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CHEMICAL AND NUTRITIONAL STUDIES ON THE HAWTHORN LEAVES AND THEIR THERAPEUTIC EFFECT ON LIVER DISORDER RATS

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ABSTRACT: Medicinal plants and herbs contain many important chemical compounds with therapeutic functions and roles. The hawthorn plant is considered one of the most important of these plants because of its effective effect in treating many different diseases because it contains many phenolic compounds and antioxidants., so the current study aims to know the chemical and nutritional properties of the hawthorn leaves and their therapeutic effect on albino rats inflicting with liver disorder. The study was conducted on thirty (30) Male albino rats of the Sprague-Dawley breed weighing 150±10g. rats were fed a basal diet for one week before the study, and they were divided into five groups, each with six rats. As control negative normal rats (C–ve). In the first group, the negative control group, normal rats were fed only the basal diet for 28 days. The remaining rats (n=24) were injected with carbon tetrachloride (Ccl_4). The second group (the positive control group) is a group of infected rats that were injected with carbon tetrachloride and were not fed on the experimental plant. The other three groups are experimental groups that were injected with carbon tetrachloride and each group was fed the basic meal on hawthorn leaves at one of three concentrations (5%, 10%, 15%). The results revealed that group (4) (rats fed on basal diet plus 10% hawthorn leaves) recorded the best treatment for decreasing alkaline phosphatase (ALP), ALT enzymes, and Total protein of hepatic CCl_4 intoxicated rats when compared to (C +ve) group. The study recommends placing hawthorn leaf powder in the meals of patients with liver disorders.

Keywords: Antioxidants - phenolic compounds – Hawthorn Leaves- liver disorder.

INTRODUCTION

Liver disease is associated with high prevalence, increased mortality, and a substantial healthcare burden. By 2017, approximately 1.5 billion people were suffering from chronic liver disease (CLD), which, along with liver cirrhosis, accounts for 2 million annual deaths worldwide (James *et al.*, 2018). Non-alcoholic fatty liver disease (NAFLD) accounts for a major component (60%) of CLD, other components of which include hepatitis B and C viruses (38%) and alcohol consumption (2%) (Tapper and Parikh, 2018). Acute liver failure is mainly a consequence of viral infection and drug-induced liver injury in the developing and developed worlds, respectively (Wong *et al.*, 2019). Although vaccination and novel antiviral agents have lowered the incidence of virus-induced liver diseases, including cirrhosis, considerable risk factors, including metabolic syndrome, obesity, and alcohol and drug misuse/overuse, still lead to various liver disorders. (Liangpunsakul *et al.*, 2016). Hawthorn *(Crataegus oxyacantha)* is a fruit-bearing shrub with a long history as a medicinal substance. Uses have included the treatment of digestive ailments, liver diseases, kidney stones, and cardiovascular disorders. Flavonoids are postulated to account for these effects. Hawthorn has shown promise in the treatment of New York Heart Association (NYHA) functional class II congestive heart failure (CHF) in both uncontrolled and controlled clinical trials. There are also suggestions of a beneficial effect on liver diseases and blood lipids (Sanchez *et al.*, 2020). Hawthorn is present

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worldwide with about 280 species, among which the most common are: *C. monogyna*, *C. laevigata*, *C. mexicana*, and *C. douglasii*, grown in Europe, North Africa, West Asia, and North America. The scientific name of hawthorn comes from the Greek word “kràtaigos” which means “strength and robustness” due to its hard and durable wood. Natural habitats of hawthorn are wooded and sunny areas on predominantly limestone soils up to 1500 m above sea level (Vieira *et al.*, 2020). Various parts of this plant in particular, the berries, flowers, and leaves are rich in nutrients and have been traditionally associated with many health, medicinal, or nutraceutical beneficial health effects (Souto *et al.*, 2020) Anti-microbial, anti-inflammatory, antioxidant, anti-cancer, and anticoagulant properties. Anti-microbial, anti-inflammatory, antioxidant, anti-cancer, and anticoagulant properties are some of the most relevant properties associated with this plant. According to its traditional use, and since it is generally recognized as safe (GRAS), the Committee for Herbal Medicinal Products of the European Medicines Agency classified hawthorn as a “traditional herbal medicinal product” (Pimente *et al.*, 2018). This wild plant has been used as a traditional medicine, herbal drug, and food supplement for centuries. According to the holistic and traditional approach, hawthorn leaves and flowers are used to prepare infusions that can be used to control palpitations, tachycardia, and can act against diarrhea, urinary retention, and intestinal cramps, liver disease. Indigenous peoples from Latin America use the berries for the preparation of a highly energetic drink called “Pennican”, and, in many parts of the world, the berries are used to prepare jams and as flavoring for dishes like white meats. Hawthorn, however, can also have a few collateral effects and contraindications; in particular, it is not recommended when blood pressure is low. Considering the multiple health properties of this medicinal wild herb, this review describes the potential use of hawthorn in therapy and as a support of some human health (Singh, 2018). Free radicals are implicated by scientists in the aging process (e.g., wrinkle formation) and the onset of numerous health issues, including cardiovascular disease and cancer. Hawthorn's antioxidants may prevent a portion of the damage caused by free radicals, particularly related to cardiovascular disease. (Daiber *et al.*, 2017). The liver is an organ about the size of a football. It sits just under your rib cage on the right side of your abdomen. The liver is essential for digesting food and ridding your body of toxic substances. Liver disease can be inherited (genetic). Liver problems can also be caused by a variety of factors that damage the liver, such as viruses, alcohol use, and obesity. Over time, conditions that damage the liver can lead to scarring (cirrhosis), which can lead to liver failure, a life-threatening condition. However early treatment may give the liver time to heal. Dehghani *et al.*, (2019).

