (14),and inflammation diseases(15).The current work was aimed to evaluate the protective efficacy of rosemary leaves extract against adverse effects, in male rabbits exposed to cypermethrin

Materials and methods

Procedure extraction of Rosemary

Leaves: in our work a Rosemary Leaves (*Rosmarinus officinalis*) were purchased from, the market in Thi-qar city /Iraq, were prepared that used a leaf extract after being cleansed, washed with distilled water, and dried. at room temperature for two days in the shade, following ground into powder and then we take weighed 50g of crushed leaves were extracted with 200ml of ethanol 70% put in the round flask, for 12 hrs using reflex extractor. The extraction was filtered by using Whatman filter paper (No.31), the final product was left in the petri-dish under the shade, The dried extract was collected and kept in a tightly closed container and stored at 4°C until use.,the final of extraction was used in the current work with dose (100and200mg/ kg), this extract was created using the technique outlined by (16).

Animals protocol and animals care: 40 male rabbits weighing (1200-1300 gms), were housed in Animal House, two rabbits in each plastic cage for 14 days before the experiment within temperature (25±2 °C) and 12hrs light / dark cycle, food access and water ad libitum to rabbits . Animals , randomly distributed into four experimental groups, each group (10 rabbits)as following; Group I negative control animals was orally administered corn oil (1ml), Group II (group CYP) animals were orally exposed to CYP with dose 66.5 mg/kg b.w of 1/10 LD50 (17)dissolved in corn oil daily, Group III (Rosemary extract+ CYP) orally administered Rosemary extract 100mg/kg b.w plus 66.5 mg/kg b.w of

CYP dissolved in corn oil daily, Group IV(Rosemary extract+ CYP): Orally administered Rosemary extract 200mg/kg b.w plus 66.5 mg/kg b.w of CYP dissolved in corn oil daily, the treatment continue for 6 weeks.

Samplings; blood samples (about 5ml) were collected following (24 hours) last dose of treatment by puncture heart from all groups. Samples were allowed to clot, then all samples were separated by centrifuge at (3000 rpm) for 10 minutes to serum was obtained and then stored at (-20°C) until used for biochemical measurements

Serum assays: The average of Alkaline phosphatase, (ALP) was estimated, according to method of (18) , The level of alanine aminotransferase (ALT) was assessed using the technique described by (19), while,the aspartate aminotransferase ,(AST) activity was evaluated by method by (20), Triglycerides and total Cholesterol (TC) Concentrations .were evaluated by method of (21 and 22) , The method described by(23) was used to test the levels of high-density ,lipoprotein (HDL-c),Low-density lipoprotein (LDL-c), and very low-density lipoprotein (VLDL-c) cholesterol. The total protein concentration was determined by using a special total protein Kit (Agappe Diagnostic LTD., India) (24). Serum Creatinine and serum urea were measured enzymatically by using a special chemical kit (Agappe Hills,Dist,Ernakulam,Kerala.India_683 562), (25) . The Statistical Analysis; Mean values of serum indices were analyzed by,

one-way analysis of variation (ANOVA), obtained mean differences and standard deviations (mean \pm SD) between treated and control groups, P-values ($P < 0.05$) are considered statistically significant

Results and discussion

The results are presented in the a table (1) showed that there was a significant increase, in the levels of AST, ALT and ALP levels in the rabbits treated with cypermethrin, this rise is brought on by exposure to cypermethrin, which damages the liver and induces cytosolic enzyme leakage from hepatocytes and other bodily organs into the blood. (25), who noticed that the elevation in the biomarkers of liver (Transaminases activity) indicator to cellular damage of liver hence released these enzymes in to circulation.. Elevation of liver enzymes may also be caused by increased gene expression as a result of long-term exposure. pesticide. (26), (27) found the elevation of AST, ALT levels and lower level total protein in rats after dermally exposed to cypermethrin for 28 days. stated that cypermethrin hepatotoxicity is probably affected in two ways firstly by the occurrence of an inflammatory state, and secondly by On the other hand Co-administration of resomary extract with

cypermethrin restored, the levels of the enzymes in the serum of the rabbits as an indication of protective, effect of rosemary extract against liver damage induced by cypermethrin. This is consistent with (28 & 29), who stated that resomary has a productive effect on the hepatotoxicity. (28) Who discovered that rosemary lowers the levels of the AST, ALT, and ALP enzymes in the blood. This reduction may be due to rosemary's high antioxidant content, which makes it a free radical scavenger. In support of this hypothesis the antioxidant activity of rosemary might be due to radicals scavenging activity and their affinity to the substrates.

