

EFFECT OF CHICORY (*CICHORIUM INTYBUS* L.) LEAVES EXTRACT TO PROTECT CERTAIN LIVER ENZYMES IN MICE AGAINST CARBON TETRACHLORIDE-INDUCED HEPATOTOXICITY

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ABSTRACT

Chicory contains dietary inulin and oligofructose compounds which have a beneficial effect on metabolism, as well as polyphenol type derivatives, which may be responsible for the antioxidant properties. The effect of different concentrations of the hydroalcoholic extract of dried powdered leaves of chicory on CCl₄-induced hepatotoxicity in vivo in mice, has been studied. Thirty-five mice (20-30 g) of age four weeks were randomly divided into 5 groups (seven in each). The mice orally received three different concentrations of chicory leaves extract (CLE) before injection for 3 consecutive days by CCl₄. The study showed that CCl₄ caused elevation in serum GOT, GPT, and ALP activities in mice, but there was no pronounced elevation in total protein and albumin. Pretreatment of mice with 75, 150, and 300 mg/kg/bwt of CLE protected the liver against CCl₄-induced cytotoxicity. CLE significantly ($p < 0.05$) reduced the elevated level of cholesterol, triglycerides, and significantly increased the levels of HDL in the group of mice pretreated with CLE (150 mg/kg); 145 ± 1.3 , 243 ± 1.3 , and 34 ± 1.2 mg/dl, respectively, compared with negative control (185 ± 1.4 , 290 ± 1.4 , and 21 ± 1.3 mg/dl, respectively). In contrast, serum fasting glucose and magnesium level in mice groups were not significantly different under the same conditions. CLE is potentially a good natural source for antioxidants and natural hepatoprotective as well as beneficial to who is suffering from hypercholesterolemia.

Key words: CCl₄, Antioxidants, Inulin, Lipid profile, Glucose, Magnesium

صدام

مجلة العلوم الزراعية العراقية - 2021- 52: (5) 1248-1253

تأثير مستخلص أوراق الهندباء لحماية بعض إنزيمات الكبد في الفئران

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المستخلص

يعد نبات الهندباء من النباتات التي تزرع في بعض مناطق مدينة بغداد بشكل بري إذا يحوي على العديد من المركبات الفعالة مثل الانثولين والاوليكوفركتوز بالإضافة الى المركبات الفينولية ومشتقاتها والتي لها تأثير ايجابي على العمليات الأيضية في الجسم. هدفت الدراسة الحالية لمعرفة تأثير مستخلص أوراق نبات الهندباء في فئران مصابة بالتسمم الكبدي عن طريق ربايع كلوريد الكربون CCl₄ المحقن IP. استخدم 35 فأراً بوزن 20-30 غم ويعمر اربع اسابيع، قسمت الفئران الى خمس مجاميع 7 فئران في كل مجموعة. أظهرت الدراسة أن CCl₄ تسبب في ارتفاع أنشطة أنزيمات الكبد GOT و GPT و ALP في الفئران ، ولكن يظهر ارتفاع واضح في إجمالي البروتين والألبومين. أظهرت النتائج أن مستخلص نبات الهندباء (CLE) يمكن أن يحمي الكبد من السمية الخلوية التي يسببها CCl₄ ويجرعات 75 و 150 و 300 ملغم / كغم. علاوة على ذلك، قلل الهندباء بشكل معنوي كبير ($p < 0.05$) من المستوى المرتفع للكوليسترول والدهون الثلاثية ، وزاد بشكل ملحوظ من مستويات انخفاض HDL الذي تسببه CCl₄ في مجموعة الفئران المعالجة بـ (CLE 150 ملغم / كغم) ؛ 145 ± 1.3 ، 243 ± 1.3 ، 34 ± 1.2 ملغم / ديسيلتر ، على التوالي ، مقارنة المصابة غير المعالجة (185 ± 1.4 ، 290 ± 1.4 ، 21 ± 1.3 ملغم / ديسيلتر ، على التوالي). لكن من جانب آخر، لم يظهر الهندباء اي تأثير على مستوى الكلوكوز ولا على عنصر المغنيسيوم في الدم. لكن بشكل عام يعد الهندباء مصدراً غنياً بمضادات الأكسدة الطبيعية والتي لها تأثير ايجابي ووقائي لأنزيمات للكبد وكذلك مفيد للأشخاص الذين يعانون من ارتفاع كولسترول الدم.