Aim of study

This study aims to know hawthorn leaves' chemical and nutritional properties and their therapeutic effect on albino rats suffering from liver disorders.

MATERIALS AND METHODS

1- Materials

1.1- Preparation leaves of Hawthorn (*Crataegus species)*

Leaves of hawthorn were acquired from the Jeddah, Saudi Arabia, local market. After being washed and dried in an oven designed for drying at a temperature of 50 C for 3 days, the hawthorn was pulverized and milled into a fine powder.

1.2. Experimental animals

In the experiment, we employed thirty male albino Sprague Dawley rats that weighed 150 plus or minus 10 grams each.

1.3- Carbon tetra chloride (CCl_4)

Carbon tetrachloride (CCl_4) was obtained from El-Gomhoryia Company for Drugs, equipments and Chemical Industries, Cairo, Egypt as a 10% liquid solution. It was dispensed in white plastic bottles (each containing one liter) as a toxic chemical material for liver poisoning according to Passmore and Eastwood (1986).

At the same time, it is mixed with paraffin oil which is obtained from the pharmacy for dilution during the induction.

2- Methods

2.1- Biological experiment

- **Basal diet composition of rats**

The basal diet comprised the following components: five percent cellulose, ten percent maize oil, 0.25 percent choline chloride, one percent vitamin mixture, 0.35 percent methionine, & four percent salt mixture (Morsi, 1992).

$CaCO_3$ (600 mg), $MgSO_4.2H_2O$ (204 mg), K2HPO$_4$ (645 mg), $CaHPO_4.2H_2O$ (150 mg), $Fe(C_6H_5O_7)$ $26H_2O$ (55 mg), ZnC_{12} (0.5 mg), $MnSO_4.4H_2O$ (10 mg), NaCl (334 mg), $CuSO_3.5H_2O$ (0.06 mg), as well as K_1 (1.6 mg) comprised the basal diet utilized in the experiment.

The standard test diet included all of the following, Vitamin E (10 Iu), Calcium pantothenic acid (0.40 mg), Thiamin (0.50 mg), Vitamin A (200 Iu), Vitamin K (0.50 Iu), Pyridoxine (1.00mg), Niacin (4.00 mg), Para-aminobenzoic acid (0.02 mg), Vitamin D (100 Iu), Folic acid (0.02 mg), Choline chloride (200 mg), Inositol (24 mg), Vitamin B12 (2.00 g) (Campbell, 1963).

Table 1: The components of the fundamental & experimental diets.

Component (g)	Basal diet	5% Hawthorn	10% Hawthorn	15% Hawthorn
Evaluate substances	---	5	10	15
Casein	20	20	20	20
Corn oil	4.7	4.7	4.7	4.7
Mineral mix	3.5	3.5	3.5	3.5
Vitamin mix	1	1	1	1
Cellulose	5	5	5	5
Cholin chloride	2	2	2	2
Sucrose	10	10	10	10
Corn starch	**Up to 100**	**Up to 100**	**Up to 100**	**Up to 100**

2.2- Design of Experiments & Animal Groups:

At the age of fourteen to sixteen weeks, 30 Male Sprague-Dawley albino rats at maturity weighed 150±10 g. The animals were housed in strain-free metallic coverings secured to plastic enclosures, and stringent hygiene protocols were adhered to. To facilitate adjusting, A basal-style diet was supplied to the rodents for 7 days before the commencement of the experiment. Specialized non-scattering feeding containers were utilized to administer diets to rodents to prevent food loss and contamination. Ad libitum water was supplied through a narrow mouth container equipped with a metallic tube securely fastened to its mouth using a rubber tube. As previously stated, organisms were acclimated to the experimental protocol by being exposed to a twelve-hour light & twelve-hour dark cycle for seven days before its commencement. Rats were separated into five groups of six. The rodent classes consisted of the following:

- Group one: Six rodents were provided with a basal diet (negative control).
- Group two: six hepatic rats consuming a basal diet (positive control)
- Group three: Six hepatic rats consumed a basal diet comprising 5% Hawthorn.
- Group four: Six hepatic rats consumed a basal diet comprising 10% Hawthorn.

- Group five: Six hepatic rats consumed a basal diet comprising 15% Hawthorn.

2.3. Biological evaluation

Throughout the twenty-eight-day experimental period, daily feed consumption as well as weekly body weight measurements were documented. The food efficiency ratio (F.E.R), body weight gain (B.W. G. percent), as well as organ weight were ascertained in accordance with the methodology outlined by Chapman *et al.* (1959).

2.3.1. Blood sampling

After the twenty-eight-day trial rodents were sacrificed under anesthesia & ether. Blood samples were collected in a sterile dry centrifuge tube using the retro-orbital method. Following twenty minutes of clotting at room temperature, they were centrifuged at 1500 rpm for fifteen minutes. Until biochemical analysis, Serum samples were obtained using a dry, sterile hypodermic, infusion into Wisserman tubes, as well as frozen at -10ºC in the refrigerator were preserved. Following this, the liver, spleen, heart, lungs & kidneys of the rats were extracted, rinsed in saline solution, desiccated, & weighed. In accordance with the methodologies outlined by Schermer, (1967).

