



Hepatoprotective, therapeutic and *in vitro* cytotoxic activities of dandelion in comparison with silymarin

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ABSTRACT

Taraxacum officinale, or dandelion, has been used in folk medicine in the treatment of several diseases. The aim of this study is to evaluate the hepatoprotective effects of dandelion extract in comparison to silymarin both *in vivo* and *in vitro*. Sixty rats were divided into six groups, G1: normal control group, G2: liver injured rats induced by carbon tetrachloride (CCl₄), G3: rats protected by dandelion extract, G4: rats protected by silymarin extract, G5: rats treated with dandelion extract, G6: rats treated with silymarin extract. To some extent, dandelion and silymarin were equally effective in reducing serum markers of liver damage as well as oxidative stress. Histopathological examination of liver sections confirmed these results. The two compounds showed cytotoxic activity on HepG2. Dandelion exerts an effective hepatoprotective property in experimental liver injury to a significant degree as standards of silymarin.

Introduction

The liver plays a pivotal role in the metabolism and detoxification of endogenous and exogenous hepatotoxicants in the body, and these metabolic reactions can also potentially lead to liver injury.

Carbon tetrachloride (CCl₄) is a widely used industrial solvent and it is a xenobiotic that can induce liver injury. It has been widely used in terrestrial animal models to investigate hepatotoxicity [1]. The most remarkable pathological characteristics of CCl₄-induced hepatotoxicity are fatty liver, cirrhosis, and necrosis, which have been thought to result from the formation of reactive intermediates such as trichloromethyl radical ($\bullet\text{CCl}_3$) and trichloromethylperoxy radical ($\bullet\text{OCCl}_3$) [2]. These free radicals lead to the peroxidation of fatty acids found in the phospholipids making up the cell membranes. Consequently, cell membrane structures and intracellular organelle membrane structures are completely broken down and finally induce corresponding health problems [3].

The general strategy for prevention and treatment of liver damage; includes reducing the production of reactive metabolites and inhibiting the generation of free radicals using antioxidants [4].

In recent years, with the speeding up of the rhythm of lifestyles and changing diets, the risk of liver disease has been increased greatly and is frequently fatal. Several

hepatoprotective agents have been applied in clinical practice, but some of them have potential adverse effects. On this basis, the use of natural antioxidant phytochemicals has surfaced as an effective and safe dietary reference for liver diseases [5].

Common dandelion (*Taraxacum officinale*, Asteraceae), a widely distributed perennial weed in the Northern Hemisphere, has been used in folklore to treat diverse ailments such as dyspepsia, heartburn, spleen disorders and liver disorders [6]. Dandelion leaf is clinically used in various preparations, including infusions and ethanolic extracts and is added to salads because of its significant source of potassium, vitamins, and minerals. Dried dandelion leaves and roots are also available as herbal teas [7].

Flavonoids and phenolic compounds (such as luteolin, caffeic acid, and chlorogenic acid) have been detected in extracts of dandelion. These compounds, which are generally found in plants, have been reported to protect cells from oxidative stress by preventing the formation of free radicals or by detoxifying free radicals, resulting in the protection from a variety of pathophysiological processes [8].

Since common dandelion is often used as herbal tea, this study used dandelion hot water extract (DWE). Hence, the protective effects of DWE were evaluated in rats treated with CCl₄. Silymarin, a natural hepatoprotective agent, was included as a reference control standard.

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Materials and methods

Materials:

Dandelion and silymarin extracts were kindly provided as a gift sample from Egypharmaceutical company, Egypt. All chemicals used in this study were purchased from Sigma Chemical Co. (St. Louis, MO, USA) and Merck (Darmstadt, Germany). All other reagents kits were obtained from Biodiagnostic Company.

Dandelion, was dissolved in sterile H₂O (100 mg/ml) at room temperature for 2 h and then extracted twice with water at 100°C for 1 h. The aqueous extracts were filtered through a 0.45 µm membrane. This aqueous dandelion extract lyophilized and the resulting light yellow powder (17% w/w yield) [9]. The hot-water extract of silymarin was prepared by boiling with distilled water for 1 h. The extract was filtered, freeze-dried, and kept at 4°C. The yield of extraction was 16.73% [10].

