Protective Effects of Chicory (*Cichorium intybus*) and Milk Thistle (*Silybum marianum*) Powder in Carbon Tetra Chloride-Induced Liver Disorder in Rats

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Abstract

The study's objective is to determine the therapeutic effects of specific plant herbs on rats that have liver impairment due to carbon tetrachloride injection. The study examined the influence of milk thistle, chicory and their combination in different ratios (1:1, 2:1 and 1:2) on hepatic rats, 42 male albino rats were segregated into 7 groups and administered with carbon tetra chloride (CCl_4) to induce hepatic

injury. Biological and biochemical variations were observed, Liver functions include Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Oxidative enzyme (SOD) and (CAT) were measured. The treated groups showed the functions of liver and kidney are reduced, when given a 5% combination of milk thistle and chicory (1:2), with significant differences ($P \leq 0.05$) observed.

In conclusion, the hepatic rats fed a 5% milk thistle and chicory (1:2) powder experienced improved liver function, oxidative enzymes and renal functions.

Keywords: Milk thistle, chicory, liver functions, carbon tetrachloride injection (CCL_4)

التأثيرات الواقية للكبد لمسحوق حليب الشوك والشيكوريا على اضطرابات الكبد فى الفئران المستحث برابع كلوريد الكربون.

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المستخلص

تم دراسة تأثير تركيز ٥٪ من حليب الشوك والشيكوريا ومخلوطهم بنسب مختلفة (١:١, ٢:١, ١:٢) على شكل مسحوق على التغيرات البيولوجية والكيميائية الحيوية فى الفئران المصابة بالكبد بواسطة رابع كلوريد الكربون. حيث تم استخدام أثنين وأربعون من ذكور الفئران من نوع الألبينو وقسمت إلى ٧ مجموعات، كل مجموعة بها (٦) فئران. تم قياس ووظائف الكبد (ALT, AST)، والإنزيمات المؤكسدة (سوبر أوكسيد ديسميوتيز . الكتاليز) ووظائف الكلى (اليوريا وحمض اليوريك والكرياتينين). أظهرت النتائج أن

المجموعات المعالجة أظهرت انخفاضًا في وظائف الكبد والإنزيمات المؤكسدة ووظائف الكلى، خاصة عند التغذية بنسبة ٥٪ من مخلوط حليب الشوك والشيكوريا بنسبة (١:٢)، مع وجود فرق معنوي ($P\leq0.05$). في الختام، أظهرت الفئران المصابة بخلل فى الكبد التي تم تغذيتها على الخليط بنسبة ٥٪ من مسحوق مخلوط حليب الشوك والشيكوريا بنسبة (١:٢) تحسن في مستوى الأنزيمات المؤكسدة ووظائف الكبد ووظائف الكلى وصورة دهون الدم. لذا توصى الدراسة بإمكانية استخدام مخلوط حليب الشوك والشيكوريا بالنسب الموصى بها لتقليل مستويات للحد من الخلل الحادث فى الكبد بالجسم.

الكلمات الدالة: الشيكوريا وحليب الشوك ووظائف الكبد

Introduction

The liver is a significant bodily organ involved in hormone formation, serum protein production, cholesterol synthesis, enzyme production, and detoxification as alcohol (1). It has been established that oxidative stress contributes to the development of liver diseases. The toxic effect of Free radicals leads to damage of the liver (2).

Carbon tetrachloride is a hepatotoxic compound that can damage the liver and is transformed into reactive metabolites (free radicals) by cytochrome P450 (3).

Medicinal plants containing terpenes alkaloids, and glycoside flavonoids for treating liver disorders are utilized (4). The therapeutic potential of Milk Thistle

(*Silybum marianum*) is attributed to the presence of silybin, which has significant biological effects due to its antioxidant and anti-inflammatory properties (5).

Chicory (*Cichorium intybus*) is a plant with medicinal significance due to its phytochemical contents, in the root (6). Chicory has anti-testicular toxicity, antioxidant, diuretic, and immune-modulatory properties (7). The uses of *Cichorium intybus* extract to reduce liver damage, enhance antioxidant activity, and alleviate liver disease (8). Therefore, our investigation aimed to determine the therapeutic impact of certain plant herbs (Chicory and Milk Thistle) on rats with impaired liver function following carbon tetrachloride injection (CCL_4).

