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Hepatoprotective effect of dandelion (*Taraxacum officinale*) against induced chronic liver cirrhosis

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Recently, it was found that dandelion flower extract may fight free radicals (chemical by-products known to damage DNA). Health care providers clinically use dandelion root to promote liver detoxification. Dandelion leaves water extract was investigated for hepatoprotective activity against carbon tetrachloride induced liver damage. The aim of the study was to evaluate the efficacy of the hepatoprotective activity of dandelion leaves water extract against carbon tetrachloride (CCl_4) intoxication in liver albino rats. Carbon tetrachloride was used as hepatotoxic agent and dandelion leaves water extract was used as a probable hepato-protective agent. Various biochemical parameters were studied to evaluate the hepatoprotective activity of dandelion leaves water extract. The study was also supported by histopathology of liver sections and DNA extraction of the studied animals to investigate the genomic DNA integrity. The results revealed that the serum markers in rats treated with CCl_4 recorded elevated concentration, indicating severe hepatic damage by CCl_4, while the blood samples from dandelion treated animals showed significant reduction in the serum markers, indicating the effect of the plant extract in restoring the normal functional ability of the hepatocytes. The present study revealed that dandelion leaf water extract could afford a significant protection against CCl_4 induced hepatocellular injury.

Key words: *Taraxacum officinale*, dandelion leaf water extract (DLWE), hepatoprotective activity, carbon tetrachloride (CCl_4) damage.

INTRODUCTION

Epidemiological studies have proved that fruits and vegetables consumption reduces risk of chronic diseases. Increased consumption of fruits and vegetables containing high levels of phytochemicals has been recommended to prevent chronic diseases related to oxidative stress in the human body (Chu et al., 2002). Hepatic malfunction due to inhalation or ingestion of hepatotoxic materials such as acetaminophen, cadmium chloride, ethanol, carbon tetrachloride (CCl_4) and ally alcohols are significantly increasing worldwide (Wolf, 1999).

Plants used in traditional medicine require detailed investigation from an ethnopharmacological approach for the treatment of liver disorders because hepatic ailments remain a serious health problem caused by drugs, chemicals and alcohol (Anju et al., 2012). Various medicinal plants and their formulations are used in the Indian traditional system of medicine for their hepatoprotective

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potential. Also, the plant-based hepatoprotective agents or drugs contains diversity of major active constituents such as phenols, coumarins, lignans, terpenoids, carotenoids, glycosides, flavonoids, organic acids, alkaloids and xanthenes. Several phytomolecules have been reported as having potent hepatoprotective principles. So, an investigation into the lead molecules that may produce better therapeutic effect, is required to overcome the pharmaceutical imbalance between remedies that protect the liver and drugs that induce hepatotoxicity (Anju et al., 2012).

Carbon tetrachloride (CCl_4) is catalysed by cytochrome P_{450} in the liver cell endoplasmic reticulum leading to the generation of an unstable complex of CCl_3 radical, which reacts rapidly with O_2 to yield highly reactive hepatotoxic trichloromethyl peroxy radical (Recknagel et al., 1989). These free radicals attack microsomal lipids leading to its peroxidation and also covalently bind to microsomal lipids and proteins, ultimately initiating a site of secondary biochemical processes (Rao and Recknagel, 1969).

Dandelion (*Taraxacum officinale*) has been used in folklore medicine and Traditional Chinese medicine in the treatment of inflammation and several women's diseases such as breast and uterine cancers (Ung-Kyu et al., 2010), and it is also acclaimed as a nontoxic medicinal herb with exceptional values for its choleretic, diuretic (Schütz et al., 2006), anti-rheumatic (Bisset and Wichtl, 1994; Newall et al., 1996) and anti-inflammatory properties (Jeon et al., 2008). Several flavonoids including caffeic acid, chlorogenic acid, luteolin, and luteolin 7-glucoside have been isolated from the dandelion (Williams et al., 1996).

Although there is little scientific support for the medicinal use of dandelion, a number of studies suggests that the herb may help lessen inflammation and kill bacteria. In a 2003 study, scientists found that dandelion flower extract can fight free radicals (Hu and Kitts, 2003). While many people think of the common dandelion as a pesky weed, herbalists consider it a valuable herb with many culinary and medicinal uses. Dandelion is a rich source of vitamins A, B complex, C, and D, as well as minerals such as iron, potassium, and zinc (Hu and Kitts, 2003). Its leaves are often used to add flavor to salads, sandwiches, and teas. The roots can be found in some coffee substitutes, and the flowers are used to make certain wines (Hudec et al., 2007). The hepatoprotective activity of dandelion aqueous extract was investigated in D-galactosamine-induced hepatitis in rats.

Dandelion was found to have a potential therapeutic material for treating chemically induced or viral hepatitis (Park et al., 2008). Dandelion hot water extract was also found to have protective effect on acute liver inflammation induced by CCl_4 in rats (Park et al., 2010). Dandelion leaves produce a diuretic effect while the roots act as an antiviral agent, appetite stimulant, digestive aid, and may help promote gastrointestinal health. Dandelion flower has antioxidant properties. Dandelion may also help improve the immune system. Health care providers clinically use dandelion root to promote liver detoxification and dandelion leaves to support kidney function (Hu and Kitts, 2003).

More studies revealed that dandelion leaf extract suppressed the production of tumor necrosis factor (TNF)-α by inhibiting interleukin-1 production from primary cultures of rat astrocytes, and also showed a protective effect against cholecystokinin octapeptide induced acute pancreatitis by significantly decreasing the pancreatic weight/body weight ratio in rats (Seo et al., 2005). Dandelion leaf extract has been shown to have stronger hydrogen peroxide scavenging activity compared with the root extract because of its high polyphenol content (Schütz et al., 2006). Dandelion leaf extract has been shown to exhibit a protective effect against cholecystokinin octapeptide-induced acute pancreatitis (Seo et al., 2005). Therefore, the current study was designed to evaluate the possible hepatoprotective activity of dandelion against carbon tetrachloride (CCl_4) intoxication in liver albino rats.