Animals:

Sixty adult male rats were obtained from the Egyptian Company for vaccines & medicines (center to produce natural toxin, Helwan farm). Rats were acclimated to the experimental facility for 1 week and housed in stainless steel cages (12 h dark/light cycle) an ambient temperature of 25° C and relative humidity of 55%. Animal care was followed as recommended by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Induction of liver injury by CCl₄:

Liver injury was induced according to the method of **Bhadoria et al.** [11] with slight modifications. Rats were injected intraperitoneally with freshly prepared CCl₄ in olive oil (1:1 V/V, 2ml/kg) twice weekly for one week.

Study design:

Rats were randomly assorted into six groups: G1) rats were injected with olive oil and served as normal controls; G2) rats were intoxicated by CCl₄ and served as positive controls; G3) rats received dandelion extract (25 mg/kg daily) [6,12] orally, for two weeks and then intoxicated with CCl₄; G4) rats received silymarin extract (50 mg/kg daily) [13,14] orally, for two weeks and then intoxicated with CCl₄; G5) CCl₄ intoxicated rats treated with dandelion extract for two weeks; and G6) CCl₄ intoxicated rats treated with silymarin extract for two weeks.

Samples collection:

At the end of the experiment, blood samples were taken by heart puncture under light ether anesthesia after a fast of 12 h. Serum was separated from the clotted blood samples by centrifugation at 5000 rpm for 5 min and then aliquot and analyzed. The liver was dissected out, rinsed in isotonic sterile saline, blotted dry on a filter paper, and a small part from the right lobe of the liver was cut and fixed in 10% buffered formalin (pH 7.2) at 4° C for histologic examination.

Preparation of liver homogenate:

Twenty percent whole liver homogenate was prepared in phosphate buffered saline (pH 7.4). Malondialdehyde (MDA) content, total protein, DNA and RNA concen-

trations were estimated in the prepared homogenate. The MDA content was estimated according to method of **Esterbauer and Cheesman** [15]. Total protein concentration was estimated in the whole tissue homogenates by the dye binding method of **Bradford** [16]. Total DNA and RNA concentrations were extracted from the whole tissue homogenate by 5% perchloric acid according to the method of **Schneider** [17] and then determined colorimetrically by the diphenylamine [18] and orcinol [19] methods, respectively.

Biochemical assays:

Serum alanine and aspartate aminotransferase activities were determined by the colorimetric method of **Reitman and Frankel** [20]. Alkaline phosphatase (ALP) activity was determined by the colorimetric method of **Kind and King** [21]. Bilirubin was estimated according to the method of **Jacobsen** [22]. 5' nucleotidase (5' NT) activity was measured according to the method of **Ellis and Goldberg** [23]. Hydroxyproline contents were estimated in the blood samples by the methods of **Domitrović et al.** [24]. Total protein concentration was estimated using the method of **Bradford** [16]. Serum albumin was colorimetrically determined using a commercial assay kit by the method of **Doumaset et al.** [25].

Histopathological examination:

Specimens were cleared in xylene and embedded in paraffin at 56 degree in hot air oven for twenty four hours. Paraffin bees wax tissue blocks were prepared for sectioning at 4 microns by slide microtome. The obtained tissue sections were collected on glass slides, deparaffinized and stained by hematoxylin and eosin stains [26] for histopathological examination under a light microscope.

Cytotoxic activity:

In vitro cytotoxic potentialities of dandelion and silymarin were carried out on liver cancer cells (Hep G2) according to method of **Skehan et al.** [27]. Cells were plated in 96-multiwell plate for 24 hours before treatment with the tested extracts, to allow attachment of cell to the wall of the plate. Different concentrations of the tested (0-100 µg/ml) extracts were added to the cell monolayer. Triplicate wells were prepared for each individual dose. Monolayer cells were incubated with the extracts for 48 hours at 37°C in 5% CO₂ atmosphere. After 48 hours, cells were fixed, washed and stained with sulforhodamine B (SRB) stain. Excess stain was washed with acetic acid and attached stain was recovered with Tris EDTA buffer. Color intensity was measured in an ELISA reader at λ max 564 nm. The relation between surviving fraction and extract concentration was plotted to get the survival curve of the tumor cell line. Also, the IC₅₀ for each tested extracts was calculated.