2.4. Biochemical analysis

2.4.1. *Quantification of Lipids in Serum*

TG: Triglycerides were determined by enzymatic calorimetry in accordance with Fassati and Prencipe's (1982) methodology.

TC: primary application of TC measurement, as described by Allain (1974).

2.4.1.1. HDL-cholesterol

According to Lopez (1977), phosphotungstic acid & magnesium ions selectively precipitating all lipoproteins other than the HDL fraction-cholesterol contained in the supernatant may be measured via a similar.

2.4.1.2. V-LDL & LDL- cholesterol: The technique developed by Lee & Nieman (1996) was utilized for the measurement of both very low-density lipoproteins (VLDL) & low-density lipoproteins (LDL).

2.4.1.3. Total Lipids

According to Young e*t al.*, (2002), the colorimetric approach was used to determine the amount of total lipids.

2.4.2. Estimation of liver functions:

2.4.2.1. Estimation of Alanine transferase (ALT): The approach proposed by Tietz (1976) was utilized for the analysis necessary to establish the ALT. Pyruvate & L-Glutamate are produced as a byproduct of the reaction that ALT catalyzes, which involves the amino group transfer from L-alanine to a-ketoglutarate.

2.4.2.2. Estimation of AST: The approach developed by Henry (1974) & Yound (1975) was utilized throughout the process of determining the (AST).

2.4.3. Complete Blood Count (CBC) Test

The CBC Test included WBC count, HB level, RBC Count, and WBC Platelet count. The results of CBC are generated by highly automated electronic and pneumatic analyzers based on aperture-impedance and/or laser beam cell sizing and counting according to Jacobs *et al.*, (2001).

2.5. HPLC identification of phenolic compounds

HPLC analysis was carried out using an Agilent 1260 series. The separation was carried out using Zorbax Eclipse Plus C8 column (4.6 mm x 250 mm i.d., 5 μm). The mobile phase consisted of water (A) and 0.05% trifluoroacetic acid in acetonitrile (B) at a flow rate of 0.9 ml/min. The mobile phase was programmed consecutively in a linear gradient as follows: 0 min (82% A); 0–1 min (82% A); 1-11 min (75% A); 11-18 min (60% A); 18-22 min (82% A); 22-24 min (82% A). The multi-wavelength detector was monitored at 280 nm. The injection volume was 5 μl for each of the sample solutions. The column temperature was maintained at 40 °C.

2.6. Chemical analysis was performed on raw materials according to the A.O.A.C. (2000). The contents of moisture, protein, fat, crude fiber, and ash of Lemongrass, Cratagaeus leaves and fruits were determined. Total carbohydrates were calculated by difference.

2.7. Statistical Analysis: One-way classification was employed to compute statistical analyses. In accordance with Snedcor & Cochran (1967), analysis of variance (ANOVA) as well as least significant variance (LSD) are utilized**.**

RESULTS AND DISCUSSION

1. Chemical results

1.1. Approximate chemical composition of Hawthorn leaves.

Data given in Table (2) showed the chemical composition of Hawthorn. To identify the polyphenols compounds, in the leaves of hawthorn, the ethanolic extract was prepared. The results indicated that the moisture, protein, fat, ash, fiber, carbohydrates, and energy value contents of Hawthorna dry weight (D/W) were 7.96, 11.40, 59.38, 11.40, 11.54, and 6.77 Kcal/100g, respectively. The moisture, protein, Lipids, carbohydrates, fiber, and ash, respectively.

Table 2: Approximate chemical composition of Hawthorn.

Samples	Moisture (%)	Approximate chemical composition (%)				
		Ash	Protein	Lipids	Fiber	Total carbohydrates
Hawthorn (Cratagaeus leaves)	7.96	6.77	11.40	11.40	11.54	59.38

1.2. Total content of polyphenols compounds of the leaves of Hawthorn

Table (3) shows the total number of 19 different phenolic compounds ~~were~~ assessed in leaves of Hawthorn., 17 of them existed, while 2 were absent. Total phenolic compounds reached as high as (10810.764 ppm). By focusing on the major phenolic compound, it was found that the highest content was recorded for gallic acid (3776.45 ppm), chlorogenic acid (976.46 ppm) & rosmarinic acid (839.02 ppm). over a hundred compounds have been isolated from hawthorn leaves, including flavonoids, terpenoids, lignans, organic acids, and nitrogenous compounds. (Wang 2020).

2. Biological effects

2.1. Effect of different levels of hawthorn leaves on food intake (FI) of hepatic CCl_4-intoxicated rats.

Table (4), indicates the effect of different levels of hawthorn leaves on food intake (FI) of hepatic CCl_4-intoxicated rats. It could be noticed that the mean value of the (C^{-ve}) group was higher than (C^{-ve}) group, being (383.75±1.03 and 355.50±2.50 g respectively) which showed a significant ($p \leq 0.05$) difference with a percent of change -7.36 for (C -ve) group when compared with (C^{+ve}) group. Only 5% of hawthorn leaves treatment groups indicated a significant decrease when compared to the (C+ve) group. Other treatments raised the FI. The values were (376.75±2.71, 405.80±1.05, and 393.00 ±4.35 g) for groups 3, 4, and 5, respectively. The percent of change were (-1.82, +5.74, and +2.41) for the Above-mentioned groups respectively. Group (4) (rats fed on basal diet plus 10% hawthorn leaves) recorded the highest feed intake and accordingly healthy appetite of hepatic CCl4-intoxicated rats when compared to (C +ve) and (C -ve) groups.