MATERIALS AND METHODS

The present research protocol was examined and approved by the scientific ethical committee in King Abdulaziz university, Saudi Arabia and Medical Research Center, Faculty of Medicine, Ain Shams University, Cairo, Egypt.

Preparation of dandelion leaves water extracts (DLWE) for rats

Dandelion leaves were obtained from the herbal medicine market (Saini, Egypt). The dried dandelion leaves were then homogenized to a fine powder and stored at room temperature ($25 \pm 2°C$) until use. Briefly, 100 g of powdered material was boiled in water (1:10 w/v) for 4 h. The water extracts were filtered through Whatman No. 1 filter paper and evaporated under a vacuum at $40°C$ and then further dried to a powder using a freeze-dryer at $50°C$ (Soo-Yeul et al., 2002).

Treatment of rats, induction of liver fibrosis and experimental design

Eighty four male Wister albino rats weighing 150 to 200 g were used as the animal model. Animals were housed in well-ventilated polypropylene cages with husk beds. All animal experiments were performed following "Principles of laboratory animal care" (NIH publication no. 85-23, revised in 1985). The animals were acclimatized to conditions in the laboratory (26 to 28°C, 60 to 80% relative humidity, 12 h light/dark cycle) for 10 days prior to the commencement of the treatment, during which they received standard diet and tap water *ad libitum* (Anupam et al., 1995). Animals were kept as 6 rats per a large cage. On day 0, rats were injected subcutaneously at a dose of 0.2 ml/100 g body weight of 40 ml/L CCl_4 (Morgan Chemical Factory, Egypt) dissolved in paraffin oil (Morgan Chemical Factory, Egypt) (Dong-Chang et al., 2005). The injection was given three times a week for 6 successive weeks. The same volume of paraffin oil alone was used as control. Liver fibrosis was determined by killing five rats with histopathology weekly.

Route of administration

Dandelion leaves water extract (DLWE) was administrated to the rats through the mouth using intragastric catheter tube to ensure the proper and secure ingestion of the extracts according to the method recommended by Dawit et al. (2006). The animals were randomly divided into two main groups. Group A (normal control group) and group B (liver injured group) which were sub-classified into:

1. Group A: Twelve rats were left to serve as normal basic control.
2. Group B: This group included seventy two rats which were all injected subcutaneously at a dose of 0.2 ml/100 g body weight of previously prepared CCl_4. Group B was divided as follows:
3. Group B1: Thirty six albino rats served as a control pollutant group (+ve control) and were subdivided into three subgroups; each subgroup containing twelve rats. Twelve rats were sacrificed at the end of the 2nd week, twelve rats were sacrificed at the end of the 4th week and the last twelve rats of group B1 were sacrificed at the end of the 6th week of the study.
4. Group B2: Thirty six albino rats were introduced with 25 ml/kg DLWE once/day and were subdivided into three subgroups; each subgroup containing twelve rats. Twelve rats were sacrificed at the end of the 2nd week, twelve rats were sacrificed at the end of the 4th week and the last twelve rats of group B2 were sacrificed at the end of the 6th week of the study.

Samples collection and biochemical assays

Blood was collected from each rat in a centrifuge tube and placed at room temperature for 20 min. Serum was then separated by centrifugation at 3,000 rpm for 10 min using cooling centrifuge (Beckman, CS-15R Centrifuge, California-USA). Serum sample was divided into two aliquots, one for determination of serum alanine transaminase (ALT) (Henry et al., 1974, 1960), serum aspartate transaminase (AST) (Henry et al., 1974, 1960), serum gamma glutamyle transferase (GGT) (Gerhard et al., 2002), serum alkaline phosphatase (ALP) (Elias et al., 1963), serum lactate dehydrogenase (LDH) (Elias et al., 1963), serum urea concentration (Chaney and Marbach, 1962) and serum albumin concentration (Pinnell and Northam, 1978), and the second for determination of AChE (acetylcholinesterase) concentration by an enzymatic rate method (Donald et al., 1978). The chemicals were purchased from (Bio-Med. Diagnostics Reagent, Egy-Chem, Egypt). The abdomen of each animal was excised immediately after scarifying and the liver was excised and divided into two portions, one portion was immersed immediately into 10% buffered neutral formaldehyde solution and processed for histopathological examination, the second portion was used for DNA extraction.

Genomic DNA extraction of rats' livers and gel electrophoresis

DNA was extracted according to purification protocol of total DNA from animal tissues by using (Spin-Column Protocol) (QIAGEN, DNeasy, RNeasy, QIAGEN Group); DU (Beckman Instruments, Inc.). Gel preparation was done according to Raj (2007). Molecular biology grade agarose was used to prepare 2% agarose gel in 1× Tris-acetate-ethylenediaminetetraacetic acid (TAE) buffer. The power supply was turned on at 100 volts for 30 to 45 min to allow separation of DNA marker bands.

Histopathological examination

Livers of the sacrificed rats were dissected, removed and one half of each liver was fixed in 10% buffered neutral formalin solution.

The fixed specimens were then trimmed, washed and dehydrated in ascending grades of alcohol. These specimens were cleared in xylene, embedded in paraffin, sectioned at 4 to 6 microns thickness and stained with Hematoxylen and Eosin (H and E) then examined microscopically for the evaluation of histopathological changes (Nehal, 2011). Portal inflammation, necrosis, fibrosis-cirrhosis and steatosis were scored by examining three randomly chosen fields of view per tissue section and estimating a score for each specific parameter. A total pathology score was calculated according to Ishak et al. (1995).