Statistical analysis:

Data were expressed as mean ± standard deviation. Results of biochemical studies were statistically analyzed using one-way analysis of variance (ANOVA). All statistics were done using SPSS 17 for

Windows (PSS, Inc., Chicago, IL, USA). Differences were considered statistically significant at $P < 0.05$.

Results

Results presented in **Tables 1-3** and **Figures 1 and 2** showed that, carbon tetrachloride intoxication in G2 caused a dramatic increase in serum conjugated bilirubin (120%), ALT (244.3%), AST (190.4%), 5' NT (123.5%), and ALP (60.6%) activities compared with normal rats. With regard to their concentrations in normal control group, serum levels of total protein, and albumin were significantly decreased in CCl₄ intoxicated rats by 41.7%, and 47.5%, respectively. In contrast, serum level of hydroxyproline was significantly increased when compared with its level in normal control group ($p < 0.001$).

Prophylactic administration of dandelion (G3) caused a decrease in conjugated bilirubin (15.6%), ALT (15.1%), AST (4.1%), 5' NT (22.2%), and ALP (10%) activities compared with CCl₄ intoxicated rats (**Table 1**). In the meantime, pretreatment by dandelion impact positively on total protein, and albumin levels where dandelion increase those levels by 21% and 26.9% respectively as compared to positive control. Oral administration of dandelion before CCl₄ intoxication caused non-significant decrease in hydroxyproline level.

Implemented dose of silymarin before intoxication (G4)

caused marked reduction in serum levels of conjugated bilirubin, ALT, AST, 5' NT, and ALP when compared with those levels of positive control rats in G2. Silymarin administration has precautionary activity where, it elevates serum levels of total protein, and albumin when compared to positive control as received before hepatic injury. Pretreatment by silymarin exhibited significant reduction in hydroxyproline level (32.3%) when compared with its level in positive control group.

The oral treatment of intoxicated rats with dandelion (G5) significantly improve the serum levels of conjugated bilirubin, ALT, AST, 5' NT, and ALP by 40.2%, 64.5%, 58.9%, 39.2% and 25.7%, respectively. Sequential administration of dandelion extract and CCl₄ to rats restored serum protein fractions to normal levels. The same approach in normalization was observed in the serum hydroxyproline which markedly decreased by 49% when compared with positive control rats (**Table 2**).

Therapeutic administration of silymarin in liver injured rats (G6) improved the averages of conjugated bilirubin, ALT, AST, 5' NT, and ALP when compared with positive control. Remedial dose of silymarin also, optimized serum levels of total protein and albumin to normal levels by 68.5% and 60.9% respectively as compared to positive control. In the same context, significant decrease in hydroxyproline level ($p < 0.001$) was observed in silymarin treatment group.

Table 1: Effect of silymarin and dandelion extracts on serum liver enzymes in different rat groups.