The results in Table 2 revealed a considerable decline in highdensity ,lipoprotein (HDL) concentration and an increase in total cholesterol, triglyceride, lowdensity, lipoprotein (LDL), and very lowdensity, lipoprotein (VLDL) concentration in the cypermethrin-treated group compared to the control group, respectively, due to the effect of cypermethrin on liver which lead to disturbance, liver functions these findings agreed with (30) they reported that the liver is the chief site for regulating internal a

chemical environment and it is an important organ in the body, liver injury induced by different hepatotoxic substance has been recognized as problems for many years. The unique metabolic functions of the liver and their relation to the gastrointestinal tract is an important target of toxicity to oxidative stress, and a toxic chemicals (antibiotics, chemotherapeutics, carbon tetrachloride, etc.

(31) who declared In the hydrophobic portion of the plasma membrane bilayer, where this pyrethroid is predominantly located, pyrethroids cause a changes in the plasma. membrane that results in increased lipid peroxidation and decreased fluidity. Changes in the levels of phospholipids, fatty acids, and cholesterol affect the fluidity of the membrane, which affects the activity of enzymes and the functionality of receptors and channels found in the a plasma membrane. Due to the exogenous effects of cypermethrin administration, there is a decrease in the synthesis and release of lipoprotein lipase (LPL) enzyme in the liver and adipose tissues. This decreases TG metabolism and raises its level in blood. (32) The results in aTable (2) showed a notable decline ($p < 0.05$) in the concentrations of lipid profile in the the treated groups with

cypermethrin and Rosmary extract in both doses and a significant increase in HDL concentration due to. the ameliorative effect of rosemary on lipid profile which inhibit the oxidation. Other studies (33, 34)

have hypothesized that the decrease in lipid levels seen after consuming a rosemary plant is due to a decrease in dietary fat absorption, which is corroborated by an elevated in fecal fat excretion. (35) founded reduction in the lipid profile was attributed to the active a components Rosemary oil contain cineole - α pinene, Camphor , borneol and Ginger oil contain Linalool, Terpeneol, Borneol, Eucalyptol. . Rosemary inhibits the activity of 3-hydroxy-3-methylglutaryl coenzyme (A(HMG-CO) reductase). This provides a significant cholesterol reduction by antioxidant (36), As well as in the treated group with(RE at dose 200mg/kw)the data showed a significant reduction ($p \leq 0.05$) in lipid profile and a significant increase in HDL concentration than other groups due to the scavenging activity of hight dose to rosemary excreat in improvement effect similar findings with which reported by(35) Treatment of diet-induced obesity mice (15-week-old) with 200 mpk of the rosemary ethanolic extract, which contains rosmarinic

acid, carnosol, and carnolic acid, lowered lipid profiles. The results of the present study in Table (3) pointed out that there was a significant, increase in kidney function tests (urea, creatinine, uric acid and blood urea nitrogen concentrations) and a significant decrease in (total protein) in the treated group with cypermethrin agreed with previous studies (37 and 33). found that this elevation in these parameters Due to cypermethrin's damaging effects on the kidney, which result in nephrotoxicity, toxic consequences are caused by one or more common pathological changes.

