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Carbon tetrachloride – induce hepatotoxicity in rats: The potential effect of bread supplemented with dandelion (*Taraxacum officinale*) on the nutritional status

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Our work interest has focused on the effect of supplementation of rat diets with leaves and roots of dandelion (*Taraxacum officinale*), on biochemical and histological parameters of rats with hepatotoxicity. Two main experimental rat groups were used in this experiment, in addition to, two-groups fed on basal diet or basal diet containing (200g unsupplemented bread), as negative control (NC) groups The first and second main groups were divided into five subgroups (6 rats each). Subgroups (1) in the first and second main groups were fed on basal diet and basal diet containing 200g unsupplemented bread respectively, as positive control (PC) groups, subgroups (2 & 3) in the first and second main groups were fed on basal diet containing 1% & 2% leaves and basal diet containing 200 g bread supplemented with (5 and 10 g leaves/ 100 g bread) respectively, subgroups (4 & 5) in the first and second main groups were fed on basal diet containing 1% & 2% roots and basal diet containing 200g bread supplemented with (5 and 10 g roots/ 100g bread) respectively. On the day of 27th of the experiment, all animals except the negative control groups were administered with 20% CCl_4 in liquid paraffin oil at a dose 5 ml/kg body weight to induce hepatotoxicity. During the experimental period (35 days), the diets consumed and body weights were recorded twice weekly. Injected rats with CCl_4 showed defectiveness in all parameters. Rats with hepatotoxicity were fed on basal diet containing the two levels from leaves or roots of dandelion (1% & 2%), or basal diet containing supplemented bread with the same levels from them led to decrease in serum (cholesterol, triacylglycerol, low & very low density lipoprotein, uric acid, urea nitrogen, creatinine, bilirubin and liver functions) significantly, while high density lipoprotein and total iron increased significantly ($p< 0.05$), as compared to the PC group. These data indicate that, groups of rats treated with supplemented diets or supplemented bread with high level from leaves or roots of dandelion offered protection against CCl_4-induced hepatotoxicity.

Key words: hepatotoxicity, dandelion, kidney and liver functions

INTRODUCTION

The liver is one of the most important organs in the biotransformation of food, drugs, endogenous and exogenous substances. Profuse supply of blood and the presence of many redox systems enable liver to convert these substances into different kinds of inactive and active metabolites (Wang et al. 2007). Providentially, the liver has a remarkable ability to restore and heal itself. Most important to liver health is a healthy diet and minimal exposure to toxic substances. Affirmed that toxins can also be naturally-occurring, resulting from normal digestion and metabolism. Supplementation with particular herbs and nutrients would also help protect and support healthy liver function and stimulate internal detoxification (Murray and Pizzorno 1999).

Taraxacum officinale (TO), commonly known as dandelion, is a nutritive herb rich in bitter principles -taraxacin and taraxacerin- throughout the roots, leaves and flowers which improve fat digestion, including the secretion of salivary and gastric juices. TO is one of the best known European medicinal plants which have been used for the treatment of various inflammatory diseases. It is used for loss of appetite, dyspepsia, flatulence, gallstones, bile stimulation, laxative, diuretic, circulatory tonic, skin toner, blood tonic, digestive tonic and as anti-viral and bacterial infections (James 1997).

Dandelion has culinary and medicinal and nutritional value which is rich source of vitamins and minerals. The leaves have the highest amount of pro-vitamin A of all greens (14,000 IU/100g raw greens) and contain ample amounts of vitamins D, B complex, and C. Dandelion is also very good source of fibre, potassium (297 mg/100mg leaf), iron, calcium, magnesium, phosphorus, zinc, manganese, copper, and sodium, besides that, contains high levels of choline, an important hepatic nutrient (Murray 1995). Dandelion roots and leaves were believed to be quite safe, with no side effects or likely risks other than rare allergic reactions. Dandelion is on the FDA's (Food Drug Administration's) GRAS (generally recognized as safe list) (Newall et al. 1996)

Dandelion contain flavonoids such as quercetin, luteolin, luteolin-7-O-gluccoside, p-hydroxyphenylacetic acid, germacranolide acids, chlorogenic acid, chicoric acid, monocaffeyltartaric acid, scopoletin, aesculetin, aesculin, cichoriin, arnidiol, faradiol, caffeic acid, taraxacoside,

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taraxasterol, and large amounts of the polysaccharide, inulin, as well as a high potassium content (Hu and Kitts 2004; Trojanova et al. 2004; Seo et al. 2005). Besides to terpene/sterol compounds include beta-amyrin, taraxasterol, and taraxerol, as well as free sterols (sitosterin, stigmasterin, and phytosterin) structurally related to bile (Duke 1989) and (Leung and Foster 1996). One of these, taraxasterol has been documented to exhibit anti-carcinogenic activity (Takasaki et al. 1999) and an anti-tumor action of the aqueous extract of TO has been reported (Baba et al. 1981).

In addition to that, dandelion was shown to have a positive effect on blood cholesterol (Murray 1996) and used to successfully treat hepatitis, liver disease, jaundice, gall bladder disease, constipation, and arthritis, prevent waste accumulation in the liver (Salmond 1997). Dandelion constituent inulin may also prove to be useful in the treatment of diabetes (Swanston-Flatt et al. 1989), it has experimental weight-loss activity due to its diuretic actions; its leaves are a rich source of potassium, this may make it the only naturally occurring potassium-sparing diuretic, although its diuretic action is likely to be different from pharmaceutical drugs (Murray 1995).

For the first time we investigated the effects of dietary non-supplemented and supplemented bread with 1% and 2% (leaves and roots) of dandelion on liver function, lipid fraction, kidney function and iron status of rats with hepatotoxicity.