Parameter Groups	ALT (U/L)	AST (U/L)	5' NT (U/L)	ALP (U/L)
Group 1 Mean ± S.D.	35.38 ± 2.09 ^b	52.05 ± 2.16 ^b	14.43 ± 1.40	114.17 ± 6.76
Group 2 Mean ± S.D % change # G1	121.83 ± 20.13 ^a 244.3%	151.16 ± 19.32 ^a 190.4%	32.25 ± 4.46 ^{a, b} 123.5%	183.37 ± 19.63 ^{a, b} 60.6%
Group 3 Mean ± S.D. % change # G1 % change # G2	103.37 ± 10.76 ^{a, b} 192.1% 15.1%	145.01 ± 26.9 ^a 178.6% 4.1%	25.10 ± 5.24 ^{a, b} 73.9% 22.2%	165.01 ± 19.92 ^{a, b} 44.5% 10.0%
Group 4 Mean ± S.D. % change # G1 % change # G2	47.18 ± 5.10 ^{a, b} 33.35% 61.3%	72.42 ± 7.10 ^{a, b} 39.1% 52.1%	17.27 ± 1.65 ^b 19.7% 46.4%	155.94 ± 14.40 ^{a, b} 36.6% 14.9%
Group 5 Mean ± S.D. % change # G1 % change # G2	43.31 ± 5.91 ^b 22.4% 64.5%	62.01 ± 5.13 ^b 19.13% 58.9%	19.60 ± 3.59 ^{a, b} 35.8% 39.2%	136.31 ± 3.91 ^{a, b} 19.4% 25.7%
Group 6 Mean ± S.D. % change # G1 % change # G2	40.42 ± 5.05 ^b 14.25% 66.8%	58.81 ± 8.74 ^b 12.9% 61.1%	18.07 ± 3.24 ^b 25.2% 43.9%	124.8 ± 11.84 ^b 9.3% 31.9%

Values are mean ± SD for 10 animals in each group.

a: Significant difference at $p < 0.05$ compared with control group (G1).

b: Significant difference at $p < 0.05$ compared with CCl₄ treated animals (G 2).

ALT: alanine transferase; AST: aspartate transferase; 5' NT: 5' nucleotidase; ALP: alkaline phosphatase.

Table 2: Effect of silymarin and dandelion extracts on serum direct bilirubin, hydroxyproline, total proteins and albumin) in different rat groups.

Parameter Groups	DB (mg %)	HYP (mg %)	TP (g %)	Alb (g %)
Group 1 Mean \pm S.D.	0.35 \pm 0.07 ^b	1.02 \pm 0.29 ^b	7.51 \pm 0.90 ^b	3.75 \pm 0.51 ^b
Group 2 Mean \pm S.D. % change # G1	0.77 \pm 0.07 ^a 120%	2.57 \pm 0.46 ^a 151.9%	4.38 \pm 0.87 ^a 41.7%	1.97 \pm 0.54 ^a 47.5%
Group 3 Mean \pm S.D. % change # G1 % change # G2	0.65 \pm 0.19 ^a 85.7% 15.6%	2.34 \pm 0.82 ^a 129.4% 8.9%	5.30 \pm 0.77 ^a 29.4% 21.0%	2.50 \pm 0.43 ^a 33.3% 26.9%
Group 4 Mean \pm S.D. % change # G1 % change # G2	0.49 \pm 0.09 ^{a, b} 40.0% 36.4%	1.74 \pm 0.74 ^{a, b} 70.6% 32.3%	6.98 \pm 0.95 ^b 7.1% 59.3%	3.07 \pm 0.50 ^{a, b} 18.1% 55.8%
Group 5 Mean \pm S.D. % change # G1 % change # G2	0.46 \pm 0.09 ^{a, b} 31.4% 40.2%	1.31 \pm 0.19 ^b 28.4% 49.0%	6.16 \pm 1.04 ^{a, b} 17.9% 40.6%	2.65 \pm 0.73 ^{a, b} 29.3% 34.5%
Group 6 Mean \pm S.D. % change # G1 % change # G2	0.41 \pm 0.10 ^b 17.1% 46.7%	1.17 \pm 0.32 ^b 14.7% 54.5%	7.38 \pm 0.57 ^b 1.7% 68.5%	3.17 \pm 0.54 ^b 15.5% 60.9%

Values are mean \pm SD for 10 animals in each group.

a: Significant difference at $p < 0.05$ compared with control group (G1).

b: Significant difference at $p < 0.05$ compared with CCl₄ treated animals (G2).

DB: direct bilirubin; TP: total proteins; HYP: hydroxyproline; Alb: albumin.

CCl₄ intoxication induced significant increases in hepatic MDA content (110.9 %); in contrast, significant decreases were recorded in total protein, RNA, and DNA concentrations (60.7 %, 54 %, and 68.1 %, respectively) compared with the normal control rats. All of the aforementioned parameters were slightly normalized in the rest groups that receiving silymarin and dandelion pre or post hepatic injury by CCl₄ (**Table 3**).