Material and methods

Materials

The source of dried milk thistle and chicory was an herbalist at an Herbal shop in Menoufia Governorate, Egypt. As for choline chloride, casein, cellulose powder, and kits such as ALT, AST, SOD, CAT, urea, Uric acid and creatinine from The Al- Gomhoria Company for Trading.

Experimental animals

42 male albino rats were acquired from the Ministry of Health in Cairo. The rats had an average weight of 140±10 g. This study was Ethically Approved by the Scientific Research Ethics Committee (Animal Care and Use), Menoufia University. Approval No. #12-SREC-07-2019.

Methods

The dried milk thistle and chicory powder were ground using the Drying device in the Agriculture Faculty, University.

The induction of liver experimental.

Rats were administered with a fresh mixture of equal volumes of paraffin oil and CCl_4 by three intraperitoneal injections (IP) at doses of 0.2 ml/100 g body weight as the CCl_4-treated control (9).

Experimental design: -

Carbon tetrachloride CCl_4 was injected twice a week for two weeks to induced liver fibrosis and hepatotoxicity in rats. The study used 42 male adult albino rats weighing (140±10g). All rats are given (casein diet) as per. For adaptation. the rats were fed a casein diet, then after the week had passed the rats were divided into 8 groups, each group containing 6 rats, Group 1 was a negative control, consuming a basal diet. Group 2 was a positive control, consuming a basal diet. Group 3 received a basal diet with 5% milk thistle powder, and Group 4 received a basal diet with 5% chicory powder. Group (5): fed on basal diet and 5% mixture (1:1) of milk thistle and chicory.

Group (6) was given a basal diet with a 5% mixture of milk thistle and chicory in a ratio of 2:1, while Group (7) was given the same diet but with a 5% mixture in a ratio of 1:2. The rats were observed for 28 days and then weighed and blood samples were taken. According to Dong *et al.* (10).

Blood sampling

Liver Function:

Rats were fasted and sacrificed after a 28-day experiment. Blood samples were taken and serum was separated using centrifugation (11). Determination of serum alanine aminotransferase (ALT), and serum aspartate aminotransferase (AST) was carried out according to the method of Bergmeyer (12). The activity of SOD was evaluated with a SOD detection kit according to the method described by Arkel *et al.* (13), and the technique of Kim *et al.* (14) was used to measure the tissue catalase (CAT) activity.

Kidney Function:

Kidney function was determined using the enzymatic method serum urea was measured (15). Serum uric acid was measured calorimetrically following the method of Patton and Crouch (16). The kinetic method was used to measure creatinine (17).

Statistical analysis

The data were analyzed using a completely randomized factorial design (18). Differences between treatments of ($P \leq 0.05$) were considered significant using Costat Program. Biological results were analyzed by On Way ANOVA (19).

Results and discussion

1- Liver function enzyme levels in rats were affected by a diet supplemented with a mixture of milk thistle and chicory.

Data given in Table (1) showed the changes in liver functions, specifically ALT and AST enzyme levels, in hepatic rats fed a diet supplemented with a mixture of milk thistle and chicory. The results showed that the ALT enzyme level in the liver of the positive control group was significantly higher compared to the negative control group, with a statistically significant difference. The mean values were 279.33 U/L and 119 U/L, respectively. Conversely, among the treated groups, the highest ALT liver enzyme level was observed in group G3, while the lowest level was recorded in group G7, with a significant difference ($P \leq 0.05$). The mean values were 219.00 U/L and 138.66 U/L, respectively.

The highest level of the AST liver enzyme among the treated groups was recorded for G3, while the lowest level was observed for G7, with significant differences ($P \leq 0.05$). The mean values were 48.33 and 28.66 U/L, respectively. Our findings support the assertions made by (20).

Liver function (ALT and AST) changes in hepatic rats fed a diet supplemented with milk thistle and chicory mixture (21). It has been demonstrated that the chicory root extract may have hepatoprotective effects against CCl4. This rustle agrees with (22) who reported that chicory plant leaves could be considered a powerful

nutraceutical therapeutic agent for the treatment of hepatotoxicity induced by CCl4 in rats. All concentrations used in this study caused improvement in hepatotoxicity in rats (23).