Statistical analysis

Analysis of data was done using STATISTICA 7. Mann Whitney Willcoxon test was used instead of unpaired t-test in non-parametric data (Standard deviation (SD) > 50% mean).

RESULTS

Serum biochemical parameters of liver and kidney functions

Several hepatic enzymes in serum were used for the biochemical markers to understand the early hepatic injury. Table 1 shows serum levels of liver profile of untreated control group and CCl_4 treated control group throughout the whole study. The mean serum levels of ALT, AST, GGT, ALP and LDH of CCl_4 treated control group at the end of the 2nd, 4th and 6th weeks were significantly ($P < 0.01$) increased compared to that of the untreated control group (negative control) at the end of the corresponding studied weeks of the study, while the mean serum levels of AChE of CCl_4 treated group showed a significant ($P < 0.01$) decrease at the end of the 2nd, 4th and 6th weeks compared to that of the untreated control group at the end of the same corresponding studied weeks of the study.

Meanwhile the mean serum levels of urea at the end of the 2nd, 4th and 6th weeks were significantly ($P < 0.01$) increased in CCl_4 treated control group compared to that of the untreated control group (negative control) at the end of the same corresponding studied weeks of the study. The mean serum levels of albumin in CCl_4 treated control group were fluctuating between insignificant ($P > 0.05$) decrease at the end of the 2nd week and significant ($P < 0.01$) decrease at the end of the 4th week compared to that of the untreated control group at the end of the same corresponding weeks of the study. While at the end of the 6th week, the mean serum level of albumin of CCl_4 treated control group showed significant ($P < 0.01$) elevation compared to that of the untreated control group at the end of the same corresponding week of the study.

Table 2 shows that the mean serum levels of ALT, AST, GGT, ALP and LDH of dandelion protected group at the end of the 2nd, 4th and 6th weeks of the study were significantly ($P < 0.01$) decreased compared to that of CCl_4 treated group (+ve control) at the end of the cor-responding studied weeks of the experiment, respectively

Table 1. Serum levels of liver and kidney profiles in CCl_4 treated & untreated groups.

Parameter	ALT (IU/L)	AST (IU/L)	GGT (U/L)	ALP (IU/L)	LDH (IU/L)	AChE (U/L)	UREA (mg/dl)	ALBUMIN (g/L)
Untreated control group	82.2±14.4	234.7±11.4	19.4±2.3	176.8±13.5	633.8±45.8	2149.0±82.0	36.9±5.6	4.3±0.6
CCl_4 treated group at the end of the 2nd week	147.5±15.8	274.3±10.8	34.6±4.6	258.1±13.8	1537.4±101.5	1837.3±32.2	60.7±7.5	3.7±0.4
Significance (P)	<0.01 H. Sig.	<0.01 H. Sig.	<0.01 H. Sig.	<0.01 H. Sig.	<0.01 H. Sig.	<0.01 H. Sig.	<0.01 H. Sig.	>0.05 Insig.
CCl_4 treated group at the end of the 4th week	276.1±17.4	677.4±132.8	47.3±5.8	492.8±26.3	2114.5±101.7	1621.2±53.9	65.0±9.8	3.1±0.3
Significance (P)	<0.01 H. Sig.	<0.01 H. Sig.	<0.01 H. Sig.	<0.01 H. Sig.	<0.01 H. Sig.	<0.01 H. Sig.	<0.01 H. Sig.	<0.01 H. Sig.
CCl_4 treated group at the end of the 6th week	347.2±32.4	1242.9±42.8	70.4±2.7	526.0±17.6	2452.2±155.2	1037.2±104.2	67.8±6.3	6.3±0.4
Significance (P)	<0.01 H. Sig.	<0.01 H. Sig.	<0.01 H. Sig.	<0.01 H. Sig.	<0.01 H. Sig.	<0.01 H. Sig.	<0.01 H. Sig..	<0.01 H. Sig.

H. Sig.= highly significant, Insig = significant.

except at the end of the 2nd week, the mean serum levels of ALT and AST of dandelion protected group were insignificantly (P > 0.05) elevated compared to that of CCl_4 treated group (+ve control) at the end of the same corresponding week, while the mean serum levels of AChE of dandelion protected group showed significant (P < 0.01) increase at the end of the 4th and 6th weeks of the experiment compared to that of CCl_4 treated group at the end of the same corresponding studied weeks of the experiment. But the mean serum levels of AChE of dandelion protected group displayed a significant (P < 0.01) decrease at the end of the 2nd week compared to that of CCl_4 treated group at the end of the same corresponding week of the study.

The mean serum levels of urea in dandelion protected group showed significant (P < 0.01) decrease at the end of the 2nd, 4th and 6th weeks of the study compared to that of the CCl_4 treated group (+ve control) at the end of the same corresponding studied weeks of the experiment. The mean serum levels of albumin in dandelion protected group were fluctuating between insignificant (P > 0.05) increase at the end of the 2nd week, significant (P < 0.01) increase at the end of the 4th week and significant (P < 0.01) decrease at the end of the 6th week of the study compared to that of the CCl_4 treated group at the end of the same corresponding studied weeks.

Results of DNA

Figure 1 showed the effect of supplementation of DLWE on the genomic DNA integrity of different CCl_4 treated rats livers in addition of the genomic DNA integrity of untreated animal liver (-ve control) as well as the genomic DNA integrity of CCl_4 treated animal liver (+ve control) at the end of the 2nd week where, lane 1 represents DNA marker, lane 2 represents the genomic DNA of untreated animal liver (-ve control) and seems to be intact, but the genomic DNA of lane 3 is disintegrated as it represents the genomic DNA of CCl_4 treated animal liver (+ve control) at the end of the 2nd week. The other nine lanes (lanes 4 to 12) represent the genomic DNA of dandelion protected animals against CCl_4 with different responses towards DLWE hepatoprotection at the end of studied weeks of the experiment.