Histologic examination of liver sections from all rats is presented in **Figure 1**, showed liver section of healthy control rats with normal hepatic lobular architecture, showing structural integrity (**Fig. 1a**). On the contrary, liver sections of CCl₄ intoxicated rats (**Fig. 1b**) showed ballooning degeneration of hepatocytes with foci of dysplasia with steatosis. Bile duct damage and infiltration by chronic inflammatory cells were also seen. Conversely, liver sections of the dandelion protective group (G3) showed nodules that vary in size in which there are many lymphocytes and plasma cells (**Fig. 1c**). In the meantime, Liver sections of the prophylactic group with silymarin (G4) showed minimal parenchymal spotty inflammation and mild portal tract inflammation (**Fig. 1d**). In contrast, Liver sections of the curative group supplemented by dandelion (G5) showed the mature fibrous tissue (**Fig. 1e**). While, liver sections of the therapeutic group treated with silymarin (G6)

showed very mild parenchymal and very mild portal tract inflammation (**Fig. 1f**).

The investigated cytotoxic activity of the tested extracts against HepG2 cell lines showed dose-dependent reduction of the IC₅₀ values by 38.04 μ g and 12.84 μ g for dandelion and silymarin, respectively (**Fig. 2**).

Discussion

In the present study, the hepatoprotective and therapeutic effects of dandelion and silymarin extracts were investigated against CCl₄-induced hepatotoxicity in rats. CCl₄ is considered a potent hepatotoxin, which is frequently used to produce an experimental model to study the mechanisms involved in the progression of hepatic disease and the impact of various drugs on this progression. CCl₄ induces cytotoxic injury through bioactivation mediated by the microsomal cytochrome P450 isozyme to trichloromethyl radical and further to a reactive metabolite. These activated radicals bind covalently to the macro-molecules and induce peroxidative degradation of membrane lipids of endoplasmic reticulum, which is rich in polyunsaturated fatty acids. This leads to the formation of lipid peroxides [28,29].

One of the most sensitive indicators of hepatocyte injury is the release of intracellular enzymes in the circulation. There is a direct correlation between rise in these serum enzyme activities and severity of damage to liver tissue [30].

Table 3: Effect of silymarin and dandelion extracts on hepatic biochemical parameters in different rat groups.

Parameter Groups	MDA (nmol/g)	TP (mg/g)	RNA (mg/g)	DNA (mg/g)
Group 1 Mean ± S.D.	21.25 ± 1.47 ^b	153.9 ± 24.21 ^b	6.93 ± 0.38 ^b	3.10 ± 0.27 ^b
Group 2 Mean ± S.D. % change # G1	44.82 ± 6.23 ^a 110.9%	60.41 ± 4.75 ^a 60.7%	3.17 ± 0.41 ^a 54.0%	0.99 ± 0.33 ^a 68.1%
Group 3 Mean ± S.D. % change # G1 % change # G2	39.23 ± 5.44 ^{a,b} 84.6% 12.5%	76.82 ± 4.32 ^{a,b} 50.1% 27.1%	3.75 ± 0.14 ^{a,b} 45.6% 18.3%	1.35 ± 0.09 ^{a,b} 56.4% 36.3%
Group 4 Mean ± S.D. % change # G1 % change # G2	32.94 ± 5.86 ^{a,b} 55% 26.5%	88.42 ± 9.74 ^{a,b} 42.5% 46.3%	4.09 ± 0.23 ^{a,b} 40.7% 29.0%	1.73 ± 0.32 ^{a,b} 44.2% 74.7%
Group 5 Mean ± S.D. % change # G1 % change # G2	27.16 ± 1.51 ^{a, b} 27.8% 39.4%	95.70 ± 10.15 ^{a,b} 37.8% 58.4%	4.32 ± 0.18 37.4% 36.3%	2.01 ± 0.40 ^{a,b} 35.1% 103%
Group 6 Mean ± S.D. % change # G1 % change # G2	23.51 ± 3.10 ^{a,b} 10.6% 47.5%	124.54 ± 12.43 ^{a,b} 19.1% 106.1%	4.87 ± 0.61 ^{a,b} 29.4% 53.6%	2.97 ± 0.25 ^{a,b} 4.2% 200%

Values are mean ± SD for 10 animals in each group.

a: Significant difference at p< 0.05 compared with control group (G1).

b: Significant difference at p< 0.05 compared with CCl₄ treated animals (G 2).