Table 3: The total content of polyphenols compounds in the leaves of Hawthorn.

No	Polyphenols compounds	Area	Conc. (µg/ml)	Conc. (µg/g)
1	**Gallic acid**	2134.73	188.82	3776.45
2	**Chlorogenic acid**	1257.85	163.22	3264.31
3	**Catechin**	0.00	0.00	0.00
4	**Methyl gallate**	15.51	0.78	15.63
5	**Coffeic acid**	258.61	20.01	400.24
6	**Syringic acid**	667.58	48.82	976.46
7	**Pyro catechol**	225.53	32.51	650.28
8	**Rutin**	0.00	0.00	0.00
9	**Ellagic acid**	124.02	12.39	247.77
10	**Coumaric acid**	35.71	1.27	25.41
11	**Vanillin**	110.21	4.10	81.92
12	**Ferulic acid**	98.67	5.73	114.64
13	**Naringenin**	129.12	11.80	236.03
14	**Rosmarinic acid**	391.27	41.95	839.02
15	**Daidzein**	6.11	0.34	6.85
16	**Querectin**	52.35	7.07	141.32
17	**Cinnamic acid**	16.63	0.30	5.96
18	**Kaempferol**	9.23	0.58	11.65
19	**Hesperetin**	17.10	0.84	16.82

Table 4: Food intake (FI) for hepatic CCl_4 - intoxicated rats fed on different levels of hawthorn leaves. (n = 6 rats)

Parameters Groups	FI (g/28 days) Mean ± SD	% Change of Control (+) group
Control -ve	355.50±2.5 ^{e}	-7.36
Control +ve	383.75±1.03 ^{c}	0.00
5% Hawthorn leaves	376.75±2.71 ^{d}	-1.82
10% Hawthorn leaves	405.80±1.05 ^{a}	+5.74
15% Hawthorn leaves	393.00±4.35 ^{b}	+2.41
LSD	4.54	

***Nots*:**

- Values denote arithmetic means ± Standard deviation of the mean.
- Means with different letters (a, b, c, d, e, f, g) in the same column differ significantly at p<0.05 using the one-way ANOVA test, while those with similar letters are non-significantly different.

2.2. Effect of different levels of hawthorn leaves on relative organs weight of hepatic CCl4-intoxicated rats

Data present in Table (5), illustrates the mean value of relative organs weight for control and different groups of hepatic CCl_4 - intoxicated rats fed on different levels of hawthorn leaves. As shown the mean value of liver weight for (C^{-ve}) group was 7.75±0.03% while in the (C^{-ve}) group was 5.38±0.03%. These results denote that there was a significant increase in the (C^{+ve}) group when compared to the (C^{-ve}) group which revealed a significant difference with a percent of change -30.58 for the (C^{-ve}) group when compared with (C +ve) group. In treated rats, there were mostly significant decreases in treated groups when compared to the (C^{+ve}) group, being (5.30±1.05, 5.00±1.00, and 6.84±0.04g) for groups 3, 4, and 5 respectively. The percent of change were (-31.61, -35.48, and -11.74) for the above-mentioned groups respectively. Group (4) (rats fed on basal diet plus 10% hawthorn leaves) recorded the best treatment for decreasing the liver weight of hepatic CCl4-intoxicated rats when compared to the (C^{+ve}) group.

The mean value of spleen weight for the (C^{+ve}) group was 0.96±0.02while the (C-ve) group was 0.88±0.02%. The results denote that there was a significant increase in (C^{+ve}) when compared to the (C^{-ve}) group which showed a significant difference with a percent of change -8.33 for the (C^{-ve}) group when compared with the (C^{+ve}) group. In treatment rats, there was a significant decrease when compared to ((C^{+ve}) group. The values were (0.60±0.05, 0.57±0.01, and 0.72±0.02) for groups 3,4, and 5 respectively. The percent of change were (-37.5, - 40.62, and -25.00) for the above-mentioned groups respectively. Group (4) (rats fed on basal diet plus 10%hawthorn leaves) recorded the best treatment for decreasing spleen weight of hepatic CCl4-intoxicated rats when compared to the (C^{+ve}) group.

The mean value of lung weight for the (C^{+ve}) group was 1.90±0.03% while the (C^{-ve}) group was 1.30±0.02%. These results show that there was a significant increase in the (C^{+ve}) when compared to the (C^{-ve}) group which revealed a significant difference with the percent of change -31.57 for the (C^{-ve}) group when compared with the (C^{+ve}) group. All treatment groups indicated a significant decrease when compared to the (C^{+ve}) group. The values were (1.63±0.04, 1.54±0.05, and 1.69±0.04) for groups 3, 4, and 5 respectively. The percent of change were (- 14.21, -18.94, and -11.05) for the above mention groups respectively. It could be noticed that groups (3 and 5)showed no significant difference. Group (4) (rats fed on basal diet plus 10% hawthorn leaves) recorded the best treatment for decreasing lung weight of hepatic CCl_4-intoxicated rats when compared to the (C^{+ve}) group.