These include crystal nephropathy, inflammation, and altered tubular cell toxicity. The increase in serum urea seen in the current study may be driven by three different factors: 1) a change in protein metabolism, 2) a increased in its synthesis due to impaired hepatic function, and 3) a decrease in its filtration rate in the kidney (38) . The elevation in blood creatinine, level was found with a harm to functioning nephrons and impaired ,renal function (39, 40) Also, The outcomes of the current investigation revealed the results a substantial reduction in the total protein in the group treated alone with cypermethrin (p

0.05), Due to cypermethrin toxicity, alterations in protein and free amino acid metabolism and synthesis in the liver may be responsible for the decrease in blood protein levels. this outcomes concur (9) might have noticed the drop in serum proteins. due in part to cypermethrin's harmful effects on liver cells, other trials that used oral cypermethrin delivery at various doses revealed similar results. (6, 41,42). From other hand co ademinsteration to rosemary with cypermethrin showed a significant decrease ($P \leq 0.05$) in (urea, creatinine, uric acid and blood urea nitrogen concentrations) whereas, , there was an enormous rise in (total protein concentrations), ,Plasma creatinine an urea, Extract from (*Rosmarinus officinalis*) has caused levels to decrease. These findings concurred with other studies, (43, 44) which claimed that the renal toxicity was improved by rosemary aqueous extract.

The study suggests that, the administration of high dose of a rosemary extract has strong effects on the normal appearance of kidneys tissues and decreased levels, of a creatinine and urea from low dose , an investigation conducted, Another study (44) found that rosmarinic acid may effectively reduce

glomerular hypertrophy, glomerulosclerosis, and reduced urea and creatinine extract depletes plasma levels of urea and creatinine. These findings concur with other studies (44) They claimed that the renal, toxicity was improved by rosemary aqueous extract. This was shown by the normal, appearance of renal tissues and the lower levels of a urea and creatinine.

Conclusion: From the results of this study , conclude that Cypermethrin toxicity causes Pathological and biochemical alteration in the liver and kidney of male rabbits and the administration of the Rosemary lead to ameliorative effect on parameters we examined in this study.

Table (1) Serum AST, ALT and ALP in the control group and treated groups with Rosemary extract plus CYP in male rabbits.

Parameters	AST unit/L	ALT unit/L	ALP unit/L
Groups			
Control (1mlcorn oil)	42.87±3.29 d	62.68 ±2.68 c	116.27 ±2.69 d
Cyp (66.5mg/kg)	86.70 ±2.59 a	142.36 ±2.61 a	234.30 ±4.37 a
RE (100mg/kg plus Cyp 66.5mg/kg)	57.48 ±2.73 b	89.53 ±1.36 b	145.80 ±3.88 b
RE(200mg/kg plus Cyp 66.5mg/kg)	48.73 ±2.25 c	67.80 ± 2.89 d	121.29±3.96 c

Data are expressed as: mean ± SD (n=10). The different letters (a,b and c)refer to significant difference (p≤ 0.05)
Cyp : Cypermethrin **RE**: Rosemary ethanolic extract.

Table (2) Serum total cholesterol ,triglyceride , ahigh density lipoprotein,low density lipoprotein ,and very low density lipoprotein in control group and treated groups with Rosemary (extract)plus CYP in male rabbits.

Parameters					
Groups	Total cholesterol (TC) mg/dl	Triglycerides (TG)mg/dl	HDL-C mg/dl	LDL-C mg/dl	VLDL –C mg/dl
Control (1mlcorn oil)	138.72±2.51 d	165.56 ±3.04 d	42.07 ±1.73 a	23.93 ±1.65 d	20.14±1.92 c
Cyp (66.5mg/kg)	229.46 ±2.29 a	272.08 ±2.24 a	24.34 ±2.60 d	45.14± 2.17 a	30.17 ±1.89 a
RE (100mg/kg plus Cyp 66.5mg/kg)	166.51 ±3.59b	194.16 ±2.74b	33.54±2.15 c	32.78±2.17 b	25.19±1.45 b
RE(200mg/kg plus Cyp 66.5mg/kg)	148.93 ±3.12c	170.68 ±1.60c	40.31 ±1.45 b	27.07 ±1.44 c	22.85±1.88 b

The data is presented as mean :SD (n=10). Significant difference ($p \leq 0.05$) is denoted by distinct letters. Cyp:Cypermethrin RE: Ethanolic extract of rosemary.

Table (3) serum Creatinine, Urea, Blood urea nitrogen, Uric acid and Total Protein Concentrations in control group and treated groups with Rosemary (extract) plus CYP in male rabbits.