الكلمات المفتاحية: كبدي ،مضادات اكسدة، انثولين ،دهون الكلية، كلوكوز، مغنيسيوم.

INTRODUCTION

Cichorium intybus, known as “chicory” has been used in folk medicine in Asia and Africa since many years ago. Chicory is used with liver diseases in different parts of Iraq. Roots, seeds, and leaves of the plant have been considered to be a hepatoprotective agent. Chicory is a plant of an herbaceous perennial plant belonging to the family compositae which grows naturally in different parts of the country and the wild type is widely spread used Iraq (5). The liver is the prime organ system contributory to the detoxification, metabolism, and excretion of various endogenous and exogenously administered. The physiological activity of the liver results in the generation of highly reactive free radicals, which covalently bonds with membrane lipids causing peroxidation of lipid consequently can lead to changes in membrane fluidity and permeability (tissue damage) (8). The liver contributes several biochemical reactions at the same time, it may be attacked by the free radicals; however, inbuilt antioxidant systems like tissue glutathione and superoxide dismutase protect the tissues from free radical attack. Strengthening of inbuilt protective mechanisms or exogenous administration of antioxidants can be useful in protecting the organs against reactive oxygen species (9, 10, 17). Herbal medicines have been used for a long time in the treatment of liver diseases due to safe hepatoprotective. Medicinal plants are significant sources of hepatoprotective drugs. Therefore, researchers worldwide are engaged to use several plants; one of these is chicory (15). Chicory has been traditionally used in folk medicine to increase the shelf life of foods inhibition bacteria, fungi, and yeasts. The tuberous root of this plant contains many of medicinally important compounds such as inulin, flavonoids, coumarins, vitamins (especially vitamin C), sesquiterpene lactones, fats, minerals, fructans, mannitol, and latex (22). The tuberous root is used as an antiulcerogenic, antihepatotoxic, anti-inflammatory, liver tonic, treat AIDS, cancer, diabetes, hypertension, antimicrobial, antcardiovascular. Inulin can be used to replace sugar and fat, reduce the calories of food, growth of microorganisms, and catalyst for immunity (18). The aim of the study is to

verify the effect of chicory (*Cichorium intybus* L.) to protect liver enzymes against CCl₄-induced toxicity that was measured by the spectrophotometric technique in the serum of mice.

MATERIALS AND METHODS

Plant material and chemicals

Fresh leaves of *Cichorium intybus* were collected and washed thoroughly in tap water during September 2018 from rural areas around the city of Baghdad, Iraq. The plant was diagnosed by professors from the Department of Field Crops, University of Baghdad, Iraq. The plant was dried at room temperature as around 20–26 °C. Carbon tetrachloride and other chemicals were purchased from BDH and Sigma Chemical Companies.

Preparation of extracts

Hundred grams of powdered leaves were macerated in 70% EtOH (400 ml) at room temperature for two days. The extract was filtered and filtered dried in oven at 45 °C. Total phenolic and total flavonoids compounds in CLE were determined using HPLC according to (3).

Experimental animals

Male albino mice (20-30 g) at the age of four weeks were obtained from the National Laboratory for Drug Control and Research / Ministry of Health. The mice were housed at room with controlled temperature (24 ± 2°C) and humidity (55± 5%), with a 12 h dark/light cycle and ad-libitum access to distilled water and food. All mice were acclimatized in metal cages for one week prior to the experiment to ensure normal behavior and growth.