Results of histopathology

The biochemical findings reported in the present study were associated with histopathological

Table 2. Serum levels of liver profile in dandelion hepato-protected groups.

Parameter	ALT (IU/L)	AST (IU/L)	GGT (U/L)	ALP (IU/L)	LDH (IU/L)	AChE (U/L)	UREA (mg/dl)	ALBUMIN (g/L)
CCl$_4$ treated group at the end of the 2nd week	147.5±15.8	274.3±10.8	34.6±4.6	258.1±13.8	1537.4±101.5	1837.3±32.2	60.7±7.5	3.7±0.4
Dandelion protected group at the end of the 2nd week	128.0±11.4	264.6±14.8	19.2±1.1	220.0±18.3	1181.8±33.3	1753.2±28.2	37.6±3.5	4.2±0.5
Significance (P)	>0.05 Insig.	>0.05 Insig.	<0.01 H. Sig.	<0.01 H. Sig.	<0.01 H. Sig	<0.01 H. Sig	<0.01 H. Sig	>0.05 Insig
CCl$_4$ treated group at the end of the 4th week	276.1±17.4	677.4±132.8	47.3±5.8	492.8±26.3	2114.5±101.7	1621.2±53.9	65.0±9.8	3.1±0.3
Dandelion protected group at the end of the 4th week	116.0±12.2	215.3±22.9	16.4±2.0	177.3±10.6	752.0±28.2	1843.0±34.7	35.3±2.7	4.1±0.3
Significance (P)	<0.01 H.Sig.	<0.01 H.Sig.	<0.01 H.Sig.	<0.01 H.Sig.	<0.01 H.Sig.	<0.01 H.Sig.	<0.01 H.Sig.	<0.01 H.Sig.
CCl$_4$ treated group at the end of the 6th week	347.2±32.4	1242.9±42.8	70.4±2.7	526.0±17.6	2452.2±155.2	1037.2±104.2	67.8±6.3	6.3±0.4
Dandelion protected group at the end of the 6th week	85.5±6.9	161.5±11.1	12.8±2.1	145.6±6.7	679.9±30.1	2087.6±63.6	23.4±2.8	3.7±0.3
Significance (P)	<0.01 H.Sig.	<0.01 H.Sig.	<0.01 H.Sig.	<0.01 H.Sig.	<0.01 H.Sig.	<0.01 H.Sig	<0.01 H.Sig.	<0.01 H.Sig

H. Sig.= highly significant, Insig = significant.

examination sections of five rats' livers out of twelve rats which constitute each subgroup of the study. The histopathological examination results of the livers of the normal rats fed on standard diet (-ve control) showed normal histological picture (Figure 2A). Liver examination of CCl$_4$ treated rats showed insignificant (P > 0.05) progress in portal inflammation, necrosis, fibrosis and steatosis at the end of 2nd and 4th weeks of the study compared to that of the negative control rats at the end of the same corresponding studied weeks. Figure 2B shows mild periportal inflammation and vacuolated hepatocytes of CCl$_4$ treated rat's liver at the end of the 2nd week, while at the end of the 6th week, portal inflammation, necrosis, fibrosis and steatosis were significantly

(P < 0.01) progressive in CCl$_4$ treated rat's liver compared to that of the negative control rats at the end of the same corresponding studied week. Figure 2C shows periportal fibrosis, congestion, steatosis of CCl$_4$ treated rat's liver at the end of the 6th week. Also, the histopathological examination of the livers sections of dandelion protected rats revealed that portal inflammation, necrosis, fibrosis and steatosis at the end of 2nd and 4th weeks of the study were insignificantly (P > 0.05) improved compared to that of the CCl$_4$ treated rats (+ve control) at the end of the same corresponding studied weeks of the experiment (Tables 3, 4, 5 and 6). Figure 3A shows periportal inflamation in dandelion protected rat liver at the end of the 2nd week. But at the end of the 6th

week, those histopathological parameters (portal inflammation, necrosis, fibrosis and steatosis) showed significant (P < 0.01) improvement compared to that of the CCl$_4$ treated rats (+ve control) at the end of the same corresponding studied week (Tables 3, 4, 5 and 6). Figure 3B shows congestion, inflammation in dandelion protected rat liver at the end of the 4th week and figure 3C shows steatosis around portal tract in dandelion protected rat liver at the end of the 6th week.

DISCUSSION

CCl$_4$ is used as hepatotoxic agent that enhance

Table 3. Portal Inflammation grades of dandelion protected groups against CCl$_4$ treated groups throughout the study.

Group / Grade of portal inflamation	0	1	2	3	4
Number of CCl$_4$ treated group at the end of the 2nd week	2	3	-	-	-
Number of Dandelion protected group at the end of the 2nd week	1	3	1	-	-
P			N.S		
Number of CCl$_4$ treated group at the end of the 4th week	-	4	1	-	-
Number of Dandelion protected group at the end of the 4th week	-	3	2	-	-
P			N.S		
Number of CCl$_4$ treated group at the end of the 6th week	-	-	1	4	-
Number of Dandelion protected group at the end of the 6th week	5	-	-	-	-
P			H.S		

*(n): Number of rats/group = 5, *$P < 0.01$ = H. Significant (H.S), *$P < 0.05$ = Significant (S), *$P > 0.05$ = Insignificant (N.S). **Mann Whitney Willcoxon test was used instead of unpaired t-test in non-parametric data (SD > 50% mean).