MATERIALS AND METHODS

Materials

Carbon tetrachloride (CCl_4), casein, vitamins, minerals, cellulose, choline chloride were purchased from El-Gomhoreya Company, Cairo, Egypt. White flour (80% extract), yeast, salt and sugar were purchased from local market, Cairo, Egypt. Dandelion (*Taraxacum officinale)* was obtained from local market (Cairo – Egypt), leaves of dandelion and roots separated into leaves and roots and dried.

Rats

Rats were obtained from the laboratory animal colony, Ministry of Health and Population, Helwan, Cairo, Egypt.

Methods

Preparation of bread

Normal bread consists of wheat flour (90gm), yeasts (5gm), salt (2.5gm), sugar (2.5gm).

Supplementation of bread

In this study, white flour supplemented with (5, 7.5, 10 and 20 g leaves/100 g also 5, 7.5, 10 and 20 g roots/100 g) and used in the preparation of bread.

Table 1: Sensory evaluation of unsupplemented and supplemented bread with leaves and roots of dandelion (20 marks scale)

Samples \ Characteristics	Sensory characteristics (20 marks)				
	Colour	Flavour	Taste	Texture	General Acceptance
USB (100 g WF)	19.4 ± 0.5[a]	19.2 ±0.8[a]	18.8 ±0.8[a]	18.2 ±1.3[a]	19.2 ±0.8[a]
SB (5 g L D+ 95 g WF)	17.4 ±1.1[ace]	17.0±1.6[adg]	17.6 ±1.5[ac]	17.2 ±0.8[ab]	17.8 ±1.6[ab]
SB (7.5 g LD + 92.5 g WF)	15.4 ±3.2 [bcd]	15.6 ±3.4[bcdef]	16.2 ±2.4[bcf]	15.4±3.2 [bcde]	16.4 ±2.6[bc]
SB (10 g LD + 90 g WF)	14.8 ±3.1[bde]	15.2 ±2.9[ceg]	15.0 ±1.8[de f g]	16.0 ±1.6[ad]	15.8 ±1.6[bcd]
SB (20 g LD + 80 g WF)	11.4 ±4.5[f]	11.6 ±4.8[h]	10.8 ±4.3[h]	13.0 ±2.5[g]	12.0±3.3[e]
SB (5 g RD + 95 g WF)	17.6 ±1.1[ab]	18.6 ±0.5[ab]	18.2 ±0.4[ab]	16.6 ±0.5[ac]	17.6 ±0.5[ac]
SB (7.5 g RD + 92.5 g WF).	16.4 ±1.1[bcd]	17.2 ±0.9[ac]	16.8 ±0.8[acdg]	16.0 ±0.5[ae]	16.6 ±1.5[bc]
SB (10 g RD + 90 g WF)	17.0 ± 0.7[ad]	16.6 ±0.8[ae]	16.4 ±1.1[ace]	16.0 ±1.2[af]	16.0 ±0.7[bc]
S B (20 g RD + 80 g WF)	12.4 ±1.1[ef]	12.6 ±1.3 [fg h]	12.2 ±1.5[h]	12.0 ±1.4[g]	13.6 ±1.1[de]

U S B =unsupplemented bread, SB= supplemented bread, LD= leaves of dandelion, RD =root of dandelion, WF= white flour); LSD: Least significant differences (P<0.05 Mean scores in each column with same letters are not significantly different.

Table 2: Chemical composition of non-supplemented and supplemented bread with leaves and roots of dandelion

Sample \ Nutrients	g / 100g					
	Moisture	Protein	Fat	Ash	Fibre	Carbohydrates
Bread	30.025	8.323	1.791	0.724	0.583	58.554
BS with 5g L/100 g WF	30.018	8.633	1.784	0.731	0.591	58.243
BS with 10g L/100 g WF	30.020	8.640	1.780	0.739	0.596	58.225
BS with 5g R/100 g WF	30.015	8.639	1.788	0.751	0.595	58.212
BS with 10g R/100 g WF	29.992	8.711	1.783	0.748	0.603	58.163

BS= Bread Supplemented, WF =Wheat Flour, L =Leaves, R= Roots; The value of each parameter is the mean of two determinations.

Sensory evaluation

Ten panelists from the staff of the Faculty of Home Economics (Helwan University) were (invited) for sensory evaluation bread. Each panelist was asked to evaluate unfortified and fortified bread samples with dandelion leaves and roots, according to colour, odour, taste, texture and general appearance (Abd El-Latif 1990).

Chemical analysis

Moisture content, total protein, fat, fibre and ash were determined in bread samples according to the methods outlined in A.O.A.C (1990). Carbohydrates content were calculated by deduction from other estimated nutrients.

Experimental design

Seventy-two male albino rats (200 - 210g) were kept in individual stainless steel cages under hygienic conditions and fed, for one week on basal diet for adaptation in the animal house of the Faculty of Home Economics, Helwan University. The basal diet in the preliminary experiment consists of 20% casein (protein > 80%), corn oil 4%, cellulose 5%, vitamin mixture 1%, salt mixture 3.5%, choline chloride 0.2% and the remainder is corn starch (Reeves et al. 1993). Vitamin mix and minerals mix prepared according to (A.O.A.C. 1975).

After the period of adaptation on basal diet; the rats were divided into first and second main groups (n=60) were subsequently divided into five subgroups (6 rats each). Subgroups (1) in the first and second main groups were fed on basal diet and basal diet containing 200g un-supplemented bread, as negative (NC) and positive (PC) control groups respectively. Subgroups (2 & 3) in the first and second main groups were fed on basal diet containing 1% & 2% leaves and basal diet containing 200 g bread supplemented with (5 and 10 g leaves/ 100 g bread) respectively. Subgroups (4 & 5) in the first and second main groups were fed on basal diets containing 1% & 2% roots and basal diet containing 200g bread supplemented with (5 and 10 g roots/100g bread) respectively. On the 27th day of the experiment, all animal except the NC group were administered 20% CCl_4 in liquid paraffin oil at a dose 5 ml/kg body weight to induce hepatotoxicity (Haimin et al. 2006). During the experimental period (35 days), the diets consumed and body weights were recorded twice weekly.

