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***IMPACT OF LEAVES AND FLOWER FROM ALFALFA AND DANDELION TO  
IMPROVE LIVER FUNCTION AND PREPARING SOME FOOD PRODUCTS***

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## **IMPACT OF LEAVES AND FLOWER FROM ALFALFA AND DANDELION TO IMPROVE LIVER FUNCTION AND PREPARING SOME FOOD PRODUCTS**

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### **Abstract**

Alfalfa and dandelion are a rich source of natural antioxidants. Therefore, this study was carried out to identify the effect of eating flowers and leaves from alfalfa and dandelion to improve liver function for rats and using them to make some foods products, such as pan bread and tea. Then sensory properties of these products were evaluated through trained arbitrators. Biochemical analysis and histopathological properties were investigated using sixty four male rats which were randomly divided into two main groups the first group: control negative group (8 rats) fed on a basal diet, while the second group :56 rats were fed on a basal diet and they were injected subcutaneous by CCl<sub>4</sub>, in paraffin oil ( viv 4ml/kg) to induce fibrosis in liver. Then divided to 7 subgroups .Subgroup (1): fed on a basal diet as a positive control group. Subgroup (2): fed on a basal diet containing 25g alfalfa leaves. Subgroup (3): fed on a basal diet containing 25g alfalfa flowers. Subgroup (4): fed on a basal diet containing 25g dandelion leaves. Subgroup (5): fed on a basal diet containing 25g dandelion flowers. Subgroup (6): fed on a basal diet containing 25g mixed leaves of alfalfa and dandelion. Subgroup (7): fed on a basal diet containing 25g mixed flowers of alfalfa and dandelion.

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The obtained results showed that the chemical composition of alfalfa and dandelion contain high percent of protein, fiber and antioxidant. and the obtained results showed significant decrease the treated groups in ALT, AST, total cholesterol, triglyceride , LDL, blood urea, Creatinine ,random blood sugar and MDA . Also increase the treated groups in HDL, GSH ,SOD and Catalase. Generally, the results of the sensory evaluation demonstrated that ,the pan bread and tea were acceptable. The study recommends the using of alfalfa and dandelion (leaves and flower ) as food additives for their nutritional and healthy benefits.

Keywords: Alfalfa leaves and flower - dandelion leaves and flower - liver enzymes- kidney function-antioxidant enzymes.

### **INTRODUCTION :**

Liver is a very important organ with a lot of functions for our bodies to have a perfect health level, then what we eat determines our level of liver health or disease. Plants food is an essential part of the human diet and comprises various compounds which are closely related to liver health , Thus food plants selection can provide nutritional and medicinal support for liver disease ( **Guan and He, 2015** ) .

Liver fibrosis, occurs as a compensatory response to the process of tissue repair in a wide range of chronic liver injures and inflammations ( **Cordero and Huch., 2018**). fibrosis is an essential part of the assessment and management of patients with chronic liver disease ( **Joseph , 2020**).

Alfalfa is considered one of the most important genera of the leguminosae family, Its flowers have a sweet and mild licorice flavor and are traditionally used garnish or ingredient in salads, soups, entrees, desserts, and drinks worldwide ( **Sabudak and Guler ,2009**). it's not used only to improve appearance of meals but also for their nutritive value ( **Kelley et al ., 2002**).

The phytochemical analysis of Alfalfa (*Trifolium alixandrinum*) showed the presence of proteins, fiber, carbohydrates, saponins, lignin, phenolic phytoestrogens, flavones and iso flavonoids phenolic compounds. The previous pharmacological investigation showed that the plant possessed

antioxidant, antidiabetic, immunological, and many other pharmacological effects (Al-, Snafi ,2021).

Dandelion have been used as folk medicines in China, India, and Russia for the treatment of chronic liver diseases (Yarnell and Abascal ,2009).

Biochemical analysis of dandelion (*Taraxacum officinale* ) compound found higher polyphenols in the leaves and flowers than in the other parts. Dandelion leaves and flowers consist of carbohydrates, carotenoids, fatty acids, fiber, minerals, sugars, choline vitamins, mucilage, and pectin (Wirngo *et al.*, 2016).

The leaves and flowers of the dandelion contain coumarin and many flavonoids and important chemical components such as organic acids, inulin, vitamins A, B, C, and D (Sharifi *et al.*, 2018).

Recently, it was found that alfalfa and dandelion extract fight free radicals and attenuate inflammatory cells activations, and decreased collagen deposition (chemical by-products known to damage DNA). Health care providers clinically use dandelion to promote liver detoxification (Al-Malki *et al.*,2013 and Hamza *et al.* , 2020).

Hence, there has been more and more interests in developing and using natural, effective, safe, and multiple biological antioxidants to replace synthetic antioxidants in many industries, such as natural plant polyphenol compounds ( Lopes *et al.* , 2016 ).

Furthermore, Mentioned that oxidative stress is a common feature observed in a wide spectrum of chronic liver diseases including viral hepatitis, alcoholic, and non-alcoholic steatohepatitis. (Jiang and Török, 2014).

The current study aims to identify the impact of leaves and flowers of alfalfa and dandelion and preparing some food products.

## **MATERIALS AND METHODS:**

### **Materials:**

- 1- Flowers and leaves of alfalfa(*Trifolium alixandrinum*) and dandelion(*Taraxacum officinale*) : were harvest from a local farmairo, Egypt.
- 2- Sixty four males albino rats (Sprague Dawley rats) weighting between (150-200 g) were used throughout the study from Faculty of medicine, Al-Mansoura University,Cairo,Egypt.
- 3-CCl<sub>4</sub> from Romil (Romil Chemicals, England), from Faculty of Medicine, Al- Mansourah University,Cairo,Egypt.
- 4- Chemicals and kits were obtained from El-Gomhoryia Company , Cairo , Egypt .

### **Methods:**

#### **Leaves and flowers preparation :**

- Alfalfa and dandelion were harvested fresh at the beginning of every experiment, then dry it in the natural way by exposing it to sun light according to (**Rubin, 2004**).