The mean value of heart weight for the (C^{+ve}) group was 1.00±0.10% while the (C^{-ve}) group was 0.88±0.03%. These results indicated that there was a significant increase in (C^{+ve}) when compared to the (C^{-ve}) group which revealed a significant difference with the percent of change -12.00 for the (C^{-ve}) group when compared with the (C^{+ve}) group. All treatment groups illustrated significant decreases when compared with the (C^{+ve}) group. The values were (0.70±0.10, 0.65±0.04, and 0.77±0.01) for groups 3, 4, and 5 respectively. The percent of change were (-30.00, -35.00, and -23.00) for the above-mentioned groups respectively. It could be noticed that groups (3, 4, and 5) showed no significant differences. Group (4) (rats fed on basal diet plus j10% hawthorn leaves) recorded the best treatment for decreasing heart weight of hepatic CCl_4-intoxicated rats when compared to the (C^{+ve}) group.

The mean value of kidney weight for the (C^{+ve}) group was 1.72±0.02% while the (C^{-ve}) group was 1.43±0.02%. These results showed that there was a significant increase in (C^{+ve}) when compared to the (C^{-ve}) group which revealed a significant difference with percent of change -16.86 for the (C^{-ve}) group when compared with the (C^{+ve}) group. All treatment groups indicated a significant decrease when compared with the (C^{+ve}) group. The values were (1.42±0.07, 1.35±0.02, and 1.51 ±0.10) for groups 3,4, and 5 respectively. The percent of change were (-17.44, -21.51, and -12.21) for the above-mentioned groups respectively. It could be observed that groups (3 and 5) showed nonsignificant differences. Group (5) (rats fed on basal diet plus 10% hawthorn leaves) recorded the best treatment for decreasing kidney weight of hepatic CCl_4-intoxicated rats when compared to the (C^{+ve}) group. found that CCl_4 injection raised the relative internal organ weight of hepato-intoxicated rats due to inflammations.

Table 5: The mean ± SD value of relative organ weight (g) for for hepatic CCl4- intoxicated rats fed on different levels of hawthorn leaves. (n = 6 rats)

Parameters		Relative organs weight (g/100 g. B.Wt.)			
Groups	**Liver**	**Spleen**	**Lungs**	**Heart**	**Kidneys**
Control -ve	5.38±0.03 b	0.88±0.02 b	1.30±0.02 d	0.88±0.03 ab	1.43±0.02 be
% Change of Control (+) group	-30.58	-8.33	-31.57	-12.00	-16.86
Control +ve	7.75±0.03 a	0.96±0.02 a	1.90±0.03 a	1.00±0.10a	1.72±0.02 a
% Change of Control (+) group	0.00	0.00	0.00	0.00	0.00
5% Hawthorn leaves	5.30±1.05 b	0.60±0.05 e	1.63 ±0.04 be	0.70±0.10 e	1.42±0.07 be
% Change of Control (+) group	-31.61	-37.50	-14.21	-30.00	-17.44
10% Hawthorn leaves	5.00±1.00 b	0.57±0.01 e	1.54±0.05 c	0.65±0.04 e	1.35±0.02e
% Change of Control (+) group	-35.48	-40.62	-18.94	-35.00	-21.51
15% Hawthorn leaves	6.84±0.04 ab	0.72±0.02 c	1.69*0.04 b	0.77±0.01 be	1.51±0.10 b
% Change of Control (+) group	-11.74	-25.00	-11.05	-23.00	-12.21
LSD	1.53	0.06	0.09	0.12	0.09

Values denote arithmetic means ± Standard deviation of the mean.

Means with different letters (a, b, c, d, e, f, g) in the same column differ significantly at $p<0.05$ using one way ANOVA test, while those with similar letters are non-significantly different.

3. Effect of different levels of hawthorn leaves on alanine aminotransferase (ALT) enzyme, alkaline phosphatase (ALP) enzyme (U/L), and total protein (g/dl) of hepatic CCl_4-intoxicated rats:

Table (6) indicates the effect of different levels of hawthorn leaves on the alanine aminotransferase (ALT) enzyme of hepatic CCl_4-intoxicated rats. As shown, the mean value of serum ALT for the (C^{-ve})group was 48.00 ±2.65 U/L while in the (C^{-ve}) group was 31.00±2.00 U/L. These results denote that there was a significant increase in the (C^{+ve}) group when compared to the (C^{-ve}) group which revealed a significant difference with a percent of change -35.42 for the (C^{+ve}) group when compared with the (C^{+ve}) group. In treatment rats; there were significant decreases in all treated groups when compared to the (C^{+ve}) group being, (25.00±2.65, 23.00±2.65, and 33.00±1.00 U/L) for groups 3,4, and 5 respectively. The percent of change were (-47.92, -52.08, and -31.25) for the above-mentioned groups respectively. The best treatment when compared to healthy was that of group (4) (rats fed on basal diet plus 10% hawthorn leaves) which recorded the best treatment and lowest alanine aminotransferase (ALT) enzyme of hepatic CCl_4-intoxicated rats when compared to (C^{+ve}) group and also when compared with (C -ve) rats.

Table (6), indicates the effect of hawthorn leaves on alkaline phosphatase (ALP) (U/L) enzyme of hepatic CCl_4-intoxicated rats. It could be revealed that the mean value of the (C^{+ve}) group was higher than the (C^{-ve}) group, being (349.00 ±2.65 and 295.00±2.65 U/L respectively) which revealed a significant difference with a percent of change -15.47 for (C^{-ve}) group when compared with (C^{+ve}) group. All treatment groups showed significant differences when compared to the (C+ve) group. The values were (320.00±2.65, 301.00±3.61, and 340.00±2.65 U/L) for groups 3, 4, and 5 respectively. The percent of change were (-8.31, -13.75, and - 2.57) for the above-mentioned groups respectively. Group (4) (rats fed on basal diet plus 10% hawthorn leaves) recorded the best treatment for decreasing alkaline phosphatase (ALP) enzyme of hepatic CCl_4-intoxicated rats when compared to the (C^{+ve}) group.