Biochemical analysis of serum

At the end of the experiment, the animals were fasted overnight, then the rats were anaesthetized and sacrificed, and blood samples were collected from the aorta. Blood samples were centrifuged and the serum was separated to estimate some biochemical parameters, i.e. serum bilirubin by (Grohmann et al. 2006), aspartate amino transferase (AST) and alanine amino transferase (ALT, Ritman and Frankel 1957), uric acid (Fossati et al. 1980), urea nitrogen (Patton and Crouch 1977), creatinine (Bohmer 1971), iron (Ramsy 1957), cholesterol (Allain et al. 1974), triacylglycerol by (Fossati and Principe 1982), HDL-C (Burstein, 1970), LDL-C and VLDL-C were

Table 3: Effect of leaves and roots of dandelion on food intake, body weight gain (%) and organs weight/ body weight (%) of rats with hepatotoxicity.

Treatments / Groups	Food intake g/day	BWG %	Organs weight/body weight (%)		
			Kidney	Heart	Liver
NC	14.7 ± 1.4^{a}	42.8 ± 1.6^{a}	0.63 ± 0.03^{d}	0.29 ± 0.02^{a}	2.81 ± 0.13^{e}
PC	13.2 ±1.3$^{a b}$	21.4 ± 2.5^{c}	0.82 ± 0.03^{a}	0.32 ± 0.01^{a}	4.15 ± 0.09^{a}
1% Leaves	12.7 ± 1.8^{b}	18.1 ± 4.6^{c}	0.68 ± 0.03^{bc}	0.31 ± 0.04^{a}	3.77 ± 0.25^{b}
2% Leaves	12.2 ± 1.2^{b}	13.4 ± 2.8^{b}	0.68 ± 0.02^{b}	0.29 ± 0.04^{a}	3.48 ± 0.24^{c}
1% Roots	12.6 ±1.5^{b}	18.7 ± 3.5^{c}	0.65 ± 0.04^{cd}	0.29 ± 0.02^{a}	3.17 ± 0.25^{d}
2% Roots	12.2 ±1.2^{b}	21.2 ± 2.2^{c}	0.64 ± 0.03^{d}	0.29 ± 0.03^{a}	3.38 ± 0.40^{cd}

NC=Negative control fed on basal diet only; PC=Positive control fed on basal diet and suffering from hepatotoxicity on the 27th day
Values are expressed as mean ± SD., Significance at $p<0.05$, BWG%: Body weight gain %.
Values which don't share the same letter in each column are significantly different.

Table 4: Effect of supplemented bread with leaves and roots of dandelion on food intake, body weight gain% and organs weight/body weight % of rats with hepatotoxicity.

Treatments / Groups	Food intake g/day	BWG %	Organs weight/body weight (%)		
			Kidney	Heart	Liver
NC	15.2±2.0^{a}	49.3 ±6.6^{a}	0.65 ±0.1^{b}	0.29±0.1^{b}	3.2±0.2^{c}
PC	14.2 ±1.1^{ab}	11.5 ±3.5^{cd}	0.75±0.2^{a}	0.34 ±0.02^{a}	3.7±0.2^{a}
BS + 1% Leaves	13.5 ±0.9^{ac}	16.8 ±5.4^{d}	0.68±0.1^{b}	0.28 ±0.1^{b}	3.6 ±0.4^{ab}
BS + 2% Leaves	13.1±2.1^{bc}	4.8 ±2.9^{b}	0.67 ±0.1^{b}	0.28 ±0.1^{b}	3.2 ±0.2^{c}
BS + 1% Roots	13.1 ±1.5^{bc}	6.5 ±5.4^{bc}	0.66 ±0.1^{b}	0.31 ±0.1^{b}	3.2 ±0.1^{c}
BS + 2% Roots	13.2 ±1.8^{bc}	4.9 ±4.7^{b}	0.65 ±0.1^{b}	0.30 ±0.1^{b}	3.4 ±0.3^{bc}

NC= Negative control fed on basal diet containing un-supplemented bread, PC= Positive control fed on basal diet containing unsupplemented bread and suffering from hepatotoxicity on the day of 27th
Values are expressed as mean ± SD., Significance at $p<0.05$., BWG %Body weight gain %.
Values which don't share the same letter in each column are significantly different

calculated by the modified formula of Friedewald et al.1972 (Francisco et al. 2008).

Histological examination

Kidneys, heart and liver were removed from each rat, careful dissection, washed with saline solution, dried with filter paper and weighed according to the method described by Drury and Wallington (1980). The liver in each group was examined histopathologically, according to Sheehan and Hrapchak (1980).

Biostatistics

The data obtained was analyzed statistically for standard deviation and one-way ANOVA test (Steel and Torri 1980).

RESULTS

Data in Table (1) showed that, bread supplemented with 5% leaves of dandelion resulted in non-significant changes in all sensory characteristics, as compared to unsupplemented bread (control). While, supplemented bread with 7.5, 10 and 20% leaves of dandelion decreased all sensory characteristics, as compared to un-supplemented bread. On the other hand, non-significant changes were observed between supplemented bread with (7.5% and 10% leaves) in all characteristics.

Results in the same table showed also, supplemented bread with 5, 7.5 and 10% roots of dandelion resulted in non-significant changes in all sensory characteristics, except, colour for 7.5% roots, as compared to unsupplemented bread (control). On the other hand, supplemented bread with 20% roots of dandelion induced significant decrease $P<0.05$ in all sensory characteristics, compared with unsupplemented bread (control).