#### **Experimental design:**

Male albino rats Sprague Dawley Strain (64 rats) weighting (150: 200 g) was kept in individual stainless steel cages underhygienic conditions and fed one week on basal diet for adaptation . The experiment on rats were carried out according to institutional Animal Ethical Committee according to (**Reeves et al., 1993**).

After that period ,animals were randomly divided into two main groups as follows:

**The first main group (8 rats):** were fed on a basal diet (as a control negative group).

**The second main group (56 rats):** positive control were treated with **CCL<sub>4</sub>**, in paraffin oil ( viv 4ml/kg) by a single subcutaneous injection to induce fibrosis in liver accordind to (**Hubner et al., 1965, Dashti et al., 1989 and Nakano et al., 1996**). After injection, AST, ALT and enzymes

activity were determined in second main group to insure the induction (Delire *et al*, 2015) . The rats in the second main group were divided to seven subgroups (n=8).

- **Subgroup (1):** fed on a basal diet positive control group.
- **Subgroup (2):** fed on a basal diet containing 25 g alfalfa leaves per 100 g diet.
- **Subgroup (3):** fed on a basal diet containing 25 g alfalfa flowers per 100 g diet.
- **Subgroup (4):** fed on a basal diet containing 25 g dandelion leaves per 100 g diet.
- **Subgroup (5):** fed on a basal diet containing 25 g dandelion flowers per 100 g diet.
- **Subgroup (6):** fed on a basal diet containing 25 g of mixed leaves of Alfalfa and Dandelion per 100 g diet.
- **Subgroup (7):** fed on a basal diet containing 25 g of mixed flowers of Alfalfa and Dandelion per 100 g diet.

**Composition of food products**

Pan bread prepared according to (A.O.A.C, 2002) .

**Table (1):** Ingredients of food products

<b>Pan bread</b>	
<b>Standard</b>	Made from wheat flour (200 g) water (110 g), dry yeast (5 g), sugar (10 g), skim milk powder (4 g), and com oil (10 g).
<b>Dried alfalfa leaves</b>	Made from adding 50 g from dried alfalfa leaves per (200 g) of wheat flours , water (110 g), dry yeast (5 g), sugar (10 g), skim milk powder (4 g), and com oil (10 g)
<b>Dried alfalfa flowers</b>	Made from adding 50 g from dried alfalfa flowers per (200 g) of wheat flours , water (110 g), dry yeast (5 g), sugar (10 g), skim milk powder (4 g), and com oil (10 g)
<b>Dried dandelion leaves</b>	Made from adding 50 g from dried dandelion leaves per (200 g) of wheat flours , water (110 g), dry yeast (5 g), sugar (10 g), skim milk powder (4 g), and com oil (10 g)
<b>Dried dandelion flowers</b>	Made from adding 50 g from dried dandelion flowers per (200 g) of wheat flours , water (110 g), dry yeast (5 g), sugar (10 g), skim milk powder (4 g), and com oil (10 g)
<b>Dried alfalfa and dandelion leaves</b>	Made from adding 50 g from Dried alfalfa and dandelion leaves per (200 g) of wheat flours , water (110 g), dry yeast (5 g), sugar (10 g), skim milk powder (4 g), and com oil (10 g)
<b>Dried alfalfa and dandelion flowers</b>	Made from adding 50 g from Dried alfalfa and dandelion flowers per (200 g) of wheat flours , water (110 g), dry yeast (5 g), sugar (10 g), skim milk powder (4 g), and com oil (10 g)

**Biological Analysis of blood serum:**

At the end of experiment period(28 days), the rats were fasted overnight then the rats were anaesthetized and sacrificed, and blood samples were collected from the aorta. The blood samples were centrifuged for 10 minutes at 3000 rpm to separate the serum. The serum was carefully separated into dry clean Wasserman tubes by using a pasteur pipette and kept frozen until analysis at 20<sup>°</sup>c , Also ,Liver and Kidney were removed

from each rat, cleaned from adhesive matter and weighed then stored in formalin solution 10% according to method mentioned by according to the method described by (**Drury and Wallington, 1980**).

Determination of aspartate amine transaminase and alanine amine transaminases activities were based on the method described by (**Reitman and Frankel, 1957 and Bergmeyer and Graßl, 1983**).

Determination of lipid profile: total cholesterol was determined according to the method described by (**Allain et al., 1974**). Triglyceride were determined according to the method described by (**Fossati and principe, 1982**). High density lipoprotein-cholesterol was determine according to the method described by (**Burstein, 1970**). Low Density Lipoprotein-Cholesterol was determined according to the method described by (**Friedwald et al., 1972**).

Determination of kidney functional : Urea nitrogen was determined according to the method described by (**Patton and Crouch, 1977**) . Creatinine was determined according to the method described by (**Bohmer, 1971**).

Determination of glucose was determined in the blood according to the method described by (**Trinder, 1969**).

Determination of antioxidant :Catalase was determined in the serum according to the method described by (**Aebi, 1974**). Superoxide dismutase determined in the serum according to the method described by(**Nishikimi et al., 1972**).). Glutathione were determined in the serum according to the method described by (**Beutler ,(1963)**)and Malondialdehyde was determined by (**Draper and Hadley, 1990**).

#### ***Sensory evaluation:***

The products supplemented with the alfalfa and dandelion and their combination were evaluated for color , odor , texture , taste, and overall acceptability by twenty people including (12) nutritionists stuff in the college of specific education , damietta university. The evaluation was carried out according to the method of (**A.A.C.C, 2002**).

### ***Histopathological Examination***

Dead animals were dissected and the abdominal cavity was exposed. The liver was irrigated several times by saline via a syringe introduced through thoracid to wash blood. The liver was dissected and put into 10% formalin solution and used for the preparation of 6  $\mu$ m thick paraffin embedded slices for histopathological examination according (sheehan and hrpachak,1980).

### ***Statistical analysis:***

The obtained data were statistically analyzed using SPSS. The results were expressed as mean  $\pm$  standard deviation “SD.” and tested for significance using one way analysis of variance “ANOVA” test to compare among groups of numerical (parametric) data followed by post-hoc tukey. P value  $\leq$  0.05 was considered statistically significant, according to (Armitage and Berry,1987).