Table 4. Necrosis grades of dandelion protected groups against CCl$_4$ treated groups throughout the study

Group / Grade of necrosis	0	1	2	3	4	5	6
Number of CCl$_4$ treated group at the end of the 2nd week	2	1	2	-	-	-	-
Number of Dandelion protected group at the end of the 2nd week	2	3	-	-	-	-	-
P				N.S			
Number of CCl$_4$ treated group at the end of the 4th week	2	2	-	-	1	-	-
Number of Dandelion protected group at the end of the 4th week	-	5	-	-	-	-	-
P				N.S			
Number of CCl$_4$ treated group at the end of the 6th week	-	-	-	2	3	-	-
Number of Dandelion protected group at the end of the 6th week	2	3	-	-	-	-	-
P				H.S			

*(n): Number of rats/group = 5, *$P < 0.01$ = H. Significant (H.S) *$P < 0.05$ = Significant (S), *$P > 0.05$ = Insignificant (N.S). **Mann Whitney Willcoxon test was used instead of unpaired t-test in non-parametric data (SD > 50% mean).

Table 5. Fibrosis and cirrhosis stages of dandelion protected groups against CCl$_4$ treated groups throughout the study.

Group / Grades of fibrosis and cirrhosis	0	1	2	3	4	5	6
Number of CCl$_4$ treated group at the end of the 2nd week	3	1	-	1	-	-	-
Number of Dandelion protected group at the end of the 2nd week	3	2	-	-	-	-	-
P				N.S			
Number of CCl$_4$ treated group at the end of the 4th week	1	4	-	-	-	-	-
Number of Dandelion protected group at the end of the 4th week	3	1	1	-	-	-	-
P				N.S			
Number of CCl$_4$ treated group at the end of the 6th week	-	-	2	-	3	-	-
Number of Dandelion protected group at the end of the 6th week	3	2	-	-	-	-	-
P				H.S			

*(n): Number of rats/group = 5, *$P < 0.01$ = H. Significant (H.S), *$P < 0.05$ = Significant (S), *$P > 0.05$ = Insignificant (N.S). **Mann Whitney Willcoxon test was used instead of unpaired t-test in non-parametric data (SD > 50% mean).

Table 6. Steatosis grades of dandelion protected groups against CCl₄ treated groups throughout the study.

Grade of steatosis Group	0	1	2	3
Number of CCl₄ treated group at the end of the 2nd week	4	1	-	-
Number of Dandelion protected group at the end of the 2nd week	4	1	-	-
P		N.S		
Number of CCl₄ treated group at the end of the 4th week	1	3	1	-
Number of Dandelion protected group at the end of the 4th week	3	2	-	-
P		N.S		
Number of CCl₄ treated group at the end of the 6th week	-	-	3	2
Number of Dandelion protected group at the end of the 6th week	4	1	-	-
P		H.S		

*(n): Number of rats/group = 5, *$P < 0.01$ = H. Significant (H.S), *$P < 0.05$ = significant (S), *$P > 0.05$ = insignificant (N.S). **Mann Whitney Willcoxon test was used instead of unpaired t-test in non-parametric data (SD > 50% mean).
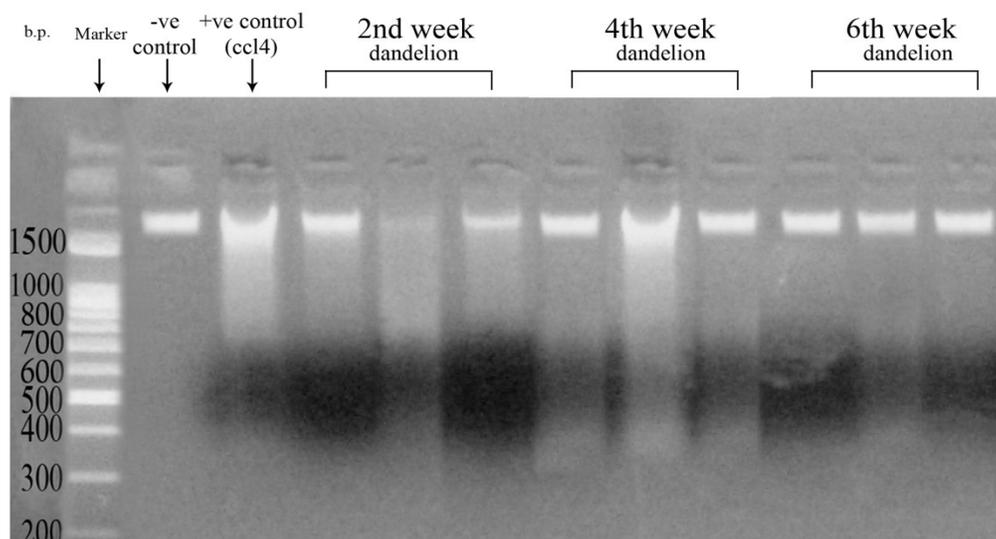
Figure 1. Effect of supplementation of dandelion leaves water extract (DLWE) on the DNA integrity of different CCl₄ treated rats' livers.
Lane 1: DNA Marker.
Lane 2: genomic DNA of untreated rat's liver (-ve control).
Lane 3: genomic DNA of CCl4 treated rat's liver (+ve control) at the end of the 2nd week.
Lane 4: genomic DNA of dandelion protected rat's liver against CCl4 at the end of the 2nd week.
Lane 5: genomic DNA of dandelion protected rat's liver against CCl4 at the end of the 2nd week,
Lane 6: genomic DNA of dandelion protected rat's liver against CCl4 at the end of the 2nd week.
Lane 7: genomic DNA of dandelion protected rat's liver against CCl4 at the end of the 4th week.
Lane 8: genomic DNA of dandelion protected rat's liver against CCl4 at the end of the 4th week.
Lane 9: genomic DNA of dandelion protected rat's liver against CCl4 at the end of the 4th week.
Lane 10: genomic DNA of dandelion protected rat's liver against CCl4 at the end of the 6th week.
Lane 11: genomic DNA of dandelion protected rat's liver against CCl4 at the end of the 6th week.
Lane 12: genomic DNA of dandelion protected rat's liver against CCl4 at the end of the 6th week.

formation of free radicals through their metabolism, causing lipid peroxidation of cellular and organelle membranes as a primary pathogenic step (Ming et al., 2006). Al-Shabanah et al. (2000) reported that CCl₄ administration to rats causes necrosis, mononuclear cell infiltration, and steatosis foamy degeneration of hepatocytes cirrhosis (Natusme et al., 1999; Naziroglu et al., 1999).