In vivo hepatoprotective activity studies

Thirty-five mice were randomly divided into 5 groups of seven animals. Group I received saline an intraperitoneal (i.p) injection of 1 ml/kg body weight (bwt) for three consecutive days as a positive control; group II received CCl₄/ olive oil (0.5: 0.5, 1 ml/kg, i.p) for three consecutive days as a negative control. Group III, IV, and V orally received chicory leave extract (CLE) (75, 150, 300 mg/kg/day for three weeks and i.p injections of 1 ml/kg bwt CCl₄/olive oil (0.5:0.5 v/v) on days 22, 23, and 24 have been done. Twenty-four hours after the third CCl₄ injection, the mice were decapitated, and blood was collected.

Determination of biochemical Parameters in the serum of mice

Glutamate Oxaloacetate Transaminase (GOT), Glutamate Pyruvate Transaminase (GPT) and alkaline phosphatase (ALP) activities were determined by colorimetric method as described by Reitman and Frankel (21) and developed by the company (Biomerux) French, as instructed by the company outfitter. Total protein and albumin were determined using kits from company (Biolabo) France. Serum total cholesterol, High-density lipoprotein cholesterol (HDL-c), and triglycerides were calorimetrically determined while VLDL was estimated as 1/5 of triglyceride in mg/dL. Low-density lipoprotein cholesterol (LDL-c) concentration was determined by the Friedewald formula (FF): $LDL-c \text{ (mg/dL)} = TC \text{ (mg/dL)} - HDL-c \text{ (mg/dL)} - TG \text{ (mg/dL)}/5$ (20).

Statistical analysis

The results were expressed as mean \pm standard error (SE). The significance of differences between means was measured by student's t-test and P values below 0.05 were considered significant by using SPSS version 25.0 for Windows 10 (SPSS, Chicago, IL, USA).

RESULTS AND DISCUSSION

CLE significantly ($p < 0.05$) reduced the elevated level of cholesterol, triglycerides, and significantly increased the levels of HDL in group of mice pretreated with CLE (150 mg/kg); 145 ± 1.3 , 243 ± 1.3 , and 34 ± 1.2 mg/dl, respectively, comparison with negative control (185 ± 1.4 , 290 ± 1.4 , and 21 ± 1.3 mg/dl, respectively) (Table 2). Treating mice with chicory was reduced cholesterol and triglycerides level to normal value. The improvement in lipid profile may be due to phenolic compounds and flavonoids (active substances of chicory) as well as inulin (water soluble fiber) which can decrease cholesterol synthesis by inhibiting hydroxymethylglutaryl-CoA reductase (1). Chicory has several health benefits such as short-chain fatty acids produced through the inulin and oligofructose fermentation in the large intestine, serves to lower cholesterol (4). Also, Wang and Cui have shown that chicory leaf contains alkaloids, saccharides, organic acids, and coumarins which may reduce serum lipid levels, uric acid, and glucose (24). Table 3

shows the effect of CLE on serum glucose and magnesium level in CCl_4 induced hepatotoxicity in mice. It has been noticed that the serum fasting glucose and magnesium level in mice groups were not significantly different after three weeks. The results of this study are contrary with Kim and Shin (1996) and Pushparaj *et al.* (2007) findings, who used roots which have higher percentage of inulin than leaves. Inulin is a soluble fiber has obvious role in decreasing blood glucose to normal level. Deficiency of Mg^{2+} may increase the incidence of diabetes mellitus and the occurrence of complications. Hypomagnesemia is a serious clinical consequence that can lead to increase the risk of complications such as developing diabetes mellitus. Moreover, magnesium is necessary for the activity of over 500 enzymes in the body (16). This is the first reported study which shows the effect of CCl_4 on serum magnesium level in mice. Compared with untreated negative control, mice treated with CCl_4 /olive oil showed increased levels of GOT (145 IU/L), GPT (261 IU/L), and ALP (97 IU/L), as an indicator of lipid peroxidation. In contrast, when the mice pretreated with CLE (75 mg/kg) before CCl_4 intoxication those values 115 IU/L, 197 IU/L, and 75 IU/L for GOT, GPT, and ALP, respectively. Meanwhile, when the mice pretreated with CLE (150 mg/kg) those values dropped down to 107 IU/L, 130 IU/L, and 51 IU/L for GOT, GPT, and ALP, respectively. The last group of mice with 300 mg/kg was most effective in reducing the levels of GOT (98 IU/L), GPT (104 IU/L), and ALP (54 IU/L) (Fig 1). Similarly, pretreatment with CLE reduced the level of serum total protein and albumin to normal level. The results showed that the dose of CLE 150 mg/kg decreased levels of total protein and albumin to 4.2 g/dL and 2.1 g/dL, respectively, when the negative control treated (CCl_4 /olive oil) those values were 5.3 g/dL and 2.7 g/dL, respectively (Fig 2). Pretreatment with CLE reduced the elevated levels of biochemical markers to near normal levels in a dose dependent manner. Acute and severe hepatic injuries can be caused by many toxic chemicals such as CCl_4 , infections and drugs; however, plant extracts have shown positive