Data present in Table (6), illustrates the mean value of total protein (g/dl) for control and different groups of hepatic CCl4 - intoxicated rats fed on hawthorn leaves. As shown the mean value of total protein (mg/dl) for the (C^{+ve}) group was 5.10±0.10(g/dl) while in the (C^{-ve}) group was 7.80±0.10 (g/dl). These results denote that there was a significant decrease in the (C^{-ve}) group when compared to the (C^{-ve}) group which revealed a significant difference with percent of change + 52.94 for the (C^{-ve}) group when compared with the (C^{+ve}) group. hepato-toxicity reduced serum T. Protein from 5.97 to 4.40 g. In hepatic rats (Table 4), there were significant increases in all treated groups when compared to the (C^{+ve}) group, being (6.60±0.26, 6.80±0.20, and 6.00±0.206 (g/dl)) for groups 3,4,5,6 and 7 respectively. The percent of change were (+29.41, +33.33, +17.64, +21.56 and +23.83) for the above-mentioned groups respectively. It could be observed that the values of groups (3 and 4) showed nonsignificant differences. Group (4) (rats fed on basal diet plus jojoba oil 5%) recorded the best treatment for increasing total protein (g/dl) of hepatic CCl4-intoxicated rats when compared to the (C^{+ve}) group. Singh, (2018), found the holistic and traditional approach, hawthorn leaves and flowers are used to prepare infusions that can be used to control palpitations, and tachycardia and can act against diarrhea, urinary retention, intestinal cramps, and liver disease.

Table 6: Effect of different levels of hawthorn leaves on alanine aminotransferase (ALT) enzyme, alkaline phosphatase (ALP)enzyme (U/L), and total protein (g/dl) of hepatic CCl_4-intoxicated rats. (n = 6 rats)

Parameters	ALT (U/L)*	% Change of Control (+) group	ALP (U/L)*	% Change of Control (+) group	Total protein(g/dl)	% Change of Control (+) group
Groups	**Mean ± SD**		**Mean ± SD**		**Mean ± SD**	
Control -ve	31.00±2.00 b	-35.42	295.00±2.65 f	-15.47	7.80±0.10 a	+52.94
Control +ve	48.00±2.65 a	0.00	349.00±2.65 a	0.00	5.10±0.10 d	0.00
5% Hawthorn leaves	25.00±2.65 be	-47.92	320.00±2.65 d	-8.31	6.60±0.26 be	+29.41
10% Hawthorn leaves	23.00±2.65 c	-52.08	301.00±3.61 e	-13.75	6.80±0.20 b	+33.33
15% Hawthorn leaves	33.00±1.00 b	-31.25	340.00±2.65 b	-2.57	6.00±0.20 c	+ 17.64
LSD	**5.85**		**5.42**		**0.44**	

Nots:

- (U/L)* means unit per liter
- Values denote arithmetic means ± Standard deviation of the mean.
- Means with different letters (a, b, c, d, e, f, g) in the same column differ significantly at $p<0.05$ using one way ANOVA test, while those with similar letters are non-significantly different.

4. Effect of different levels of hawthorn leaves on glutathione peroxides (GPX) (ng/nil), super oxidize dismutase (SOD), and catalase mmol/L of hepatic CCl_4-intoxicated rats

Table (7) indicates the effect of different levels of hawthorn leaves on glutathione peroxides (GPX) of hepatic CCl_4- intoxicated rats. It could be revealed that the mean value of the (C^{-ve}) group was higher than (C^{+ve}) group, being (40.05±0.93 and 11.32±1.02 ng/ml respectively) which showed significant differences with percent of change +253.79 for (C^{-ve}) group when compared with (C^{+ve}) group. All treatment groups showed a significant increase when compared to the (C^{+ve}) group. The values were (30.14±1.02, 33.68±2.74, and 15.36±3.62 ng/ml) for groups 3,4, and 5 respectively. The percent of change were (+166.25, +197.52, and +35.68,) for the above mention groups respectively. It could be noticed that groups (3 and 4), (2 and 5) showed nonsignificant differences between each pair. Groups (3 & 4) (rats fed on basal diet plus 5%

and 10% hawthorn leaves) recorded the best treatment for increasing (GPX) of hepatic CCl4-intoxicated rats when compared to diseased rats (C +ve).

Also, data in Table (7) showed the effect of super oxidized dismutase (SOD) on super oxidized dismutase (SOD) of hepatic CCl_4-intoxicated rats. The mean value of SOD for the (C^{+ve}) group was 34.30±2.52 while (C^{-ve}) for the group was 48.42±2.67 U/L. The results denote that there was a significant decrease (C^{+ve})when compared to the (C^{-ve}) group which revealed a significant difference with the percent of change +41.16 for the (C^{-ve}) group when compared with the (C^{+ve}) group. All treatment groups illustrated significant differences when compared to the (C^{+ve}) group. The values were (39.15+2.69, 44.12±3.66, and 36.40±2.20, U/L) for groups 3,4 and,5 respectively. The percent of change were (+14.14, +28.63, and +6.128) for the above-mentioned groups respectively. It could be observed that groups (3, and 5) showed non-significant differences between them. Group (4) (rats fed on basal diet plus 15% hawthorn leaves) recorded the best treatment for increasing SOD of hepatic CCl4-intoxicated rats when compared to healthy rats.