It has been reported that CCl₄ treatment causes increase serum levels of ALT, AST, LDH and ALP (Teocharis et al., 2001). In another study, Schmidt et al. (1975) stated that a damage to the structural integrity of the liver mirrors an increase in the level of serum
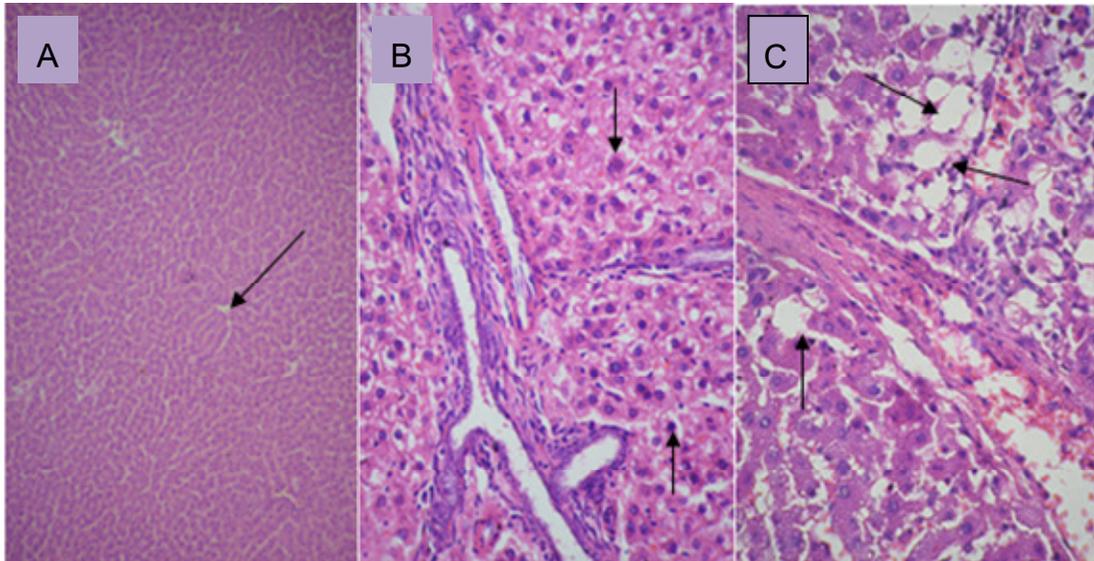
Figure 2. (A) The negative control group with normal hepatic architecture (central vein ↑), (B) *L.P mild periportal inflammation (lymphocytes), vacuolated hepatocytes of CCl$_4$ treated rat liver at the end of the 2nd week, (C) periportal fibrosis, congestion, moderate steatosis (fat vacuoles) of CCl$_4$ treated rat liver at the end of the 6th week.
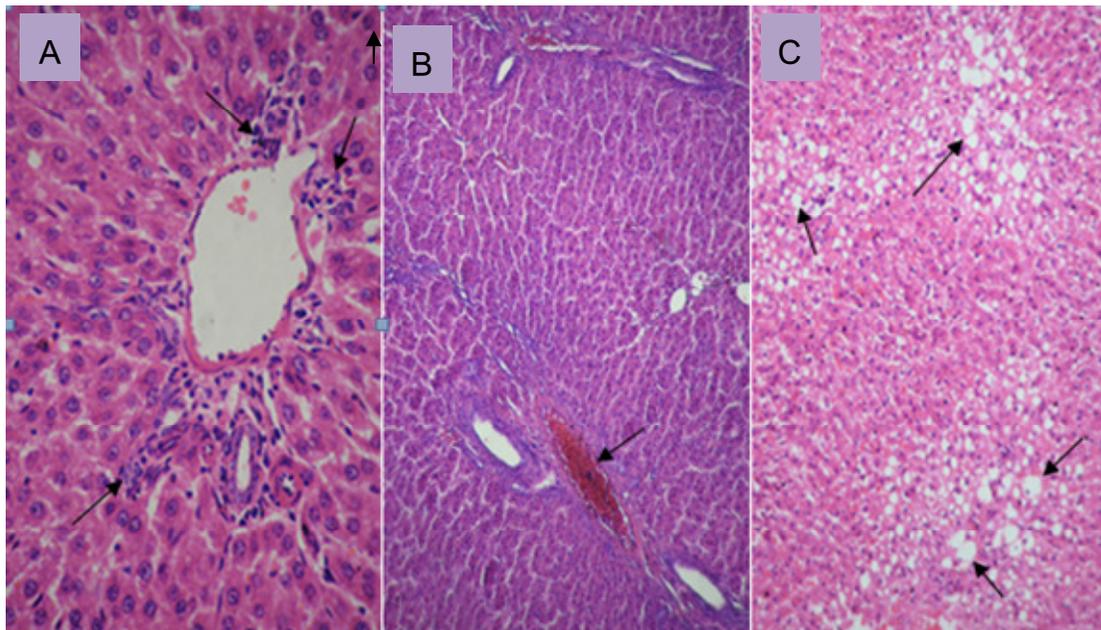
Figure 3. (A) Periportal inflamation (lymphocytes ↑) in dandelion protected rat liver at the end of the 2nd week, (B) *L.P., congestion (↑↑), inflamation in dandelion protected rat liver at the end of the 4th week, (C) *L.P.,steatosis (fat vacuoles ↑) around portat tract in dandelion protected rat liver at the end of the 6th week.
*L.P: low power

transaminases, as these are located in the cytoplasm and released into the circulation after cellular damage (Sallie et al., 1991). In the current study, treatment with CCl$_4$ caused significant (P < 0.01) elevation in the serum levels of ALT, AST, GGT, ALP, LDH and urea as well as significant

(P < 0.01) decrease in the serum level of AChE at the end of the 2nd, 4th and 6th weeks compared to that of the untreated control group at the end of the corresponding studied weeks of the study. While, due to the exposure to CCl$_4$ serum, albumin levels fluctuated between insignificant

(P > 0.05) decrease at the end of the 2nd week, significant (P < 0.01) decrease at the end of the 4th week and significant (P < 0.01) increase at the end of the 6th week compared to that of the untreated control group at the end of the corresponding studied weeks.