MDA: malondialdehyde; TP: total proteins.

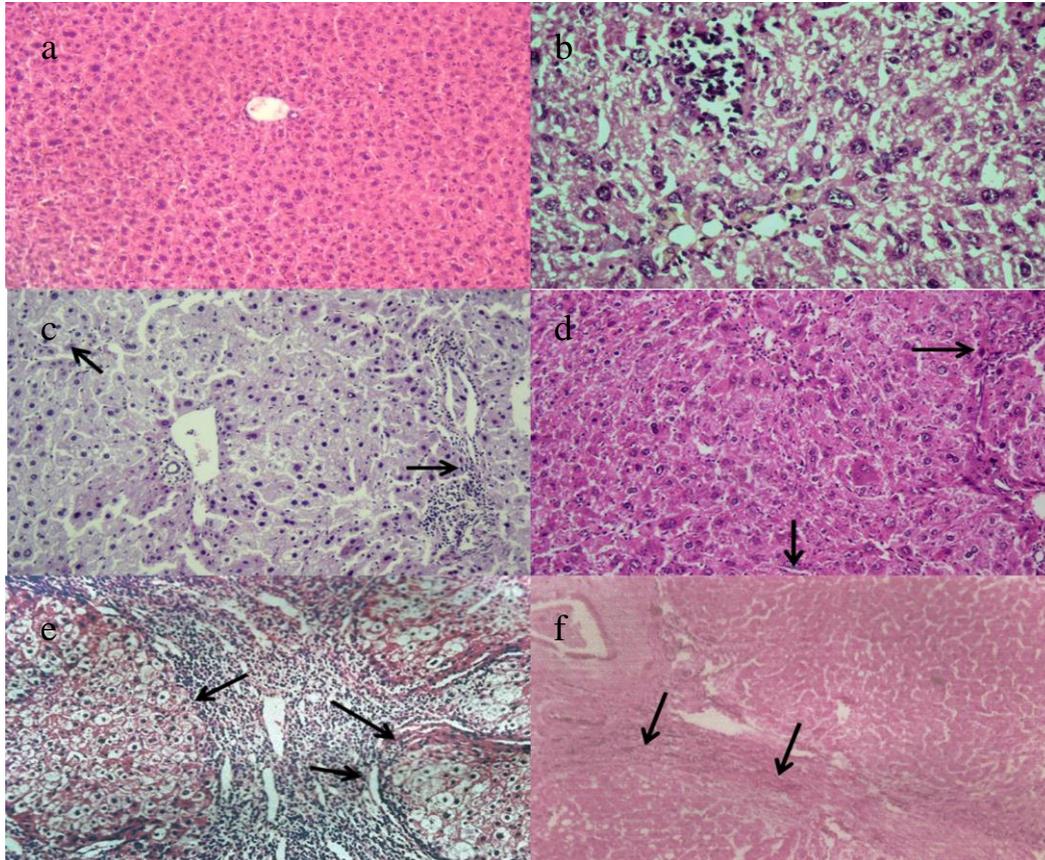


Fig. 1: Histopathological examination of liver tissues. (a) normal control, (b) positive control, (c) prophylactic group receiving silymarin, (d) therapeutic group receiving silymarin, (e) prophylactic group receiving dandelion and (f) therapeutic group receiving dandelion.