effect in treating hepatic injuries due to severe oxidative stress (13). CLE was used in this study due to its high content of polyphenolic compounds as well as other phytochemicals such as alkaloids, flavonoids, terpenoids, steroids, tannins, anthraquinones, and saponins. It has been reported previously that chicory extract had high content of phenolics compounds (58.1 mg/g), flavonoids (7.23 mg/g) and carotenoids (0.52 mg/g) (2, 17, 25). The mechanism of hepatotoxicity by CCl₄ is metabolized by cytochrome p450 in the liver to a highly reactive trichloromethyl free radical (CCl₃) which can start a chain of reactive free radical formation resulting in peroxidation of lipids and damage of the cells leading to cell lyses. (11, 12, 19). Necrosis, cirrhosis, and fatty liver are the pathological features of CCl₄ that induced hepatotoxicity which was increased the levels of biochemical framework prominently in mice. The antioxidant functions of chicory in current study are in agreement with the previously reported study (12). Also, it has been reported that alcoholic and aqueous extracts of chicory protected liver against CCl₄ induced hepatocellular damage in rats (26). It is

apparent from the present results that the protective effect of CLE against CCl₄-induced hepatic oxidative damage may be due to the presence of antioxidant compounds such as flavonoids and other polyphenols as well as Vit C which may be contributed to the protective effect of CLE against free radical generation of CCl₄ and reducing tissue damage (6). CLE at concentrations of 75, 150 and 300 mg/ kg protected the mice liver against CCl₄ – induced hepatotoxicity and lowered the level of serum liver enzymes to be normal. Since toxicity of CCl₄ is believed to be due to free radical formation and oxidative stress, therefore protective effect of the chicory extract is possibly by one or many mechanisms such as: inhibition of cytochrome P450 activity and/ or scavenging free radicals responsible for cell damage.

Table 1. Chemical composition of chicory leaf extract (CLE).

Components	CLE (mg/g)
Protein	58.2
Fat	12.1
Ash	59.3
Total phenolics	39.9
Total flavonoids	9.1

Table 2. Effect of chicory leaves extract (CLE) on serum lipid profile in protected groups in mice (n = 7 mice).

Treatments	TC (mg/dl)	TG (mg/dl)	HDL-c (mg/dl)	LDL-c (mg/dl)	VLDL-c (mg/dl)
Positive Control	140± 1.3	220± 1.5	42± 1.1	54± 1.4	44± 1.5
Negative Control	185± 1.4	290± 1.4	21± 1.3	106± 1.2	58± 1.4
CCl ₄ +CLE (75 mg/kg)	179± 1.6	280± 1.4	27± 1.5	96± 1.3	56± 1.4
CCl ₄ +CLE (150 mg/kg)	145*± 1.3	243*± 1.3	34*± 1.2	62.4*± 1.4	48.6*± 1.3
CCl ₄ +CLE (300 mg/kg)	174± 1.4	270± 1.5	30± 1.6	90± 1.6	54± 1.5

Note. VLDL-c = very low-density lipoprotein cholesterol; LDL-c = low-density lipoprotein cholesterol; HDL-c = high-density lipoprotein cholesterol; TC = total cholesterol; TG = triglycerides.

Each value is the mean of 7 animals ± SE.