Parameters Groups	Creatinine mg/dl	Urea mg/dl	BUNmg/dl	Uric acid mg/dl	Total protein mg/dl
Control (1mlcorn oil)	0.54 ±0.02c	23.53 ± 2.23 c	10.92±1.0c	2.87 ±0.19c	7.10 ±0.12 a
Cyp (66.5mg/kg)	2.11 ±0.17 a	46.08 ±2.56 a	21.38 ±1.19 a	5.35 ±0.40a	4.16 ±0.12 c
RE (100mg/kg plus Cyp 66.5mg/kg)	1.10 ±0.17 b	31.31±3.91 b	14.52 ±1.81b	3.37 ±0.39b	5.28 ±0.55b
RE(200mg/kg plus Cyp 66.5mg/kg)	0.57 ±0.01c	27.76±1.80 b	12.88 ±0.83b	3.03 ±0.10c	6.71±0.60 a

The data is presented as mean SD (n=10). Significant difference ($p \leq 0.05$) is denoted by distinct letters.
Cyp: Cypermethrin RE: Ethanolic extract of rosemary .

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دراسة قابلية مستخلص اكليل الجبل الايثانولي لحماية كبد وكلية ذكور الارانب ضد التسمم بالسيبرمثرين

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فرع الفلسفة والأدوية والكيمياء البيطري، كلية الطب البيطري ، جامعة البصرة

الخلاصة

يوفر أكليل الجبل إمكانات طبية ضد السمية الكبدية والكلية نظراً لخصائصه المضادة للأكسدة والمضادة للسمية. الهدف من هذا البحث هو استكشاف مستخلص الإيثانول من أوراق أكليل الجبل ومدى فعاليته في تقليل تأثير السمي لمبيد السبرمثرين في الكبد والكلية لدى ذكور الأرانب. تم توظيف أربعين ذكراً من الأرانب في هذه التجربة ، المجموعة الأولى (دون التعرض). تلقت الأرانب زيت الذرة (1 مل) المجموعة الثانية ؛ تعرض الأرانب عن طريق الفم للسيبرمثرين بجرعة (66.5 مجم / كجم / وزن حي (10/1 من الجرعة المميتة 50) مذابة في زيت الذرة ، المجموعة الثالثة (المجموعة الوقائية) تلقت مستخلص إكليل الجبل بجرعة 100 مجم / كجم بالإضافة إلى سايبيرمثرين بجرعة 66.5 مجم / كجم / وزن حي. المجموعة الرابعة: تلقت المجموعة الرابعة خلاصة إكليل الجبل بجرعة 200 مجم / كجم بالإضافة إلى سايبيرمثرين بجرعة 66.5 مجم / كجم / وزن حي بالجرعات المذكورة أعلاه مرة واحدة يومياً لمدة 6 أسابيع على التوالي. وفقاً لنتائج الدراسة الحالية ، زادت مستويات إنزيمات الكبد بشكل ملحوظ. ALT ، (ALS and ALP) ، مستويات الدهون (الكوليسترول الكلي ، الكليسيردات الثلاثية ، بروتين الدهون المنخفض الكثافة وبروتين دهون العالي جدا للكثافة واختبارات وظائف الكلى (اليوريا ، والكرياتينين ، وحمض اليوريا واليوريا في الدم ، وتركيزات النيتروجين) ولكن انخفاض كبير في (إجمالي البروتين وقيمة البروتين المنخفض الكثافة) في المجموعة التي عولجت بالسيبرمثرين ، بينما تلقت الحيوانات مستخلص إكليل الجبل في كلتا الجرعة بالإضافة إلى سايبيرمثرين أدى إلى انخفاض ملحوظ في مستويات المعايير الحيوية للكبد والكلية بالمستوى الطبيعي. ظهرت الدراسة الحالية أن نبات أكليل الجبل له أنشطة مضادة للتسمم كما يظهر من خلال تقليل إنزيمات الكبد ، واختبارات مستوى الدهون ، واختبارات وظائف الكلى مصحوبة بارتفاع مستويات البروتين الكلي.

الكلمات المفتاحية: سيبرمثرين ، الكبد، اكليل الجبل.