Statistical analysis showed non-significant changes in general acceptability (GA) between all supplemented bread with the different levels from leaves of dandelion, except the sample, which supplemented with the high level (20g leaves + 80g WF). On the other side, GA of supplemented bread with 5g leaves recorded non-significant changes, as compared to the control sample, while GA of other supplemented samples (7.5, 10 and 20 g leaves of dandelion) decreased significantly, compared with the control. Also non-significant difference was observed in GA between the supplemented samples with 7.5 and 10 g root. From the above-maintained data, it could be concluded that, supplemented bread with 5g leaves and roots, recorded the best results, followed by 7.5g and 10g, while the levels of 20g leaves or roots resulted in unaccepted characteristics. Then we investigated the chemical composition and the biological effects of bread supplemented with 5 and 10g leaves and roots of dandelion.

Chemical composition of bread

In the present study, bread analysed for its content and illustrated in Table (2). The percent content of moisture, protein, fat, ash, fibre and carbohydrates of un-supplemented bread were 30.025, 8.323, 1.791, 0.724, 0.583 and 58.554, respectively. Supplemented bread with 5g and 10g dandelion leaves and roots led to slight increase in the content of protein, ash and fibre, whereas, little decrease in fat, and carbohydrates occurred than that of un-supplemented bread (control).

Food intake

Table (3 & 4) show the effect of supplemented basal diet and bread with leaves and roots of dandelion on the mean values of food intake, body weight gain% and organs weight/body weight% of rats with hepatotoxicity.

The results in Table 3 and 4 indicated to a significant difference in food intake in rat groups as compared to NC.

Body weight gain % (BWG %)

The mean values of BWG% decreased significantly ($p<0.05$) in the PC rat groups compared to NC groups (21.4±2.5 and 11.5±3.5 vs. 42.8 ±1.6 and 49.3±6.6). BWG % of all treated groups with (1% and 2% leaves or roots) recorded a significant decrease ($p<0.05$), compared to NC group. It can be noticed from Table (3&4) that rat groups which were fed on basal diet containing 2% leaves or supplemented bread with leaves decreased significantly.

Organs weight/body weight percentage

Table (3 and 4) showed that, weights of kidney and liver were changed by CCl_4 injection. Statistical analysis showed a significant increase in kidney and liver weight/body weight % for PC group, compared to NC. Addition of leaves and roots of dandelion to the basal diet at levels (1% and 2%), decreased kidney and liver weight/body weight% significantly at $p<0.05$ in the rats with hepatotoxicity, as compared to PC group. On the other hand, kidney weigh/body weight % of rats fed on basal diet containing the two levels of roots recorded non-significant difference, as compared to NC group.

Tables (5) and Fig. (1) show the effect of dandelion (leaves or roots) and bread supplemented with them on serum bilirubin (mg/dl), Aspartate Amino Transferase (AST) and Alanine Amino Transferase (ALT) (IU/l) of rats with hepatotoxicity.

The values of serum bilirubin, AST and ALT were changed by CCl_4 injection, results showed a significant increase in PC group compared to NC group. Rats with hepatotoxicity and with diets containing dandelion (1% and 2% leaves or roots) or bread that provide the diets with (1% and 2% leaves or roots) led to improving values of bilirubin, AST and ALT, especially with high levels 2%.

Values of serum bilirubin (mg/dl) decreased gradually with increasing the amount of leaves or roots. The ratios of decreasing bilirubin/ percent were about 16% & 20%, when treated rats with 2% leaves or root of dandelion respectively, compared with PC group (Table 5). Additionally, the mean values of AST and ALT decreased with increasing the levels of dandelion, particularly roots, AST and ALT decreased by about 15% and 29%, when rats fed on 2% roots of dandelion, respectively. The obtained results that are displayed in Fig. (1) revealed that, supplemented bread with 2% roots lowered the mean values of serum bilirubin, AST and ALT by about 18%, 13% and 29% respectively, than that of CCl_4 group (PC).

Findings in Tables (6 and 7) presented the effect of dandelion (leaves or roots) and supplemented bread with them on total cholesterol, triacylglycerol, high density lipoprotein (HDL-C), low and very low density lipoprotein (LDL-C and VLDL-C) of serum rats suffering from hepatotoxicity.

The mean values of serum cholesterol, triacylglycerol, LDL-C and VLDL-C (mg/dl) increased significantly at $p<0.05$, whereas, the mean value of serum HDL-C decreased significantly in PC group in comparison with NC group. Feeding rats with hepatotoxicity on diets containing 1% and 2% leaves and roots of dandelion or bread that provided the diets with the same ratios enhanced the values of serum lipids, particularly, with high level of ingredients (2%).

Table (6) revealed that, all CCl_4 groups which received basal diet containing 1% and 2% leaves or roots from dandelion showed a significant decrease in serum cholesterol, triacylglycerol, LDL-C and VLDL-C ($p<0.05$), while, the level of serum HDL-C increased in comparison with the PC group. The group of rats fed on basal diet containing 2% roots of dandelion recorded significant decrease $p<0.05$ in cholesterol, triacylglycerol, LDL-C and VLDL-C by about 18.9%, 16.3%, 38.7% 16.3%, respectively, while HDL-C increased by about 13.5% compared to PC group. On the other hand, supplemented bread which provided the diets with high concentration of leaves or roots (2%) induced significant decrease $p<0.05$ in the mean values of serum cholesterol, triacylglycerol, LDL-C and VLDL-C, compared to the rest of groups Table(7)..

Kidney function

Data presented in Table (8) uric acid, urea nitrogen and creatinine (mg/dl) for PC group showed a significant increase $p<0.05$, compared to NC group. All groups which were fed on basal diet containing leaves or roots of dandelion showed significant reduction in the mean values of uric acid and urea nitrogen at ($p<0.05$), except group of rats which treated with 1% leaves of dandelion, compared with PC group. On the other hand, the lowest mean value of serum uric acid concentration was (1.6 ± 0.06 mg/dl) obtained from feeding hepatotoxic rats with basal diet containing 2% roots, this treatment induced non-significant differences in uric acid, as compared to NC group and recorded the best results. Feeding rats with hepatotoxicity on basal diet containing 2% roots showed a significant decrease in creatinine by about (9.5%) compared to the rest of treated groups and PC.