* significantly different ($p < 0.05$)

Table 3. concentrations of serum glucose and magnesium after 3 weeks of administration with CLE

Treatments	Glucose (mg/dl)	Magnesium (mg/dl)
Positive Control	146± 1.3	1.70± 0.2
Negative Control	150± 1.4	1.75± 0.3
CCl ₄ +CLE (75 mg/kg)	142± 1.6	1.70± 0.2
CCl ₄ +CLE (150 mg/kg)	140± 1.3	1.68± 0.1
CCl ₄ +CLE (300 mg/kg)	141± 1.4	1.66± 0.2

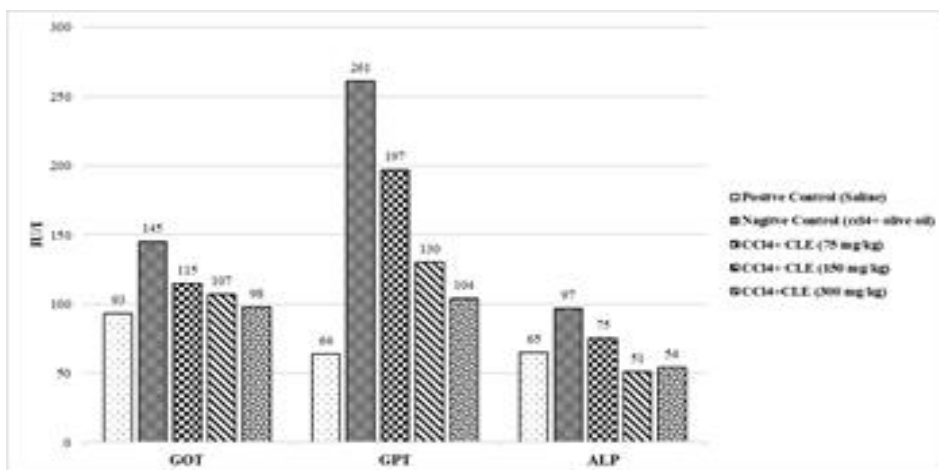


Fig. 1. The protective effect of different doses of the chicory leaf extract (CLE) on CCl₄ induced liver injury in mice (hepatic enzyme level elevation); biomarkers GOT, GPT, and ALP.

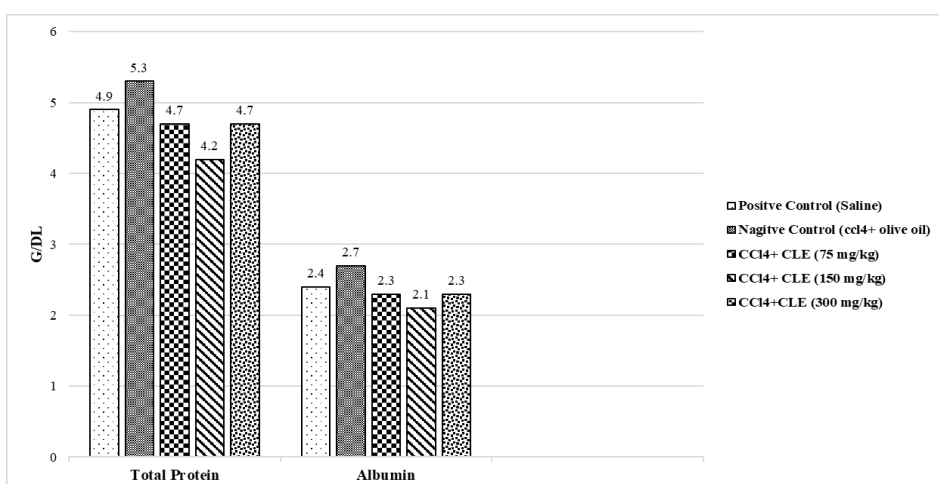


Fig. 2. The protective effect of different doses of the chicory leaf extract (CLE) on CCl₄ induced mice level; total protein and albumin

Conclusions

The findings of this study research show that the leaves extract of chicory has potent antioxidant and hepatoprotective activities due to the presence of flavonoids, polyphenolics, and other active compounds which are known to exhibit hepatoprotective function. This plant could be used in the pharmaceutical and food industries.

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