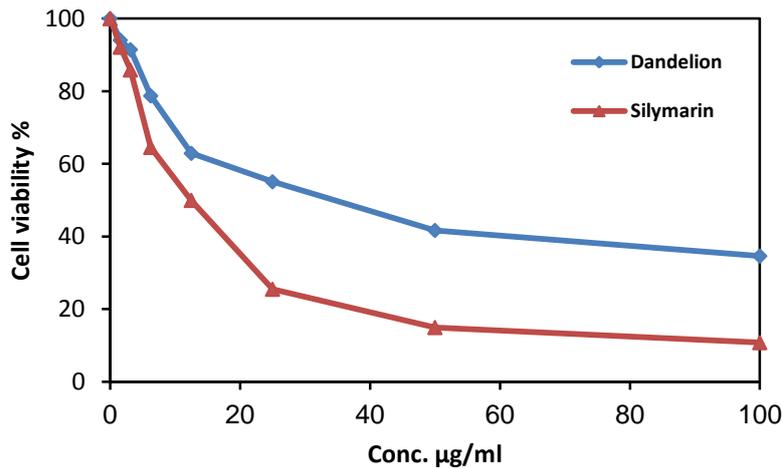


Fig. 2: Cytotoxic activity of silymarin and dandelion extracts against hepatocellular carcinoma (HepG2) cells.

Direct evidence of CCl_4 hepatotoxicity was noted in the occurrence of marked increase in the level of serum conjugated bilirubin and also in ALT, AST and ALP activities. This dramatic increase provided evidence of intrahepatic biliary obstruction, which was confirmed by histopathological examination of liver sections. These results are in agreement with many findings [31,32]. Prophylactic administration of dandelion and silymarin substantially attenuated the hepatotoxic effect of CCl_4 as evident by the normalization of serum bilirubin, ALT, AST and ALP activities. In addition, examination of liver sections showed slight alterations in the hepatocytes. On the same line, Therapeutic administration of dandelion and silymarin extracts improves the averages of the mentioned parameters but by greater extent. The hepatic improvement may be due to the prevention of the leakage of intracellular enzymes by stabilizing membrane activity. This is in agreement with **Palanivel et al.** [33]. The author reported that the healing of hepatic parenchyma and the regeneration of hepatocytes is implicated in liver improvement.

Several overviews have covered the multiple pathophysiological roles of 5'-nucleotidase. They reveal novel insight into its functional involvement in endothelial and fluid transport; endothelial barrier function; adaption to hypoxia and ischemic preconditioning; the cardiovascular system; lung, liver, and kidney function [34]; the airways [35]; immunity and inflammation [36]; leucocyte trafficking [37]; the nervous system [38], including a novel role of 5'-nucleotidase in inhibiting nociception [39]; or the immune control of cancer [40]. 5'-nucleotidase is a component of the multi-resistance machinery of tumor cells against immune surveillance and/or anticancer therapy [41].

Forms of released 5'-nucleotidase may have different cellular origins. The serum form presumably is derived from the liver [42]. The elevation in its level in CCl_4 intoxicated rats could be due to the hepatic fibrosis, where **Peng et al.** [43] reported that extracellular forma-

tion of adenosine by the action of 5'-nucleotidase plays a critical role in the development of hepatic fibrosis.

Since the metabolism of proteins, synthesis of amino acid and urea occurs in the liver; we anticipated alterations in the concentrations of these materials in liver disease. Presumably, these alterations are more marked in chronic conditions, as in liver cirrhosis. Reduced level of albumin usually follows the severity of the condition, and it is a reliable index of the prognosis [44]. Generally, the concentration of serum proteins can be used as a measurement of liver function, especially when they are compared with proteins that are not produced in the liver. CCl_4 intoxication significantly decreased serum total protein and albumin concentrations. Comparable results were previously reported and explained [45,46]. The more valuable observations in our study are the normalization of serum total protein, and albumin in different groups receiving dandelion or silymarin before or after liver injury by CCl_4 . these results are in matching with previous researches [14,47]. We suggest that, the hepatoprotective activities of the two extracts are due to their content of phenolic acids. Phenolic acids have been reported to induce phase II detoxifying enzymes, which suppress CCl_4 -active metabolites.