### ***Results and Discussion***

Data in Tables( 2) showed that Protein, Carbohydrates, Fats, Ash, Calcium and Sodium in alfalfa leaves were higher than flowers with (29.6 , 29.4, 0.9 , 5.2 , 2.54 and 2.4) (g/100 g, based on dry weight) respectively. On the other side , Iron, Manganese and Zinc concentrations were higher in alfalfa flowers than alfalfa leaves with (15.2 , 5.2 , and 3.9) respectively. Also the results added that Protein, Fibers, Carbohydrates, Fats, Ash, Phosphorus and Potassium in dandelion leaves were higher than flowers with (24.4 , 37.4, 28.7 , 0.7 , 9.4, 0.67 and 5.41 ) (g/100 g, based on dry weight) respectively.

In this regard( **Butkutè et al ., 2016**) said that Chemical composition of alfalfa flowers (Ash , Calcium, Phosphorus ,Magnesium and Potassium) were (7.15,.71,.41,.31and 2.4) (g/100 g, based on dry weight) respectively. On the other hand ( **Apostol et al., 2017**) found that alfalfa leaves (carbohydrates, Ash , Calcium, Phosphorus ,Magnesium and Potassium) were (57.1, 9.67, 2.4, 2.1 ,0.54 and 3.1) (mg/100 g, based on dry weight) respectively. Also (**Al-Snafi ,2021**) mentioned that Iron, Zinc in

alfalfa flowers were (12.3,4.2) and in alfalfa leaves were (13.4,3.1) (g/100 g, based on dry weight) respectively .

**Table (2)** showed the different Units of Nutrient in dried alfalfa , dandelion :

Nutrient	Unit	Alfalfa		Dandelion	
		Leaves	Flowers	Leaves	Flowers
Moisture	g/100gm	0.07	0.05	0.10	0.9
protein	g/100gm	29.6	28.6	24.4	23.9
Fibers	g/100gm	35.4	34.8	37.4	35.9
Carbohydrates	g/100gm	29.4	28.8	28.7	28.4
Fats	g/100gm	0.9	0.5	0.7	0.4
Ash	g/100gm	5.2	4.6	9.4	8.5
Calcium	g/100gm	2.54	1.1	0.19	0.23
Phosphorus	g/100gm	0.39	0.41	0.67	0.04
Magnesium	g/100gm	0.48	0.42	0.0004	0.0005
Potassium	g/100gm	2.27	2.34	5.41	0.58
Sodium	mg/100gm	2.4	0.23	0.082	0.12
Copper	mg/100gm	1	1.2	0.7	0.5
Iron	mg/100gm	18	15.2	23	25.7
Manganese	mg/100gm	4.2	5.2	3.4	4.8
Zinc	mg/100gm	2.8	3.9	2.1	0.7
Vitamin A	IU/100gm	13268	15875	9161	10248
Vitamin E	mg/100mg	8.2	7.3	3.7	4.6
total antioxidant	mg/100mg	2430	3780	٢١٠٠	٣٤٠٠

Results in the table (3) revealed that treating groups of rats which were suffering from liver fibroses diseases lead to decrease significant in serum of (ALT +AST) (U/L) enzyme as compared to the positive control groups . Treating group of rats which suffering from liver fibroses diseases with (alfalfa and dandelion flowers 25% ) recording the best result in **ALT** because this group showed significant decrease as compared to other treated groups .

This results are in agreement with ( **Park et al., 2010** and **Fortea et al., 2018** ) showed that heap toprotective effects of dandelion against hepatotoxicity induced by several chemicals , This led to a significant decrease in alanine transaminase (**ALT**) after ingestion with CCl<sub>4</sub>.

**Table (3):** Effect of alfalfa leaves, alfalfa flowers , dandelion leaves, dandelion flowers and their combination on liver enzymes of rats suffering from liver fibrosis diseases .

Parameters	ALT	AST
Groups	(U/L)	(U/L)
(-)Control	21.61±2.01 <sup>f</sup>	29.96±3.91 <sup>f</sup>
(+)Control	89.78±3.90 <sup>a</sup>	85.58±4.78 <sup>a</sup>
Alf. leaves 25%	63.89±5.82 <sup>b</sup>	66.60±24.33 <sup>b</sup>
Alf. flowers 25%	40.40±2.69 <sup>d</sup>	54.88±3.52 <sup>bc</sup>
Dand. leaves 25%	58.00±4.38 <sup>b</sup>	62.84±3.92 <sup>c</sup>
Dand. flowers 25%	47.44±1.78 <sup>cd</sup>	50.82±4.8 <sup>b</sup>
Mix( Alf. + Dand. leaves)	49.84±3.75 <sup>c</sup>	55.57±2.53 <sup>d</sup>
Mix( Alf. + Dand.flowers)	32.50±1.13 <sup>e</sup>	38.99±3.2 <sup>e</sup>

**Alf.** (alfalfa) , **Dand.** (dandelion)

**Values are expressed as mean± SD ( standard deviation) .**

**P:Probability \*:significance <0.05**

Data in the table (4) showed that the mean value of serum cholesterol in all liver disease group which were treated were decrease Total serum cholesterol (mg/dl) and serum triglyceride significantly (  $p \leq 0.05$ ) as compared to positive control group . on the other hand treating group of rats which suffering from liver fibroses diseases with alfalfa flowers 25% and alfalfa and dandelion flowers 25% recording the best result in total serum cholesterol and serum triglyceride because this group showed significant decrease as compared to other treated groups .

The mean value of HDL-c in all liver treated group were increased significantly (  $p \leq 0.05$ ) as compared to positive control group . treating group of rats which suffering from liver fibroses diseases with ( alfalfa and dandelion leaves 25%) and (alfalfa and dandelion flowers 25% ) recording the best result in HDL-c because this group showed significant increase as compared to other treated groups. On the other side ,The mean value of LDL-C increased significantly (  $p \leq 0.05$ ) in the positive control group

(groups of rats suffering from liver fibrosis diseases as compared to the negative control group (healthy group).