The results in table (9) demonstrated that, addition of bread supplemented with two levels from dandelion leaves or roots (1% & 2%) achieved lower values of serum uric acid and

Table 5: Effect of leaves and roots of dandelion on liver functions of rats with hepatotoxicity

Treatments / Groups	mg/dl	IU/l	
	Bilirubin	AST	ALT
NC	30.8 ± 4.6^d	77.3 ± 4.1^e	23.6 ± 3.8^d
PC	85.2 ± 3.5^a	156.2 ± 7.3^a	63.4 ± 4.9^a
1% Leaves	77.8 ± 2.8^b	149.7 ± 4.4^b	60.5 ± 4.1^a
2% Leaves	71.5 ± 2.8^c	140.3 ± 4.1^c	53.1 ± 3.3^b
1% Roots	75.3 ± 2.0^b	143.1 ± 2.9^c	52.2 ± 4.6^b
2% Roots	68.5 ± 1.7^c	133.5 ± 3.9^d	45.3 ± 3.2^c

NC= negative control, PC= positive control, Values are expressed as mean ± SD, Significance at $p<0.05$; AST=Aspartate Amino Transferase; ALT=Alanine Amino Transferase; Values which don't share the same letter in each column are significantly different ($p<0.05$).

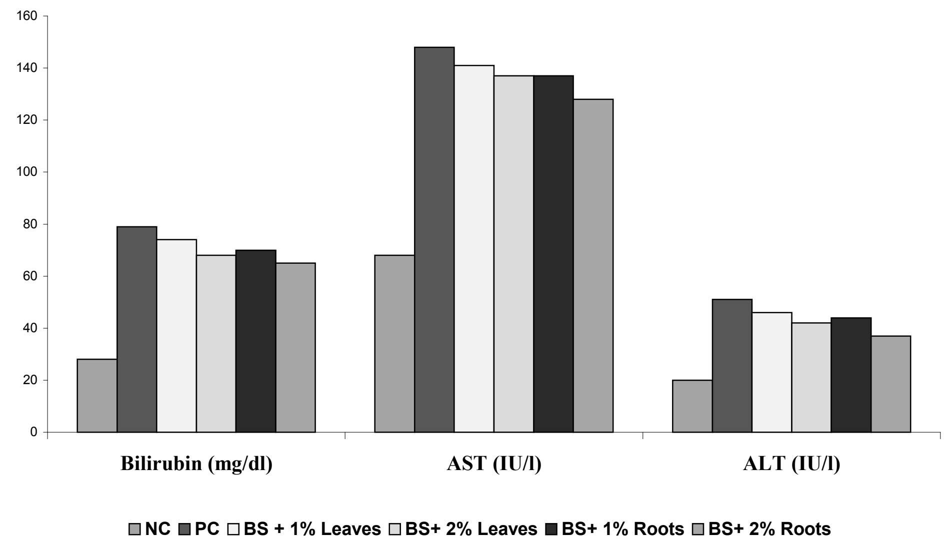

Fig 1: Effect of supplemented bread with leaves and roots of dandelion on liver

urea nitrogen than PC group. Furthermore, feeding hepatotoxic group on basal diet containing bread supplemented with 2% roots and leaves of dandelion did not differ the mean value of serum uric acid and urea nitrogen significantly when compared them with NC group and recorded best results. In respect to creatinine, the results showed that, all tested diets decreased the mean value of serum creatinine, except the diet containing supplemented bread with 1% leaves and roots, as compared to PC group.

Total iron

As presented in table (8 & 9), there were significant decreases in serum total iron (µg/dl) for PC group, compared to NC group. Feeding groups with hepatotoxicity on basal diet containing the lowest level of leaves or roots from dandelion (1%) showed non-significant changes in the mean values of serum total iron, as compared to PC group. On the other hand, group of rats, which received basal diet containing the highest levels of dandelion leaves or roots (2%), showed a significant increase (158.2 ± 3.3 and 161.3 ± 2.4 µg/dl) respectively, compared to PC group. Addition of supplemented bread with leaves or roots of dandelion to the diet at high levels 2% resulted in significant increase $p<0.05$ in the mean values of serum total iron, compared to PC group and recorded the best results.

Histology of liver of rats

Microscopically, liver of NC revealed the normal histology of hepatic lobule (Fig. 2A). Meanwhile, liver of rats from PC showed centrilobular hepatic lipidosis with appearance of homogenous eosinophilic apoptotic bodies (councilman's bodies) (Fig. 2B & 2C). However, apparent normal hepatocyte was noticed in liver of rats for the control group fed on basal diet containing (200 g. unsupplemented bread) (Fig. 2D). Vacuolar degeneration of hepatocytes was observed in examined liver of rat from PC group (Fig. 2E).

Moreover, liver of rat from hepatotoxic group fed on basal diet containing 1% leaves of (TO) revealed lipidosis of hepatocytes Fig. (2F). However, slight improvement in the histopathological picture was noticed in liver of rat from hepatotoxic groups fed on basal diets containing 2% leaves and 1% root of (TO) as the examined sections showed vacuolar degeneration of some hepatocytes Fig. (2G, 2H respectively). Moreover, liver of rat from hepatotoxic group fed on basal diet containing 2% root revealed apparent normal hepatocytes Fig. (2I). Liver of rat from hepatotoxic group fed on basal diet containing 200 g. bread which supplemented with 1% leaves of TO revealed apparent normal hepatocytes Fig. (2J). Vacuolar degeneration of some hepatocytes was recorded in liver of rat from hepatotoxic group fed on basal diet containing 200 g. bread supplemented with 2% leaves of TO Fig. (3K). However, liver of rat from hepatotoxic group fed on basal diet containing 200 g. supplemented bread with 1% root of TO revealed lipidosis of hepatocytes Fig. (2L). Apparent normal hepatocytes was noticed in liver of rat from hepatotoxic group fed on basal diet containing 200 g. supplemented bread with 2% root of TO Fig. (2M).