Liver fibrosis involves abnormal synthesis and the accumulation of extracellular matrix proteins, in particular collagen, in the liver parenchyma by activated hepatic stellate cells [48]. Matrix metalloproteinases (MMPs) comprise a family of zinc-dependent enzymes that specifically degrade extracellular matrix components like collagen. The activity of MMPs is regulated by tissue inhibitors of metalloproteinases (TIMPs). Sustained TIMPs expression is a key factor resulting in the progression of fibrosis [49]. TIMPs are produced by Kupffer cells, hepatic stellate cells and myofibroblasts in liver, but they are mainly produced by activated hepatic stellate cells. Injured liver tissue expresses more TIMP-1, which in turn causes interstitial fibrils to accumulate. Hydroxyproline is an amino acid unique to all collagens

and its level reflects the amount of collagen present and therefore can be used to determine the extent of fibrosis [50]. CCl₄ intoxication caused a dramatic increase in hepatic hydroxyproline content compared with normal controls, which was supported by the presence of fibrosis. Parallel findings have been reported previously [51]. Implemented prophylactic and therapeutic doses of dandelion and silymarin normalized the hepatic hydroxyproline content and decreased the liver fibrotic pattern.

The polyphenolic compounds, in the two extracts, have a high scavenging activity for free radicals and active oxygen species produced by CCl₄ intoxication, thus suppressing hepatic stellate cells activation. Also, these results suggest that the anti-fibrotic effect of both dandelion and silymarin is related to increased removal of deposited collagen, that is, enhanced collagenolytic activity due to decreased TIMP-1 levels.

The estimation of MDA, a byproduct of peroxidation of biological membrane polyunsaturated fatty acids, is used as an indicator of oxidative stress [52]. The CCl₄ intoxication induced an intense oxidative stress as evident by the increased hepatic MDA. Comparable findings have been reported previously [53]. The effect of the two extracts (in both protected and treated groups) on the suppression of free radicals is evident by the decreased MDA level.

The human body contains many molecules (lipids, DNA, protein, vitamins, and carbohydrates) that are susceptible to attack by reactive oxygen species, causing many human diseases. Antioxidants inhibit lipid peroxidation but may not protect other molecules, such as nucleic acids and protein, against oxidative damage. In the present study, the CCl₄-induced oxidative stress caused significant decreases in liver DNA, RNA, and total protein concentrations. The significant decreased nucleic acids suggest that ROS produced by CCl₄ intoxication caused tissue DNA oxidative damage. In the meantime, the significant decrease in hepatic total protein concentration might be due to hypertrophy of the liver, a smaller number of functioning hepatocytes owing to fibrosis, and fatty liver. In the protective and therapeutic groups, hepatic total protein, DNA, and RNA contents were normalized to lesser extent. The significant improvement at the level of biochemical parameters indicates the beneficial effects of the selected plant extract in protecting the liver.

Cancer-related research is conducted worldwide every day, since cancer is a leading cause of death. These studies often involve the investigation of the effects of biologically active substances on cancer cells, and they frequently originate from plants. There is a great need to examine reliable and inexhaustible sources of natural substances. In addition, it is important to understand the mechanisms of anticancer agents for future application in cancer therapy.

Consumption of foods that contain phenolics and flavonoids has been shown to reduce the risk of cancer [54, 55]. In addition, several studies have indicated high cytotoxic and anticancer activity of flavonoids [56, 57].

Dandelion and silymarin extracts shown cytotoxic activity on Hep G2 cells. The best cytotoxic activity was observed with silymarin (IC₅₀=12.84 µg) when compared with dandelion which showed antiproliferative activity with IC₅₀=38.04 µg. The results indicate that, both dandelion and silymarin extracts are considered to be a particularly valuable source of effective anti-proliferative and cytotoxic substances that having anticancer activities may include inhibition of cell growth, inhibition of protein kinase activities, and induction of apoptosis.

In conclusion dandelion extract in comparison to silymarin extract to some extent exhibited significant hepatoprotective activity against CCl₄ induced liver injury in rats, demonstrated by an amelioration of liver function tests and substantial improvement in the hepatocellular architecture. The hepatoprotection could be attributed to their contents; active phenolic compounds with antioxidant and antiproliferative activities. So we recommend that, dandelion could be used as a suitable alternative to silymarin as hepatoprotective agent or may be used in combination in the treatment of hepatocellular pathologies.

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