Data present in Table (7), illustrate the mean value of catalase for control and different groups of hepatic CCl_4 - intoxicated rats fed on hawthorn leaves. As shown the mean value of catalase for (C^{+ve})group was 25.32±2.52 while in (C^{-ve}) group was 83.94±2.67 mmol/L. These results denote that there was a significant decrease in the (C^{+ve}) group when compared to the (C^{-ve}) group which revealed a significant difference with percent of change + 231.51 for the (C^{-ve}) group when compared with the (C^{+ve})group. In rats given Ccl4 and then fed on jojoba, lavender oils, and their mixture, there were significant differences in all treated groups when compared to the (C^{+ve}) group, being (66.70±1.05, 69.52±3.62, and 57.81±2.01 mmol/L) for groups 3,4, and 5 respectively. The percent of change were (+163.43, +174.56, and +128.32) for the above-mentioned groups respectively. Group (4) (rats fed on basal diet plus 10% hawthorn leaves) recorded the best treatment when compared to (C^{+ve}) rats. AEman *et al.,* (2021). found that hepatic intoxication lowered the anti-oxidation enzymes namely GPX, CAT & SOD, which were improved by rat's diets containing Q10 sources, being soybean oil, jojoba seeds oil, and broccoli. Also showed that essential oils from hawthorn.raised SOD, GPX & CAT, while reducing MDA, indicating lowering of oxidative changes. Due to phenolis and flavonoids that found in hawthorn.

Table 7: Effect of different levels of hawthorn leaves on glutathione peroxides (GPX) (ng/nil) , super oxidize dismutase (SOD), and catalase mmol/L of hepatic CCl_4-intoxicated rats (n = 6 rats)

Parameters	glutathione peroxides (GPX) (ng/nil)	% Change of Control (+) group	Super oxidize dismutase (U/L)	% Change of Control (+) group	catalase mmol/L	% Change of Control (+) group
Groups	**Mean ± SD**		**Mean ± SD**		**Mean ± SD**	
Control -ve	11.32±1.62 c	+253.79	48.42±2.67 a	+41.16	83.94±2.67 c	+231.51
Control +ve	40.05±0.93a	0.00	34.30±2.52 c	0.00	25.32±2.52 a	0.00
5% Hawthorn leaves	30.14±1.02b	+ 166.25	39.15±2.69b	+ 14.14	66.70±1.05b	+ 163.43
10% Hawthorn leaves	33.68±2.24 a	+197.52	44.12±3.66 a	+28.63	69.52±3.62 a	+174.56
15% Hawthorn leaves	15.36±3.06 b	+35.68	36.40±2.20 b	+6.12	57.81±2.01 b	+128.32
LSD	3.66		4.55		4.55	

Nots:

- Values denote arithmetic means ± Standard deviation of the mean.
- Means with different letters (a, b, c, d, e, f, g) in the same column differ significantly at p<0.05 using one way ANOVA test, while those with similar letters are non-significantly different.

5. Effect of different levels of hawthorn leaves on total cholesterol (mg/dl), HDLc. (mg/dl) and LDLc. (mg/dl) of hepatic CCl4-intoxicated rats

Table (8) indicates the effect of hawthorn leaves on the total cholesterol (mg/dl) of hepatic CCl_4-intoxicated rats. It could be revealed that the mean value of the (C^{+ve}) group was higher than (C^{-ve}) group, being (137.00±2.64 and 90.00±2.64 mg/dl respectively) which showed significant differences with a percent of change -34.30 for (C^{-ve}) group when compared with (C^{+ve}) group. All treatment groups showed a significant decrease when compared to the (C^{+ve}) group. The values were (115.00±2.64, 103.00±2.64, and 124.00±2.64 mg/dl) for groups 3,4, and 5 respectively. The percent of change were (-16.06, -24.81, and -9.48) for the above-mentioned groups respectively. It could be noticed that groups (4 and 5) showed no significant difference. Group (4) (rats fed on basal diet plus 10% hawthorn leaves) recorded the best treatment for decreasing total cholesterol (mg/dl) of hepatic CCl4-intoxicated rats when compared to the (C^{+ve}) group.

Data present in Table (8), illustrates the mean value of HDLc. (mg/dl) for control and different groups of hepatic CCl_4-intoxicated rats fed on hawthorn leaves. As shown the mean value of HDLc. (mg/dl) for the (C^{+ve}) group was 59.00±2.64 mg/dl while in the (C^{-ve}) group was 76.00 + 2.64 mg/dl. These results denote that there was a significant decrease in the (C^{+ve}) group when compared to (C^{-ve}) group which revealed a significant difference with percent of change + 28.81 for the (C^{-ve}) group when compared with the (C^{+ve}) group. In rats given Ccl4 injection and then fed on hawthorn leaves, there was a significant increase in all treated groups when compared to the (C^{+ve}) group, being (70.00±10.00, 75.00±2.64, and 69.00 ± 2.64 mg/dl) for groups 3,4, and 5 respectively. The percent of change were (+18.64, +27.12, and +16.95) for the above-mentioned groups respectively. It could be observed that groups (2, 3, and 5) showed no significant differences between them. Group (4) (rats fed on basal diet plus 10% hawthorn leaves) recorded the best treatment for increasing HDLc. (mg/dl) of hepatic CCl4-intoxicated rats when compared to the healthy rats and (C^{+ve}) group.