This fluctuation of albumin values may be due to interaction of CCl_3 with protein molecules leading to an impairment of cellular processes (Chung et al., 2010). The results obtained in previous studies (Dwivedi et al., 1990; Braide, 1991) agreed with that of the current study which showed a similar rise in the levels of ALT, AST, ALP and LDH after injecting rats subcutaneously with CCl_4 three times per week to induce liver injury. The results of Bahar et al. (2003) agreed with that of the present study, as they reported that a marked elevation in the serum levels of liver enzymes ALT, AST and ALP in CCl_4 treated animals compared to that of the normal control animals.

El-Dosuky et al. (1982) findings showed a significant elevation in serum values of ALT and AST in rats exposed to a single toxic non-fatal dose of CCl_4. Anupam et al. (1995) reported that a significant elevation in serum levels of transaminases, lactate dehydrogenase and alkaline phosphatase within a full day exposure of the mice to one dose of CCl_4 led to considerable hepatocellular damage. Agarwal and Meheadle (1983) stated that exposure to CCl_4 caused increase of ALT and AST values in a dose dependent manner.

It has been reported that dandelion supplements has antioxidant activity (Soo-Yeul et al., 2002). Many investigations have attempted to isolate some compounds in dandelion and evaluate their bioactive roles because this plant has been used for a long time as a folklore medicine; these bioactive compounds were identified as luteolin, chicoric acid, chlorogenic acid, and chrysoeriol (Hu and Kitts, 2005). Among these compounds, luteolin and chicoric acid are antioxidants and play diverse roles for the prevention of inflammation (Chen et al., 2007). The anti-inflammatory activity of Luteolin has been shown to be active via NF-$_K$B and activator protein-1 modulation in LPS-stimulated RAW 264.7 cells (Chen et al., 2007). It has been shown that chicoric acid, a derivative of caffeic acid, has the most powerful antioxidant activity among reference compounds such as echinacoside, caffeic acid, and rosmarinic acid (Dalby et al., 2005).

In the current study, we further evaluated the protective effect of DLWE against CCl_4-induced hepatotoxication in albino rats. Pretreatment with DLWE attenuated the significant increase in liver enzyme activities caused by CCl_4 administration compared to that of the CCl_4 treated group (+ve control) at the end of the 2nd, 4th and the 6th weeks of the study and subsequent recovery towards normalization of these enzymes suggests that dandelion extract is capable to condition the hepatic cells with subsequent acceleration of parenchymal cells regeneration, thus protecting against membrane fragility consequently, and minimizing the leakage of liver enzymes into

enzymes into the blood circulation. The highly significant (P < 0.01) decrease of urea levels in the dandelion protected group compared to that of the CCl_4 treated group at the end of the 2nd, 4th and the 6th weeks of the study is a further clear evidence of the improvement of the functional status of the liver cells. Meanwhile, we could not explain the fluctuation of the serum albumin of the dandelion protected group compared to that of the CCl_4 treated group at the end of the studied weeks of the present study. The results of the current study came in agreement with that of Chung et al. (2010) who reported that serum levels of AST, ALT and LDH in previously exposed Sprague-Dawley rats to CCl_4 -induced heptotoxication significantly decreased after treatment with common DLWE in a dose dependent manner.

The effect of supplementation of dandelion on the genomic DNA integrity of different CCl_4 treated rats livers in addition of the genomic DNA integrity of untreated animal liver (-ve control) as well as the genomic DNA integrity of CCl_4 treated animal liver (+ve control) 2 weeks post CCl_4 treatment, were demonstrated in Figure 1, where lane 1 represents DNA marker, lane 2 represents the genomic DNA of untreated animal liver (-ve control) and seems to be intact, but the genomic DNA of lane 3 is disintegrated as it represents the genomic DNA of CCl_4 treated animal liver (+ve control) 2 weeks post CCl_4 treatment. The other nine lanes represent the genomic DNA of dandelion protected animals against CCl_4 with different responses towards dandelion hepatoprotection at the end of the studied weeks of the experiment.

The results of the present study revealed that the genomic DNA of CCl_4 treated group (+ve control) at the end of the 2nd week (Figure 1, lane 3) showed a massive degradation compared to the untreated (-ve control) group (lane 2). This indicates that CCl_4 administration led to DNA damage to this group at the end of the 2nd week. This result came in agreement with Chung et al. (2010) who reported that CCl_4 is metabolized to trichloromethyl radical ($\cdot CCl_3$) by liver microsomal cytochrome P450 isozymes in the endoplasmic reticulum, and this radical can interact with critical target molecules (nucleic acids, proteins, lipids and fatty acids) with subsequent impairment of cellular processes such as DNA and lipid metabolism.