This results are in agreement with (Farsani *et al* .,2016) who said that, Alfalfa leaves and flower led to a significantly decreased in total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), triglycerides (TG), very low-density lipoprotein (VLDL), glucose

In this regard ( Hamza *et al* ., 2020) evaluated the effects of whole plant powder of dandelion (*Taraxacum officinale*) on liver fibrosis. and the Results showed improve in the liver histology as evidenced by histopathological scoring with hematoxylin-eosin staining. In this respect (Al-Dosari , 2012 and Mahboubi and Mahboubi , 2020) reported that Pre-treatment with alfalfa for three weeks prior to administration of CCl<sub>4</sub> reduced levels of LDL, VLDL and reduced oxidative stress.

**Table (4 ):** Effect of alfalfa leaves, alfalfa flowers , dandelion leaves, dandelion flowers and their combination on lipid profile of rats suffering from liver fibrosis diseases .

Parameters	cholesterol	Triglyceride	HDL	LDL
Groups	(mg/dl)	(mg/dl)	(mg/dl)	(mg/dl)
(-)Control	59.40±4.91 <sup>f</sup>	42.00±4.67 <sup>f</sup>	48.8±2.88 <sup>a</sup>	20.20±1.33 <sup>g</sup>
(+)Control	142.6±9.22 <sup>a</sup>	114.00±6.30 <sup>a</sup>	20.7±1.25 <sup>e</sup>	84.80±9.42 <sup>a</sup>
Alf. leaves 25%	120.3±7.33 <sup>bc</sup>	97.00±4.70 <sup>b</sup>	30.5±1.63 <sup>d</sup>	54.4±6.89 <sup>b</sup>
Alf. flowers 25%	83.4±8.22 <sup>d</sup>	72.00±4.33 <sup>d</sup>	34.9±2.38 <sup>c</sup>	37.2±1.44 <sup>e</sup>
Dand. leaves 25%	109.8±8.11 <sup>cd</sup>	91.00±9.56 <sup>bc</sup>	33.6±1.75 <sup>d</sup>	48.20±2.33 <sup>c</sup>
Dand. flowers 25%	101.4±7.22 <sup>d</sup>	86.00±8.78 <sup>c</sup>	35.4±2.06 <sup>c</sup>	44.30±2.31 <sup>cd</sup>
Mix( Alf.+ Dand. leaves)	111.7±6.33 <sup>c</sup>	90.00±5.30 <sup>b</sup>	38.12±1.88 <sup>cd</sup>	51.30±6.2 <sup>b</sup>
Mix( Alf.+ Dand. flowers)	71.2±6.30 <sup>e</sup>	56.00±4.60 <sup>e</sup>	42.20±1.78 <sup>b</sup>	29.20±1.78 <sup>f</sup>

**Alf.** (alfalfa) , **Dand.** (dandelion)

Values are expressed as mean± SD ( standard deviation) .

P:Probability \*:significance <0.05

data in table (5) showed that injected rats with  $ccl_4$  led to significant increase in serum urea nitrogen and serum creatinine, as compared to non-injected rats.

Treating group of rats which suffering from liver fibroses diseases with (dandelion flowers 25% , alfalfa and dandelion flowers 25% ) recording the best result in urea nitrogen ( $46.90 \pm 3.47$  mg/dl ,  $44.65 \pm 2.20$  mg/dl ) respectively. on the other hand treating group of rats which suffering from liver fibroses diseases with (alfalfa and dandelion flowers 25%) recording the best result in serum Creatinine ( $0.45 \pm 0.033$  mg/dl ) because this group showed significant decrease as compared to other treated groups .

The mean value of serum glucose in Injected rats with  $ccl_4$  increased as compared to non-injected rats . The mean values were ( $110.05 \pm 10.32$  mg/dl ,  $87.03 \pm 9.25$  mg/dl ) respectively. On the other hand treating group of rats which suffering from liver fibroses diseases with (alfalfa and dandelion flowers 25%) recording the best result in serum glucose ( $92.76 \pm 8.40$  mg/dl ) because this group showed significant decrease as compared to other treated groups .

More recently, (**Ahsan et al., 2009 and Baliga et al ., 2013**) noted that taking the plant powder (flowers, leaves, and roots) orally dried (0.50 g / kg wt) for seven consecutive days protected rats from  $CCL_4$ -induced hepatotoxicity. When compared to the  $CCL_4$  group alone.

Moreover, (**Farsani et al ., 2016**) noted that Mice treated with alfalfa showed significantly lower levels of blood glucose. In this regard (**Mahboubi and Mahboubi ,2020**) added ,After taking the dandelion powder, blood glucose was monitored before and during the treatment periods. The results showed that dandelion leaf and root powder significantly reduced the blood glucose levels compared with  $CCL_4$  group .

In this respect (**Adewole et al., 2007 and Hismiogullari et al., 2015**) found that Injected rats with  $ccl_4$  to induced acute damage in the liver induced significant increase in serum uric acid, urea nitrogen and creatinine, as compared to the negative control group.

(Rana *et al.*, 2010) added, evidence has also found that alfalfa contains phenols and flavonoids, has antioxidant activity, and has an effective role in lowering creatinine levels. In this regard, dandelion is frequently used to preventor treat various liver diseases due to its formula rich in beneficial phytochemicals with a pronounced effect against cirrhosis (Martinez *et al.* , 2015 and Pfingstgraf *et al.* , 2021).