Table (8): Effect of different levels of hawthorn leaves on total cholesterol (mg/dl), HDLc. (mg/dl) and LDLc. (mg/dl) of hepatic CCl_4-intoxicated rats: (n = 6 rats)

Parameters	Total cholesterol (mg/dl) Mean ± SD	% Change of Control (+) group	High-density lipoprotein fractions (mg/dl)	% Change of Control (+) group	Low-density lipoprotein fractions (mg/dl)	% Change of Control (+) group
Groups	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**	**Mean±SD**	**Mean ± SD**	**Mean± SD**
Control -ve	90.00+2.64 f	-34.30	76.00±2.64 a	+28.81	6.00±2.64 a	-89.65
Control +ve	137.00+2.64 a	0.00	59.00+2.64 b	0.00	58.00±1.0 c	0.00
5% Hawthorn leaves	115.00+2.64 cd	-16.06	70.00±10.00ab	+ 18.64	37.80±1.02.31b	-34.83
10% Hawthorn leaves	103.00+2.64 b	-24.81	75.00±2.64 a	+27.12	22.00±2.00 a	-62.06
15% Hawthorn leaves	124.00+2.64 b	-9.48	69.00=1=2.64 ab	+16.95	46.20±0.20 b	-20.34
LSD	5.54		8.21		4.55	

Nots:

- Values denote arithmetic means ± Standard deviation of the mean.
- Means with different letters (a, b, c, d, e, f, g) in the same column differ significantly at $p<0.05$ using one way ANOVA test, while those with similar letters are non-significantly different.

Also, data in Table (8) indicated the effect of hawthorn leaves on LDLc. (mg/dl) of hepatic CCl_4-intoxicated rats. It could be revealed that the mean value of the (C^{+ve}) group was higher than (C^{-ve})group, being (58.00±2.64 and 6.00±1.00 mg/dl respectively) which showed significant differences with percent of change -89.65 for (C^{-ve}) group when compared with (C^{+ve}) group. All treatment groups showed significant decreases when compared to the (C^{+ve}) group. The values were (37.80±2.31, 22.00±2.00, and 46.20±0.20, mg/dl) for groups 3,4, and 5 respectively. The percent of change were (-34.83, -62.06, and -20.34) for the above-mentioned groups respectively. Group (4) (rats fed on basal diet plus 10% hawthorn leaves) recorded the best treatment for decreasing LDLc. (mg/dl) of hepatic CCl4-intoxicated rats when compared to (C +ve) group. Daiber *et al.*, (2017), showed that free radicals are implicated by scientists in the aging process and the onset of numerous health issues, including cardiovascular disease and cancer. Hawthorn's antioxidants may prevent a portion of the damage caused by free radicals, particularly in relation to cardiovascular disease.

CONCLUSION

The extensive literature survey revealed that hawthorn leaves are an important medicinal plant due to their traditional uses to treat diseases and the presence of many active chemical constituents which are responsible for various medicinal and pharmacological properties. Further evaluation needs to be carried out on hawthorn to confirm its medicinal uses and the development of formulations containing this plant for their practical clinical applications, which can be used for the welfare of mankind.

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دراسات كيميائية وغذائية على أوراق نبات الزعرور وتأثيرها العلاجي لدى الفئران المصابة باضطربات الكبد

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الملخص العربى

النباتات والأعشاب الطبية تحتوي علي العديد من المركبات الكيميائية الهامة والتي لها وظائف وادوار علاجية عديدة. ويعتبر نبات الزعرور من أهم هذه النباتات لما له من تأثير فعال في علاج العديد من الأمراض المختلفة لإحتوائة على العديد من المركبات الفينولية ومضادات الأكسدة. لذلك تهدف الدراسة الحالية إلى معرفة الخواص الكيميائية والغذائية لأوراق الزعرور وتأثيرها العلاجي على الفئران المصابة باضطراب الكبد. استخدمت الدراسة ثلاثون فأر ألبينو ذكرًا من سلالة سبراغ داولي بوزن ١٥٠± ١٠ جرام، تم تغذية جميع الفئران بنظام غذائي أساسي لمدة أسبوع قبل الدراسة و تقسيمهم إلى خمس مجموعات، كل مجموعة بها ست فئران حيث المجموعة الأولى و هي المجموعة فئران طبيعية وهى المجموعة الضابطة السالبة و المجموعة الثانية مجموعة فئران مصابه تم حقنها برابع كلوريد الكربون و لم يتم تغذيتها على نبات التجربة و تسمى المجموعة الضابطة الموجبة ,و ثلاث مجاميع تجريبية مصابة تم حقنها برابع كلوريد الكربون وتم تغذيتها في الوجبة الأساسية و ثلاث تراكيز متفاوتة (٥%، ١٠%، ١٥% من أوراق نبات الزعرور). اظهرت النتائج ان المجموعة الرابعة التي تغذت على الوجبة الأساسية و ١٠% أوراق نبات الزعرور أظهرت افضل انخفاض في مستوى انزيمات الفوسفاتيز القلوي و البروتين الكلى وذلك عند مقارنتها بالمحموعة الضابطة الموجبة وعلية، توصى الدراسة بوضع مسحوق أوراق نبات الزعرور في وجبات المراضى المصابين باضطربات في الكبد .

الكلمات المفتاحية :- مضات الاكسدة– المركبات الفينولية – اوراق الزعرور- اضطربات الكبد.