Table (1): Changes in liver functions enzyme level of hepatic rats fed diet supplemented with a mixture of milk thistle and chicory

Variable / Groups	ALT (U/L)	AST (U/L)
G1 C (-)	119.00 ^{d}±5.29	21.66 ^{d}±2.51
G2 C (+)	279.33 ^{a}± 32.95	74.00 ^{a}±8.18
G3(5% Milk thistle)	219.00 ^{b}± 60	48.33 ^{b}±3.05
G4 (5% Chicory)	142.00 ^{cd}±6.50	34.66 ^{c}±1.52
G5 (5% mixture milk thistle+ chicory) (1:1)	162.66 ^{c}± 2.08	44.00 ^{b}±4.35
G6 (5% mixture milk thistle+ chicory) (2:1)	152.33 ^{cd}±3.05	46.33 ^{b}±6.42
G7 (5% Mixture milk thistle+ chicory) (1:2)	138.66 ^{cd}±2.51	28.66 ^{cd}±2.51
LSD (P≤0.05)	24.655	7.463

ALT=Alanine Aminotransferase.

AST=Aspartate Aminotransferase

Each value is expressed as mean ± standard deviation (n = 3). Means denoted by the same letters within the same row are not significantly different at P≤0.05.

2- Changes in oxidative enzyme level of hepatic rats fed diet supplemented with a mixture of milk thistle and chicory.

The data presented in Table (2) illustrate the changes in oxidative enzyme levels (SOD and CAT) in hepatic rats fed a diet supplemented with a mixture of milk thistle and chicory. The results indicated that the SOD level in the positive control group was significantly

lower than in the negative control group (*P≤0.05*). The mean values were 46.66 u/mg and 64.33 u/mg, respectively.

Conversely, among the treated groups, the highest SOD level was observed in G6, while the lowest SOD level was recorded in G3, with a significant difference (*P≤0.05*). The mean values were 57.66 U/mg and 49.33 U/mg, respectively. These results agree with (24). who showed the potential effect of milk thistle on liver disorders induced by carbon tetrachloride.

Regarding the CAT enzyme level, the positive control group had a significantly lower value compared to the negative control group (P≤0.05). The mean values were 605.33 U/mg and 690.33 U/mg, respectively. Our results agree with (25) who noted that dietary antioxidants (silymarin) improve CCl_4 hepatotoxicity in rats.

The highest CAT level was observed in G4, while the lowest level was recorded in G6, with a significant difference (*P≤0.05*). The mean values were 664.66 U/mg and 616.33 U/mg, respectively. These findings concur with those of (26) who showed that CCl_4 is linked to lower SOD and catalase activity. It was observed that the antioxidant activities of SOD and catalase were lower in Group G2 compared to the control group. Additionally, the serum ALT and AST levels of rats treated with CCl4 were found to have significantly increased. This rustle agrees with (27), who noted that CCl4 is linked to lower

SOD. the fall in antioxidant activity are signs of oxidative stress brought on by CCl_4.

Data in Table (2) It has been analyzed the impact of milk thistle and chicory on oxidative enzymes in rats. Results showed significant differences in SOD levels between control positive and negative groups with mean values of 46.66 and 64.33 U/mg, respectively.

The treated group G6 recorded the highest SOD level and G3 had the lowest. The control positive group had lower CAT enzyme levels than the control negative group, with significant differences.

The highest CAT of treated groups was recorded for G4. while the lowest CAT was recorded for G6. This result agrees with (28) who noted the effects of silymarin on the resolution of liver fibrosis induced by (CCL_4) in rats.

Table (2): Changes in oxidative enzyme level of hepatic rats fed diet supplemented with a mixture of milk thistle and chicory.

Variable / Groups	SOD (u/mg)	CAT (u/mg)
G1 C (-)	64.33 ± 4.16^{a}	690.33 ± 10.06^{a}
G2 C (+)	$46.66^{e}\pm2.51$	$605.33^{f}\pm3.51$
G3(5% Milk thistle)	$49.33^{de}\pm0.57$	$646.66^{c}\pm2.88$
G4 (5% Chicory)	$50.66^{d}\pm0.57$	$664.66^{b}\pm5.03$
G5 (5% mixture milk thistle+ Chicory) (1:1)	$52.33^{cd}\pm1.52$	$634.33^{d}\pm4.04$
G6 (5% mixture milk thistle+ chicory) (2:1)	$57.66^{b}\pm0.57$	$616.33^{e}\pm1.52$
G7 (5% Mixture milk thistle+ chicory) (1:2)	$55.00^{bc}\pm1.0$	$626.66^{d}\pm5.13$
LSD ($P\leq0.05$)	3.189	7.463

SOD=Superoxide dismutase
CAT=Catalase
Each value is expressed as mean ± standard deviation (n = 3). Means denoted by the same letters within the same row are not significantly different at P≤0.05.