**Table ( 5 ) :**Effect of alfalfa leaves, alfalfa flowers , dandelion leaves ,dandelion flowers and their combination on kidney functions and glucose of rats suffering from liver fibrosis diseases

	Urea(mg/dl)	Creatinine(mg/dl)	Glucose(mg/dl)
(-)Control	42.25±2.32 <sup>d</sup>	0.40±0.02 <sup>e</sup>	87.03±9.25 <sup>d</sup>
(+)Control	71.38±4.59 <sup>a</sup>	0.81±.035 <sup>a</sup>	110.05±10.32 <sup>a</sup>
Alf. leaves 25%	61.76±3.93 <sup>b</sup>	0.64±.052 <sup>b</sup>	95.35±6.15 <sup>bc</sup>
Alf. flowers 25%	58.86±3.07 <sup>b</sup>	0.58±0.03 <sup>cd</sup>	94.84±8.03 <sup>bc</sup>
Dand. leaves 25%	52.61±2.81 <sup>bc</sup>	0.55±.046 <sup>c</sup>	97.43±6.36 <sup>b</sup>
Dand. flowers 25%	46.90±3.47 <sup>c</sup>	0.52±.046 <sup>d</sup>	94.92±6.52 <sup>c</sup>
Mix(Alf. + Dand. leaves)	47.14±2.82 <sup>c</sup>	0.50±0.06 <sup>d</sup>	93.43±5.57 <sup>bc</sup>
Mix(Alf. + Dand. flowers)	44.65±2.20 <sup>c</sup>	0.45±0.033 <sup>f</sup>	92.76±8.40 <sup>c</sup>

Alf. (alfalfa) , Dand. (dandelion)

Data expressed as mean ±SD,

Different letters indicate significance in means (significance ≤0.05)

Data presented in table (6) showed The mean values of GSH , SOD, catalase and MDA in  $CCL_4$  groups it was decreased significantly ( $p < 0.05$ ) (2.28±.22 NM/ml , 13.98 and 492.51 NM/ml/min ) respectively , as compared to the healthy group with ( 5.41±.43 NM/ml , 34.10±1.52 and 2463.49±212.88 NM/ml/min ) respectively . On the other hand, all tested groups showed significant increase ( $p < 0.05$ ) in these antioxidant enzymes, as compared to the positive control group. Among the alfalfa flowers 25% and (alfalfa and dandelion flowers 25% ) showed the best effect on activity of glutathione , super oxide dismutase and catalase . On the other side the

mean values of malondialdehyde in CCl<sub>4</sub> group increased significantly ( $p < 0.05$ ) ( $7.05 \pm 2.24$  NM/mL), as compared to the healthy group with ( $4.00 \pm 1.15$  NM/ml). On the other hand, all tested groups showed significant decrease ( $p < 0.05$ ) in MDA, as compared to the positive control group. Among the (alfalfa and dandelion flowers 25%) showed the best effect on MDA, because this group showed significant decrease as compared to other treated groups.

Coincide with that of (Chen *et al.*, 2020) showed that alfalfa increased total antioxidant capacity (T-AOC) and glutathione peroxidase (GSH-PX) level of three treated groups against the normal control group (NC) fed with basal diet.

Also, (Dal Bosco *et al.*, 2015) added, Alfalfa flavonoids have been usually used as an additive added in animal feed to promote the antioxidant activity of serum and liver, meat quality, growth, and production performance. Regarding (Jing *et al.*, 2015) reported that the flavonoids derived from alfalfa had exhibited very strong antioxidant activity.

(Hamza *et al.*, 2020) evaluated the effects of whole plant powder of dandelion on liver fibrosis. and the results showed improve in the liver histology, dandelion also have a great effect on fibrosis and inflammation induced by CCl<sub>4</sub>. It is also frequently used in many nutritional products and supplements (Pfingstgraf *et al.*, 2021).

Also, (Chen *et al.*, 2020) confirmed that alfalfa extract decreased malondialdehyde (MDA) by: 18.27%. Compared with the NC. On the other hand (Hamza *et al.*, 2020) evaluated the effects of whole plant powder of dandelion (*Taraxacum officinale*) on liver fibrosis.

**Table ( 6 ):** Effect of alfalfa leaves, alfalfa flowers , dandelion leaves ,dandelion flowers and their combination on Antioxidant enzymes of rats suffering from liver fibrosis diseases .

	Serum GSH (NM/ml)	Serum SOD (inhibition % )	Serum Catalase (NM/ml/min)	Serum MDA (NM/ml)
(-)Control	5.41±.43 <sup>a</sup>	34.10±1.52 <sup>a</sup>	2463.49±212.88 <sup>a</sup>	4.00±.15 <sup>e</sup>
(+)Control	2.28±.22 <sup>d</sup>	13.98±1.00 <sup>d</sup>	492.51±19.57 <sup>f</sup>	7.05±.24 <sup>a</sup>
Alf. leaves 25%	4.026±.086 <sup>c</sup>	21.73±1.21 <sup>bc</sup>	1150.94±88.42 <sup>d</sup>	6.06±.31 <sup>bc</sup>
Alf. flowers 25%	4.76±.30 <sup>b</sup>	22.98±.16 <sup>b</sup>	1314.14±50.86 <sup>b</sup>	5.21±.12 <sup>c</sup>
Dand. leaves 25%	3.89±.27 <sup>d</sup>	19.34±.84 <sup>c</sup>	1066.65±83.75 <sup>e</sup>	6.1±.46 <sup>bc</sup>
Dand. flowers 25%	4.24±.09 <sup>c</sup>	21.43±1.57 <sup>bcd</sup>	1145.99±91.71 <sup>d</sup>	5.69±.20 <sup>cd</sup>
Mix(Alf.+ Dand. leaves)	4.215±.64 <sup>b</sup>	20.8±1.76 <sup>c</sup>	1140.30±22.76 <sup>d</sup>	5.66±.24 <sup>c</sup>
Mix(Alf.+ Dand. flowers)	4.8±.38 <sup>bc</sup>	22.9±1.8 <sup>b</sup>	1250.44±127.49 <sup>c</sup>	4.81±.23 <sup>d</sup>

**Alf.** (alfalfa ) , **Dand.** (dandelion )

**Data expressed as mean ±SD.**

**Different letters indicate significance in means (significance ≤0.05)**

Data in table (7) showed the sensory evaluation to roasted pan bread, results showed the highest score of color , odor, texture, taste and over all Acceptability was recorded for the roasted pan bead fortified with dandelion flowers 25%) .

**Table (7):** Sensory evaluation of pan bread fortified with different levels of alfalfa , dandelion and their combination.