Table 6: Effect of leaves and roots of dandelion on lipid profile of rats with hepatotoxicity

Treatments / Groups	mg/dl				
	Cholesterol	Triacylglycerol	HDL-C	LDL-C	VLDL-C
NC	76.4 ± 3.9^{d}	40.2 ± 3.3^{e}	53.1 ± 4.5^{a}	15.3 ± 1.5^{e}	8.1 ± 0.6^{e}
PC	133.5 ± 5.4^{a}	63.0 ± 5.8^{a}	45.2 ± 3.8^{d}	75.7 ± 1.6^{a}	12.6 ± 1.2^{a}
1% Leaves	122.1 ± 5.3^{b}	58.4 ± 2.8^{b}	47.6 ± 1.5^{cd}	62.7 ± 3.9^{b}	11.7 ± 0.6^{b}
2% Leaves	117.9 ± 3.1^{b}	54.7 ± 3.0^{bd}	49.5 ± 2.3^{bc}	57.5 ± 3.4^{c}	10.9 ± 0.6^{bd}
1% Roots	119.6 ± 3.8^{b}	55.9 ± 2.9^{bc}	48.6 ± 1.8^{bd}	59.8 ± 2.9^{bc}	11.2 ± 0.9^{bc}
2% Roots	108.3 ± 5.3^{c}	52.8 ± 3.2^{cd}	51.3 ± 1.3^{ab}	46.4 ± 3.9^{d}	10.6 ± 0.6^{cd}

NC= negative control, PC= positive control; Values are expressed as mean ± SD, Significance at $p<0.05$; HDL-C: High Density Lipoprotein Cholesterol, LDL-C: Low Density Lipoprotein Cholesterol, VLDL-C: Very Low Density Lipoprotein Cholesterol; Values which don't share the same letter in each column are significantly different ($p<0.05$)

Table 7: Effect of supplemented bread with leaves and roots of dandelion on lipid profile of rats suffering from hepatotoxicity.

Treatments / Groups	mg/dl				
	Cholesterol	Triacylglycerol	HDL-C	LDL-C	VLDL-C
NC	73.9 ± 3.6^{e}	42.7 ± 2.8^{e}	55.8 ± 2.1^{a}	9.6 ± 1.2^{f}	8.5 ± 0.6^{e}
PC	124.0 ± 5.0^{a}	70.3 ± 5.1^{a}	46.7 ± 2.6^{d}	63.3 ± 2.1^{a}	14.1 ± 1.0^{a}
BS + 1% Leaves	115.6 ± 5.9^{b}	67.1 ± 3.9^{ab}	46.9 ± 5.1^{cd}	55.2 ± 2.7^{b}	13.4 ± 0.7^{ab}
BS + 2% Leaves	104.9 ± 4.3^{c}	60.2 ± 4.5^{cd}	50.8 ± 4.1^{bc}	42.1 ± 0.9^{d}	12.0 ± 0.9^{cd}
BS + 1% Roots	110.3 ± 4.5^{bc}	63.7 ± 2.5^{bc}	48.2 ± 3.6^{bd}	49.3 ± 4.5^{c}	12.7 ± 0.5^{bc}
BS + 2% Roots	97.6 ± 3.7^{d}	58.7 ± 3.7^{d}	52.1 ± 1.2^{ab}	33.7 ± 3.6^{e}	11.7 ± 0.7^{d}

Values are expressed as mean ± SD; Significance at $p<0.05$; HDL-C: High Density Lipoprotein Cholesterol; LDL-C: Low Density Lipoprotein Cholesterol; VLDL-C: Very Low Density Lipoprotein Cholesterol; Values which don't share the same letter in each column are significantly different.

DISSCUSION

The investigated dandelion is commonly part of Egyptian diet. Supplementation with this herb was conducted to maximize the beneficial effects of herbs in order to combat the toxic effects of CCl_4 as model of chronic liver disease. Herbal medicine is based on the premise that plants contain natural substances that can promote health and lessen illness. The elevation of free radical levels seen during the liver damage specifically in hepatotoxicity which is owing to enhanced production of free radicals and decreased scavenging potential of the cells. In this study, we investigated the bread supplemented with different concentration of leaves and roots from dandelion, in protection of hepatotoxicity in rats.

Body weight gain% of all treated groups with dandelion (1% and 2% leaves or roots) or bread supplemented with high level of leaves or roots from dandelion (2%) recorded significant decrease $p<0.05$, compared to NC group. In this respect, Kemper (1999) reported that, dandelion leaves extracts had diuretic effects as potent as furosemide (a loop diuretic) in rats and mice. The diuretic effect accounted for 100% of the weight loss found in these animal studies. However, there is little number of studies evaluating the diuretic effects of dandelion leaves or roots in humans or comparing it to standard diuretic medications. In addition, dandelion appears to have cascara acts as a laxative (Saper et al. 2004).

Treatment with dandelion (1% and 2% leaves or roots) or bread that provide the diets with (1% and 2% leaves or roots) led to remarkably prevented the elevation of serum bilirubin, AST and ALT in rats with hepatotoxicity.

Superoxide dismutase (SOD) is ubiquitous cellular enzyme that dismutates superoxide radical to H_2O_2 and oxygen and is one of the chief cellular defense mechanisms (Abdel-Moemin 2004).