3- The changes in kidney function (urea, uric acid, and creatinine levels) in hepatic rats fed a diet supplemented with a mixture of milk thistle and chicory.

Data presented in Table (3) show the changes in kidney function (urea, uric acid, and creatinine levels) in hepatic rats fed a diet supplemented with a mixture of milk thistle and chicory were observed. The data indicated that the urea level in the positive control group was significantly higher compared to the negative control group (*P≤0.05*).

The mean urea levels were 55 mg/dl for the positive control group and 23.66 mg/dl for the negative control group. Conversely, among the treated groups, the highest urea level was observed in G3, while the lowest was recorded in G7, with significant differences (*P≤0.05*). The mean values were 38.33 mg/dl and 27.00 mg/dl, respectively.

It is noteworthy that the uric acid level in the positive control group was significantly higher than in the negative control group, with mean values of 4.55 mg/dl and 2.50 mg/dl, respectively.

Among the treated groups, the highest uric acid level was observed in G3, while the lowest level was

recorded in G4, both showing significant differences. The most favorable results were seen in G4 (5% chicory), with a mean uric acid level of 2.96 mg/dl.

Regarding creatinine levels, the positive control group had a significantly higher value compared to the negative control group.

($P \leq 0.05$), with mean values of 0.79 mg/dl and 0.34 mg/dl, respectively.

Among the treated groups, the highest creatinine level was observed in G3, with a mean value of 0.61±0.01 mg/dl, respectively.

Our results agree with (29), they indicated that increased serum creatinine and urea levels may reflect renal dysfunction.

It has been proposed that silymarin increases cell replication by 30%, a property attributed to two key components: silybin and silychristin.

Also, our results agree with (30). The study presented in Table (3) displays alterations in kidney function levels of rats fed a diet supplemented with a mixture of milk thistle and chicory. The group treated with the supplement had the highest urea level, as observed in G3.

Table (3): Changes in creatinine level of hepatic rats fed diet supplemented with a mixture of milk thistle and chicory

Variable / Groups	Urea mg/dl	Uric acid mg/dl	Creatinine mg/dl
G1 C (-)	$23.66^{c}\pm3.21$	$2.80^{e}\pm0.08$	$0.34^{d}\pm0.04$
G2 C (+)	$55.00^{a}\pm6.08$	$4.41^{a}\pm 0.33$	$0.79^{a}\pm0.09$
G3(5% Milk thistle)	$38.33^{b}\pm1.52$	$3.86^{b}\pm0.22$	$0.61^{b}\pm0.01$
G4 (5% Chicory)	$33.00^{b}\pm1.0$	$2.96^{de}\pm0.02$	$0.43^{cd}\pm0.04$
G5 (5% mixture milk thistle+ Chicory) (1:1)	$35.00^{b}\pm1.0$	$3.51^{C}\pm0. 32$	$0.53^{bc}\pm0.01$
G6 (5% mixture milk thistle+ chicory) (2:1)	$34.33^{b}\pm0.57$	$3.26^{cd}\pm0.03$	$0.52^{bc}\pm0.05$
G7 (5% Mixture milk thistle+ chicory) (1:2)	$27.00^{c}\pm2.0$	$3.07^{de}\pm0.11$	$0.50^{bc}\pm0.05$
LSD ($P\leq0.05$)	5.118	0.326	0.090

Ur=Uric acid

Each value is expressed as mean ± standard deviation (n = 3). Means denoted by the same letters within the same row are not significantly different at $P\leq0.05$.

Conclusion

Rats suffering from liver problems exhibited improved health after receiving a mixture of 5% milk thistle and chicory in a 1:2 powder ratio, which improved liver and kidney functions, and oxidative enzymes.

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