The genomic DNAs of dandelion protected animals against CCl_4-induced hepatotoxicity at the end of the 2nd week (Figure 1, lanes 4, 5 and 6) suffer from variable degrees of degradation and seem to be less disintegrated compared to CCl_4 treated group (+ve control). While, the degradation of the genomic DNAs of dandelion protected animals against CCl_4 intoxication continued to increase at the end of the 4th week (Figure 1, lanes 7, 8 and 9) leading to almost complete degradation of the genomic DNA of this group. But, at the end of the 6th week of the study, the picture has been changed, where the genomic DNAs of dandelion protected animals against CCl_4 intoxication (Figure 1, lanes 10, 11 and 12) have been

significantly improved and repaired compared to the genomic DNAs of dandelion protected animals at the end of both 2nd and 4th weeks, and compared to the genomic DNA of CCl_4 treated animal liver (+ve control) as well. This DNA repair could be due to the cumulative hepatoprotective effect of dandelion. Oral administration of DLWE enhanced the rate of DNA recovery following exposure to CCl_4 hepatotoxication (Van Loon et al., 1993).

The enhanced recovery of the genomic DNAs following DLWE administration mirrors results that we have obtained on the gel. This indicates that the hepatic protection of DLWE on the molecular level was effective and stronger at the end of the 6th week of the study, while at the end of both the 2nd and 4th weeks of the study, the hepatic protection of DLWE was not efficient enough and this may be due to excessive oxidative damage caused by CCl_4 in the absence of proper DNA repair (Van Loon et al., 1993).

The results of the current study revealed that the efficacy of dandelion (*T. officinale*) leaves water extract as a hepatoprotective agent on DNA integrity against CCL4 hepatotoxic effect was insignificant at the end of the 2nd and 4th weeks as it is demonstrated through the partial degradation of genomic DNA on the agarose gel electrophoresis (Figure 1, lanes 4 to 9), while at the end of the 6th week, the genomic DNA integrity of dandelion protected animals livers was significantly improved and the genomic DNA became more intact as it is demonstrated on the agarose gel electrophoresis (Figure 1, lanes 10 to 12). This indicates that dandelion (*T. officinale*) had time-dependent hepatoprotective effect against the hepatotoxic effect of CCl_4. Schütz et al. (2006) reported that dandelion extract has powerful hydrogen peroxide scavenging ability due to its high polyphenol content.

Hu and Kitts (2004) reported that dandelion flower extracts has the capability to scavenge reactive oxygen species (ROS) and prevent DNA from ROS-induced damage *in vitro*. Suppression of oxidative stress by dandelion has been attributed to luteolin and luteolin-7-Oglucoside. Hu and Kitts (2004) stated that the concentrations of luteolin and luteolin-7-Oglucoside lower than 20 μm could significantly suppress inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) protein expression in lipopolysaccharide (LPS) activated RAW 264.7 cells. These investigations support that dandelion being an antioxidant agent and an anti-inflammatory could be a potent hepatoprotective material. The dietary antioxidant is able to modulate DNA repair via redox-sensitive pathways that ultimately influence nucleotide or base excision repair, or transcription-coupled repair (Lunec et al., 2002).

The biochemical results of the present study were associated with histopathological examination sections of five rat livers. The histopathological examination results of the livers of the normal rats fed on normal standard diet (-ve control) showed normal histological picture. Liver examination of CCl_4 treated rats showed insignificant (P > 0.05) progress in the grades of portal inflammation, necrosis, fibrosis and steatosis at the end of the 2nd and 4th weeks of the study compared to that of the negative control rats at the end of the same corresponding studied weeks. While, at the end of the 6th week, the grades of portal inflammation, necrosis, fibrosis and steatosis showed highly significant (P < 0.01) increase in CCl_4 treated rats compared to that of the negative control rats at the end of the same studied week. These results came in agreement with that of Maurizio et al. (1992) who stated that livers of rats fed on normal standard diet and treated with CCl_4 for long periods revealed the typical cirrhotic appearance including extensive remodeling associated with the existence of fibrous septa which contain a heterogeneous population of non-parenchymal cells. Also, Maurizio et al. (1992) reported massive and severe cells injury, coagulative necrosis, inflammatory infiltration and fatty metamorphosis were clearly observed, and associated with a variable degree of hyperplasia of biliary epithelium.

The present histopathological results of necrosis came in accordance with that of Weber et al. (2003) who reported that during the metabolism of CCl_4, it generates free radicals that attack microsomal lipids and proteins resulting in necrosis of hepatocytes as a consequence of lipid peroxidation.

Liver examination of dandelion protected animals' livers showed insignificant (P > 0.05) improvement in portal inflammation, necrosis, fibrosis and steatosis at the end of 2nd and 4th weeks of the study compared to that of the CCl_4 treated rat livers (+ve control) at the end of the same corresponding studied weeks. While, at the end of the 6th week of the study, portal inflammation, necrosis, fibrosis and steatosis showed highly significant (P < 0.01) improvement in dandelion protected animals' livers compared to that of the CCl_4 treated rats (+ve control) at the end of the same studied week. These histopathological findings came in agreement with that of Chung et al. (2010) who reported that DLWE protection against hepatic damage induced by CCl_4 is achieved through the modulation of inflammatory responses and oxidative status, thus DLWE seems to be an efficient therapeutic agent that prevents and treats CCl_4-induced hepatic injury.

Another study by Soo-Yeul et al. (2002) agreed with the current findings of steatosis, as they stated that dandelion supplementation could be beneficial for improvement of the lipid metabolism. The mechanism underlying the protective action against oxidation and the lipid-lowering action of a DWE supplement remains to be defined. Also, the present findings of the hepatic steatosis matched with that of Ung-Kyu et al. (2010) who proved that treatment with dandelion root and leaf markedly changed plasma antioxidant enzyme activities and lipid profiles in cholesterol-fed rabbits, and thus may have potential

hypolipidemic and antioxidant effects.

Dandelion root and leaf could protect against oxidative stress linked atherosclerosis and decrease the atherogenic index (Ung-Kyu et al., 2010). The current findings suggested that a DLWE supplement was associated with beneficial effects on the improvement of increased liver enzyme activities produced by CCl_4 administration.

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