Groups	Color	Odor	Texture	Taste	Over all acceptability	Total
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Standard	19.50 <sup>a</sup> ± 0.69	19.35 <sup>a</sup> ± 0.88	18.95 <sup>a</sup> ± 1.15	18.89 <sup>a</sup> ± 0.95	19.55 <sup>a</sup> ± 0.60	96.24 <sup>a</sup> ± 3.23
Alf. Leaves 25%	18.38 <sup>bd</sup> ± 1.06	17.74 <sup>d</sup> ± 1.99	18.34 <sup>ab</sup> ± 0.87	18.08 <sup>bc</sup> ± 1.17	18.48 <sup>b</sup> ± 0.91	91.01 <sup>b</sup> ± 3.58
Alf. Flowers 25%	18.44 <sup>bd</sup> ± 0.87	17.98 <sup>bcd</sup> ± 1.59	18.40 <sup>ab</sup> ± 0.94	18.31 <sup>abc</sup> ± 1.26	18.73 <sup>bc</sup> ± 1.14	91.85 <sup>bc</sup> ± 4.11
Dand. Leaves 25%	18.20 <sup>bcd</sup> ± 0.71	18.10 <sup>bcd</sup> ± 1.41	18.08 <sup>b</sup> ± 0.98	18.14 <sup>bc</sup> ± 1.12	19.00 <sup>abc</sup> ± 0.56	91.52 <sup>bc</sup> ± 3.31
Dand. Flowers 25%	18.57 <sup>d</sup> ± 1.19	18.80 <sup>c</sup> ± 0.85	18.40 <sup>ab</sup> ± 1.06	18.71 <sup>ac</sup> ± 1.17	19.13 <sup>ac</sup> ± 0.76	93.60 <sup>c</sup> ± 3.65
Mix (Alf. +Dand. Leaves)	17.70 <sup>bc</sup> ± 1.08	18.13 <sup>bcd</sup> ± 1.07	18.20 <sup>b</sup> ± 1.01	18.19 <sup>abc</sup> ± 0.94	18.60 <sup>bc</sup> ± 0.94	90.82 <sup>bc</sup> ± 3.35
Mix (Alf. +Dand. Flowers)	17.85 <sup>b</sup> ± 1.09	17.93 <sup>b</sup> ± 1.08	17.80 <sup>b</sup> ± 1.11	17.95 <sup>b</sup> ± 1.42	18.50 <sup>b</sup> ± 1.36	90.03 <sup>b</sup> ± 4.95
<b>F</b>	<b>7.25</b>	<b>3.76</b>	<b>2.41</b>	<b>1.79</b>	<b>3.55</b>	<b>6.27</b>
<b>Sig.</b>	<b>0.001</b>	<b>0.002</b>	<b>0.03</b>	<b>0.105</b>	<b>0.003</b>	<b>0.001</b>

***Histopathological examination of liver:***

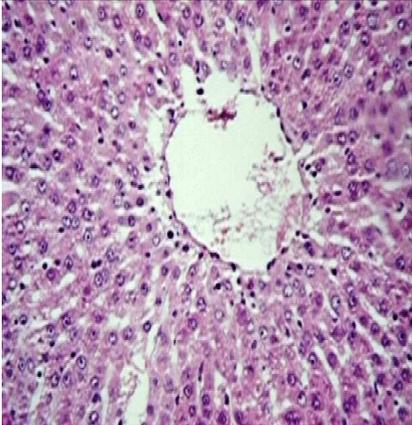
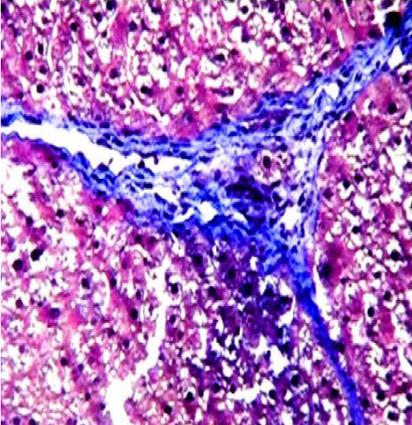
Microscopically, a section of a normal rat liver (H&E x200) showed the normal histological structure of the hepatic lobule (photo. 1). In contrast, the liver section of positive control rats (CCl<sub>4</sub> model of cirrhosis) showed a foamy vacuole of hepatocytes located primarily around the center and extending as a central septa to encircle the portal regions of photo. 2.. While the herbal diet groups (photo. 3, 4, 5, 6) show similar characteristics to those of the CCL<sub>4</sub> positive control groups photo. 2 with little improvement observed. A greater improvement was observed in group 7 and 8 (photo 7, 8) presented as slight portal vein congestion, mild activation of kupfer cells and partial restoration of hepatic function characteristics.

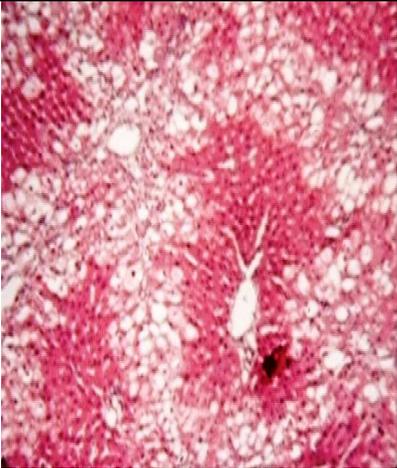
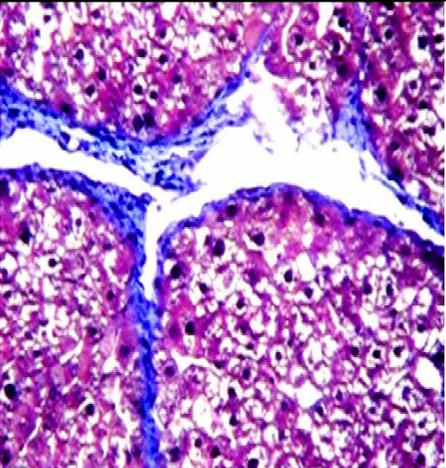
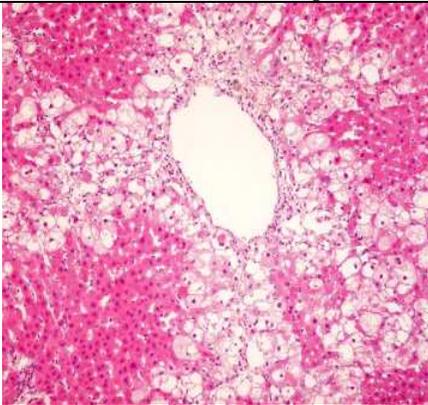
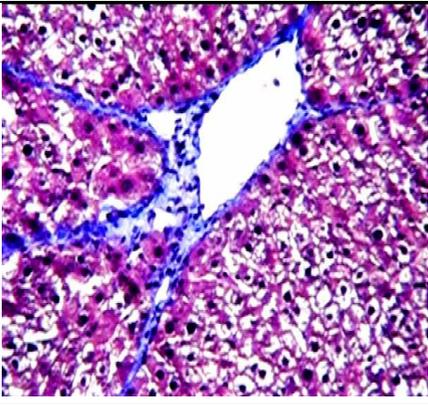
In this respect, (Li *et al.*, 2015) indicated that CCL<sub>4</sub> causes acute hepatotoxicity and also causes oxidative injury, and that the liver protective effects of (alfalfa and dandelion) may be due to inhibition of lipid peroxidation and increased antioxidant activity.