On the other hand, CCl_4 treatment decreased superoxide dismutase (SOD), catalase, glutathione, and peroxidase and increased lipid peroxidation, treatment with 100 mg/kg of (TO) extract improved the SOD, catalase, glutathione, and peroxidase levels significantly and reduced lipid peroxidation. They conclude that the hydroalcoholic extract from the root of dandelion possesses antioxidant activity, confirming the traditional use of the plant in treatment of liver disorders (Sumanth and Rana 2006).

In our results bilirubin has significantly reduced compared to PC group that may be explained as the presence of sesquiterpene in both leaves and roots of dandelion, increase bile production in the gallbladder; this makes them a particularly useful tonic for persons with lethargic liver function (Kuusi et al. 1985) and (Wichtl 1994). Also, increases of bile secretion in rats (40% or more) have been attributed to activity of bitter sesquiterpene lactones in the root (Leung and Foster 1996).

Table 8: Effect of supplemented bread with leaves and roots of dandelion on some kidney function and total iron of rats with hepatotoxicity.

Treatments / Groups	mg/dl			µg / dl
	Uric acid	Urea nitrogen	Creatinine	Iron
NC	1.6 ± 0.04^{e}	28.6±1.2^{e}	0.4 ± 0.04^{e}	176.4 ±3.6^{a}
PC	1.8 ± 0.16^{a}	38.5±3.0^{a}	0.6 ± 0.05^{a}	143.2 ±4.5^{e}
1% Leaves	1.7± 0.07^{ab}	35.2±2.2^{b}	0.6 ± 0.02^{ab}	147.1 ±3.5^{de}
2% Leaves	1.6 ± 0.05$_{bc}$	31.9±2.7^{cd}	0.5 ± 0.05^{bd}	152.3 ±3.0^{c}
1% Roots	1.7 ± 0.07$_{bd}$	33.5±2.6^{bc}	0.6 ± 0.0^{4bc}	149.9 ±4.9^{cd}
2% Roots	1.6 ± 0.09$_{cde}$	30.1±2.0^{de}	0.5 ± 0.06^{cd}	159.8 ±3.3^{b}

NC= Negative control, PC= Positive control, Values are expressed as mean ± SD. Significance at $p<0.05$.Values which don't share the same letter in each column are significantly different.

Table 9: Effect of supplemented bread with leaves and roots of dandelion on some kidney function and total iron of rats with hepatotoxicity

Treatments / Groups	mg/dl			µg/ dl
	Uric acid	Urea nitrogen	Creatinine	Iron
NC	1.6 ± 0.04^{e}	28.6 ± 1.2^{e}	0.4 ± 0.04^{e}	176.4 ± 3.6^{a}
PC	1.8 ± 0.16^{a}	38.5 ± 3.0^{a}	0.6 ± 0.05^{a}	143.2 ± 4.5^{e}
BS with 1% Leaves	1.7 ± 0.07^{ab}	35.2 ± 2.2^{b}	0.6 ± 0.02^{ab}	147.1 ± 3.5^{de}
BS with 2% Leaves	1.6 ± 0.05^{bc}	31.9 ± 2.7^{cd}	0.5 ± 0.05^{bd}	152.3 ± 3.0^{c}
BS with 1% Roots	1.7 ± 0.07^{bd}	33.5 ± 2.6^{bc}	0.6 ± 0.04^{bc}	149.9 ± 4.9^{cd}
BS with 2% Roots	1.6 ± 0.09^{cde}	30.1 ± 2.0^{de}	0.5 ± 0.06^{cd}	159.8 ± 3.3^{b}

NC= negative control, PC= positive control; Values are expressed as mean ± SD; Significance at $p<0.05$; Values which don't share the same letter in each column are significantly different.

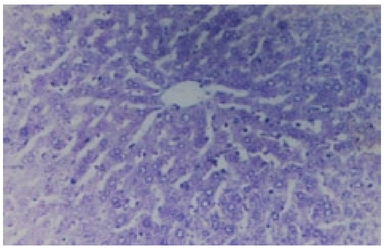

Fig. (2A): Liver of control, (untreated rat) showing the normal histology of hepatic lobule (H and E × 200)

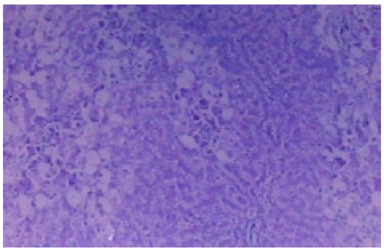

Fig. (2B): Liver of hepatotoxic group (control positive), fed on basal diet showing centrilobuler lipidosis (H and E × 100).

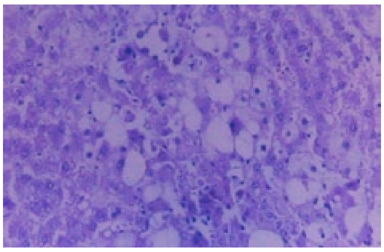

Fig. (2C): Liver of hepatotoxic group (control positive), fed on basal diet showing lipidiosis with appearance of apoptotic bodies (councilman's bodies) (H and E × 200)

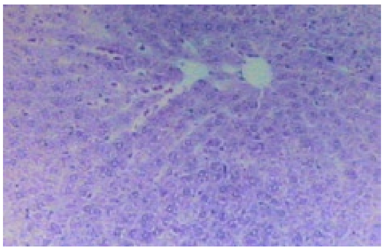

Fig. (2D): Liver of rat from control group fed on basal diet containing 200g. unsupplemented bread showing apparent normal hepatocytes (H and E × 200)

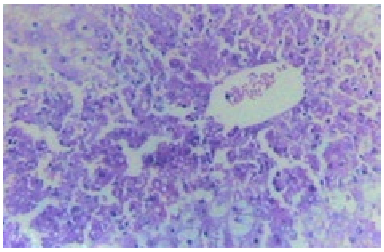

Fig. (2E): Liver of rat from the control positive group fed on basal diet containing 200 g. unsupplemented bread showing vacuolar degeneration of hepatocytes (H and E × 200)