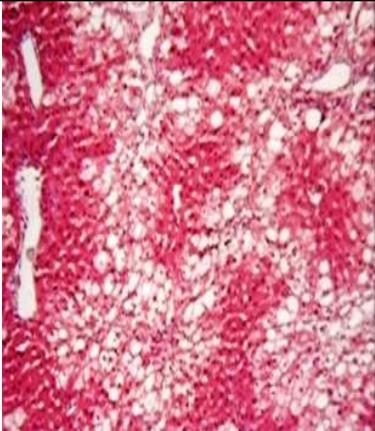
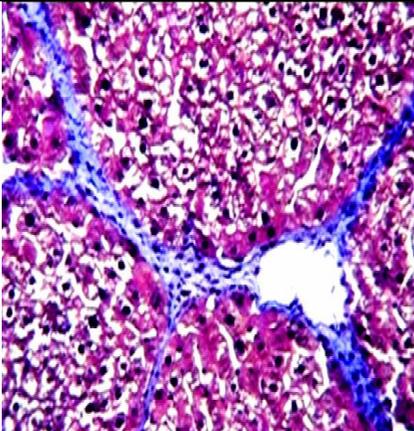
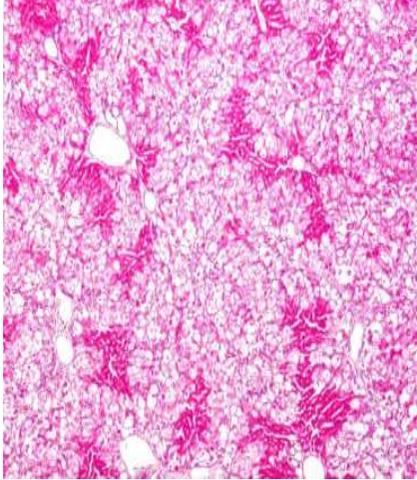
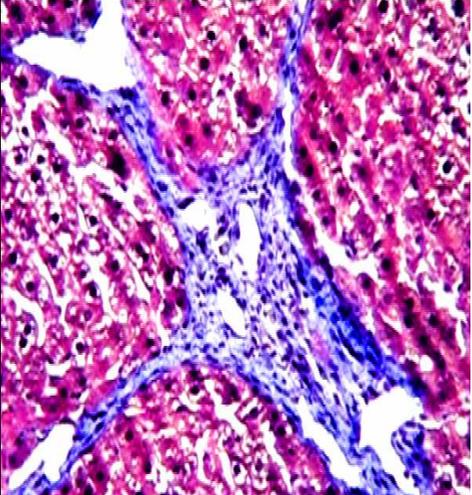
Furthermore ,(Al-Dosari ,2012) reported that the histopathological examination of the livers also showed that the alfalfa extract reduced the incidence of liver lesions induced by CCl<sub>4</sub>. The *in vitro* antioxidant

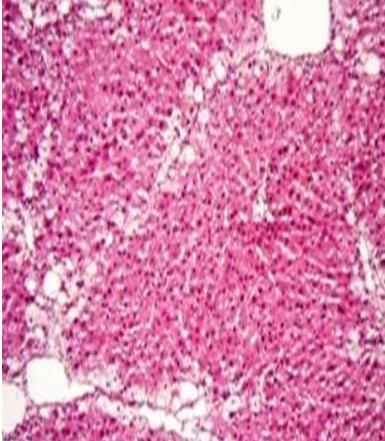
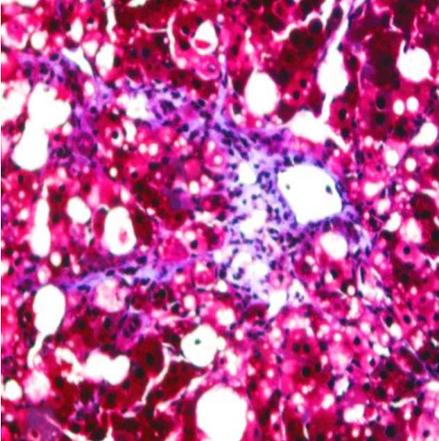
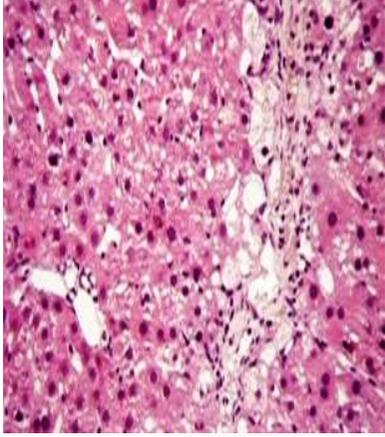
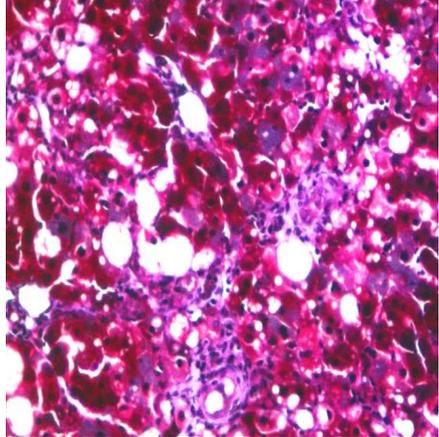
assessment of alfalfa extract on DPPH and carotene-linoleic assays demonstrated a moderate antioxidant potential.