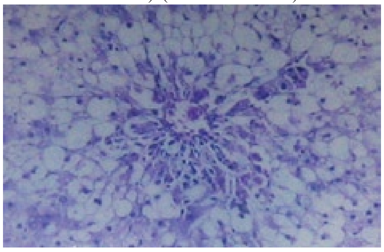

Fig. (2F): Liver of rat from hepatotoxic group fed on basal diet containing 1% leaves of (TO) showing lipidosis of hepatocytes (H and E × 200)

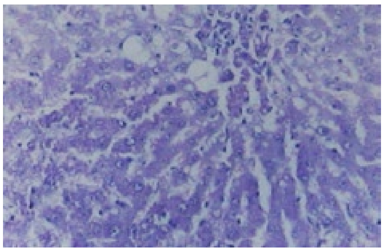

Fig. (2G): Liver of rat from hepatotoxic group fed on basal diet containing 2% leaves of (TO) showing vacuolar degeneration of some hepatocytes (H and E × 200)

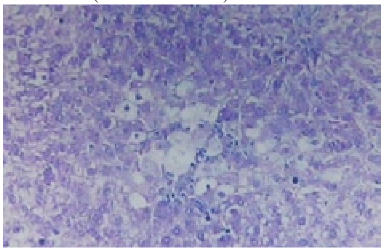

Fig. (2H): Liver of rat from hepatotoxic group fed on basal diet containing 1% root of (TO) showing vacuolar degeneration of some hepatocytes (H and E × 200)

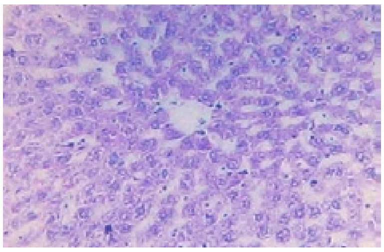

Fig. (2I): Liver of rat from hepatotoxic group fed on basal diet containing 2% root of (TO) showing apparent normal hepatocytes (H and E × 200)

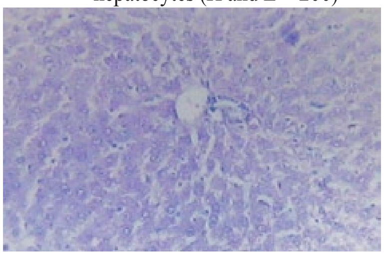

Fig. (2J): Liver of rat from hepatotoxic group fed on basal diet containing 200 g. bread supplemented with 1% leaves of (TO) showing apparent normal hepatocytes (H and E × 200)

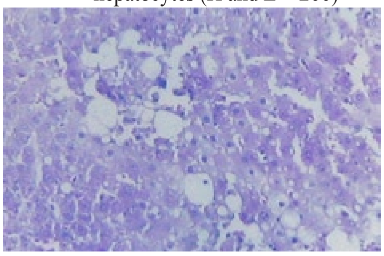

Fig. (2K): Liver of rat from hepatotoxic group fed on basal diet containing 200g bread supplemented with 2% leaves of (TO) showing vacuolar degeneration of some hepatocytes (H and E × 200)

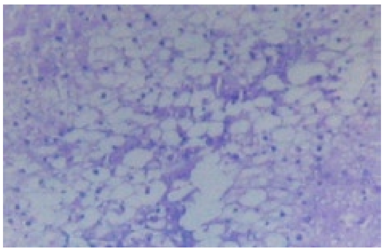

Fig. (2L): Liver of rat from hepatotoxic group fed on basal diet containing 200g supplemented bread with 1% root of (TO) showing lipidosis of hepatocytes (H and E × 200)

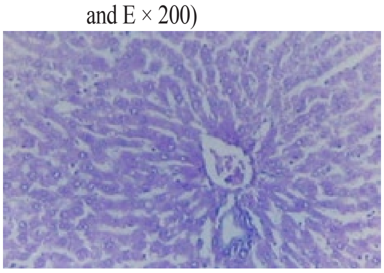

Fig. (2M): Liver of rat from hepatotoxic group fed on basal diet containing 200g, supplemented bread with 2% root of (TO) showing apparent normal hepatocytes (H and E × 200)

The addition of dandelion to the diet (1% and 2% leaves or roots or bread) improved values of total cholesterol, triacylglycerol, high density lipoprotein (HDL-C), low and very low density lipoprotein (LDL-C and VLDL-C), especially, with high levels 2%, that clearly noticed in rats with hepatotoxicity. Moreover, (Cho et al. 2002) found that an extract of dandelion lowered triacylglycerol, total cholesterol, and low-density lipoproteins (LDL-C) while increasing high-density lipoproteins (HDL) in rats with diabetes.

Dandelion has also been identified as one of the most potent herbs in lowering serum uric acid, urea nitrogen and creatinine in rats suffering from hepatotoxicity, and fed on diets containing a higher percentage of dandelion (2% leaves or roots) or bread. Markell (1997) suggested that, many of herbs that have traditionally been used to treat kidney disorders in patients whose kidneys are failing or who are on dialysis, have diuretic properties and some like dandelion leaf are high in potassium. A preliminary report suggests that dandelion root reduces the recurrence rate of urinary tract infections in women (Zaffani et al. 2006).

Iron of all treated groups with high level of leaves or roots from dandelion (2%) or bread supplemented with the same level of dandelion; recorded a significant increase $p<0.05$, as compared to PC group. Dandelion is a rich source of vitamins, minerals and flavonoids Bradley (1992). This makes dandelion an invaluable herb to help keep these nutrient levels up in the body. There is scarce data on the effect of dandelion herbs on human health specifically liver disease. However, future implications of this study may be used to examine the benefits of dandelion in human hepatic diseases.

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