According to (Hamza *et al.*, 2020) declared that dandelion inhibited the development of  $CCL_4$ -induced hepatic fibrosis. Dandelion's anti-fibrosis effects can be attributed to its ability to search for free radicals and reduce inflammatory cell activation. Tri-color partitions were made by H&E.  $CCL_4$  caused acute liver damage.

	H&E	MTC
photo. 1: (control normal group)		
	Section of a liver of a normal rat (H&E x200) showing no pathological changes.	Section of a liver of a normal rat (MTC x200) showing no positive stain for fibrosis.
photo. 2: (positive control group)		
	Foamy vacuolization of liver cells found mainly pericentral and extend as septa central central to encircle the portal areas	High area of positively stained fibrotic areas.

<p>photo. 3: Alfalfa leaves 25%.</p>		
	<p>Foamy vacuolization of liver cells found mainly pericentral and extend as septa central central to encircle the portal areas</p>	<p>Hight area of positively stained fibrotic areas</p>
<p>photo. 4: Alfalfa flowers 25%.</p>		
	<p>Foamy vacuolization of liver cells found mainly pericentral and extend as septa central central to encircle the portal areas</p>	<p>Hight area of positively stained fibrotic areas</p>

<p>photo. 5: Dandelion leaves 25%</p>		
	<p>Foamy vacuolization of liver cells found mainly pericentral and extend as septa central central to encircle the portal areas</p>	<p>Hight area of positively stained fibrotic areas</p>
<p>photo. 6: Dandelion flowers 25%</p>		
	<p>Foamy vacuolization of liver cells found mainly pericentral and extend as septa central central to encircle the portal areas</p>	<p>Hight area of positively stained fibrotic areas</p>

<p>photo. 7: Alfalfa and dandelion leaves 25%</p>		
	<p>Foamy vacuolization of liver cells found mainly pericentral and extend as septa central central to encircle the portal areas</p>	<p>Slight enhancement presented in reduction of fibrotic area.</p>
<p>photo. 8: Alfalfa and dandelion flowers 25%</p>		
	<p>Foamy vacuolization of liver cells found mainly pericentral and extend as septa central central to encircle the portal areas</p>	<p>Slight enhancement presented in reduction of fibrotic area.</p>

**Conclusion:**

The current study found that the intake of leaves and flower from alfalfa and dandelion effectively reduces liver enzymes, cholesterol, triglycerides, urea, creatinine and random sugar. Also, the flowers of alfalfa and dandelion have susceptibility, according to the sensory evaluation of all products, to histopathological studies: the results of the study reported that alfalfa and dandelion have good effects on the liver .

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## تأثير أوراق البرسيم والهندباء على تحسين وظائف الكبد ونخضير بعض المنتجات الغذائية

### الملخص

البرسيم والهندباء مصدر غني بمضادات الأكسدة الطبيعية. لذلك أجريت هذه الدراسة للتعرف على تأثير تناول أوراق وأزهار البرسيم والهندباء لتحسين وظائف الكبد في الفئران واستخدامهم في صناعة بعض المنتجات، مثل الخبز والشاي، وتم تقييم الخصائص الحسية لهذه المنتجات من خلال محكمين مدربين. تم دراسة التحليل الكيمائي والخصائص النسيجية المرضية باستخدام أربعة وستون فأر ذكر تم تقسيمهم عشوائياً إلى مجموعتين رئيسيتين، المجموعة الرئيسية الأولى: ٨ جردان تم تغذيتها على النظام الغذائي الأساسي كمجموعة ضابطة سالبة، والمجموعة الرئيسية الثانية: ٥٦ فأر تم تغذيتها على النظام الغذائي الأساسي و تم حقنها تحت الجلد بواسطة (CCL<sub>4</sub>) في زيت البارافين (٤ مل /كجم) للبحث على التليف في الكبد، تم تقسيم المجموعة الرئيسية الثانية الي سبع مجموعات فرعية. المجموعة الفرعية (١) تتغذي علي نظام غذائي أساسي كمجموعة ضابطة إيجابية، المجموعات الفرعية (٢:٧) تتغذي علي نظام غذائي أساسي يحتوي علي ٢٥ جم من أوراق البرسيم، ٢٥ جم من زهور البرسيم، ٢٥ جم من أوراق الهندباء، ٢٥ جم من زهور الهندباء، ٢٥ جم من أوراق مختلطة من البرسيم والهندباء و ٢٥ جم من زهور مختلطة من البرسيم والهندباء علي التوالي. أظهرت النتائج التي تم الحصول عليها أن التركيب الكيمائي للبرسيم والهندباء يحتوي علي نسبة عالية من البروتين واللايف و مضادات الأكسدة وأظهرت النتائج التي تم الحصول عليها انخفاضاً معنوياً في نسب إنزيمات الكبد (ALT -AST) - الكوليسترول الكلي - الدهون الثلاثية - البروتينات منخفضة الكثافة - البيوريا - الكرياتينين - سكر الدم العشوائي والمالونداهايد (MDA) في المجموعات المعالجة. وتم أيضاً زيادة معنوية في نسب البروتين الدهني عالي الكثافة - الجلوتاثيون (GSH) - ديسموتاز فوق أكسيد (SOD) والكاتاليز (CAT) في المجموعات المعالجة. وعموماً أظهرت نتائج التقييم الحسي ان الخبز والشاي المدعم بأوراق وزهور البرسيم والهندباء كانت مقبولة. وتوصي الدراسة باستخدام مستخلصات البرسيم والهندباء كمضافات غذائية لفوائدها الغذائية والصحية.

الكلمات المفتاحية : أوراق و أزهار البرسيم -أوراق و أزهار الهندباء - إنزيمات الكبد- وظائف الكلي- مضادات الأكسدة الأنزيمية .