Hepatoprotective of *Taraxacum officinale* against liver damage induced by carbon tetrachloride in male rats

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ABSTRACT

Several phytomolecules have been reported as hepatoprotective agents against many xenobiotics. In this work, we study the efficacy of the hepatoprotective activity of dandelion (*Taraxacum officinale*) against carbon tetrachloride induced liver damage in Wistar rats. Forty male albino rats were divided into five groups (8 rats each). Various liver dysfunction biomarkers were studied also deals with histopathology of liver sections. Results revealed that liver biomarkers in CCl₄-treated rats were significantly increased. In contrast, administration of alcoholic extract of dandelion herb revealed significant reduction indicating the effect of alcoholic dandelion extract in restoring the normal functional ability of hepatocytes. Histopathological parameters showed significant improvement compared to that of CCl₄ group. In the meantime, dandelion alcoholic extract contains luteolin, Leteblin glycoside, Chlorogenic acid and Cinnamic acid. In conclusion, the results obtained suggest a remarkable antihapatotoxic activity against carbon tetrachloride induced hepatotoxicity.

INTRODUCTION

The interest in phytomedicine by the professional and lay public is increasing steadily; several recent surveys from Europe and the US have demonstrated a sharp rise in the use of botanical drugs within a few years[¹, ²]. About 21% of patients attending an outpatient liver clinic had taken herbal preparations and 13% used herbs to treat liver diseases. Another survey assessed the use of complementary medicine in the USA revealed a 330% rise in the use of herbas between 1990-1997 and estimated out-of-pocket expenditures for these medicines increased from 1.8 billion in 1990 to 5.1 billion $ in 1997[³]. In Europe the expenses for production of silymarin, an herbal preparation used to treat chronic liver diseases amounts to 180 million $ in Germany, alone[⁴].

The belief that these treatments are natural and therefore safe, a feeling of better control of the disease and its management, and a holistic philosophy behind complementary medicine that pay tribute to the patients desire for wellness and quality of life in nature's womb which apparently is not provided by conventional health care [⁵]. Recently, in a review summarizing the plants with hepatoprotective activity from Iran Samani et al.[⁶] reported that ethanolic extract of dandelion was effective on decreasing serum ALT levels. Hydro alcoholic acid extract of the root enhanced levels of superoxide dismutase and catalase. You et al. [⁷] study the hepatoprotective effect of aqueous extract of dandelion root against alcoholic-induced oxidative stress. It has been reported that the complete prevention of alcoholic-induced hepatotoxicity as evidenced by the significant reduction in aspartateaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) compared to ethanol alone administered mice [⁸]. These results suggest that aqueous extract of dandelion root has protective action against toxicity in liver by elevating antioxidant potential and decreasing lipid peroxidation.
Stagos et al. [7] in their paper entitled chemoprevention of liver cancer by plant polyphenol focused their study on plant polyphenols and discuss the molecular mechanisms accounting for this activity. Moreover, Ivanov [8] demonstrated clearly that T. officinale leaves are rich source of polyphenols which possess high antioxidant properties. The author also concluded that the high yield of cichoric acid make this plant valuable source of commercial production of natural antioxidant. This antioxidant will offer many future applications in herbal medicine and nutrition fields in order to produce healthy food having healthy effect. In this context dandelion, Taraxacum officinale was first mentioned by the Arabian physicians of the tenth and eleventh centuries to treat liver and spleen ailments [9]. Germany has provided the most extensive records of the application of Taraxacum in the western world. Its uses were described to medicate gout, diarrheah, spleen and liver complaints. Therefore, the aim of this study was designed to evaluate the hepatoprotective activity of dandelion, Taraxacum officinale against liver damage induced by CCl₄ in male rat, with special reference to its major ingredients.

EXPERIMENTAL SECTION

Plant material
The seeds of dandelion were purchased from Jelitto Company Germany, then sown directly in the soil, October 2011. Samples were taken when the plant are in full blooming. Collective fresh herb was collected oven dried, powdered then extracted.

In vitro studies
Extraction
Plant samples from dried powdered plant (herb) were successively extracted with solvents with increasing polarity i.e. pet. ether (40-60), dichloromethane, ethyl acetate, then methanol. Each extract was evaporated till dryness under reduced pressure. All the extracts were weighed, kept in refrigerator for chemical and biological investigation. Extracts obtained were assayed for its polyphenolic content and its free radical scavenging activity by DPPH method [10]. The extract with high polyphenolic percentage and that produced higher scavenging activity was considered active and consequently was chemically analyzed to detect its constitutes.

Determination of polyphenolic content
Total polyphenolic content of plant extracts were evaluated using Folin-Ciocalteu method, which measure the redox properties of polyphenols in extracts, gallic acid was used as standard. The concentrations of phenolic compounds were calculated according to the following equation that was obtained from the standard gallic acid graph.

Extraction and isolation of the active constituents
Dried herb of Taraxacum officinale 1 kg were extracted with methanol 70%. The combined methanolic extracts were concentrated under reduced pressure at 45°C to yield 82g. The crude methanolic extract was suspended into hot water and successively partitioned with chloroform then n-butanol. The butanol part was evaporated till solvent free to give 3.2g. It was subjected to fractionation on polyamide column (5 x 30 cm). Elution of column started with water 100% followed by increasing methanol till 100%. The fraction eluted with methanol 70% was subjected to further purification using small sephadex LH₂₀ column eluted with 20% methanol to afford compound I and II in pure state, weighed 5.5 and 4.9 mg respectively.

The fractions eluted with 80 and 90% methanol from the polyamide column were found to be similar, so they were combined together and was further purified by preparative paper chromatography using 3MM paper and solvent system of BuOH: Acetic acid: H₂O in the ratio 4:2:1 two main zones III, IV were detected under UV light (366nm). Each zone was cut from the paper and then eluted with 80% methanol (10 sheets of 3MM paper chromatography). Each compound passed through small sephadex LH₂₀ column. Each compound was eluted with butanol saturated with water to give two pure compounds, amounted 6 and 4 mg respectively. The four compound isolated were subjected to Mass and NMR spectroscopic analyses to elucidate their structures.

HPLC analysis of the methanol extract
Methanol extract was fractionated by HPLC to detect its constituents of phenolic acids using the method of Antonella et al. [10]. The quantitation was integrated by chemostation chromatogram to a personal computer.

Estimation of IC₅₀ values
The concentrations of the extract induced 50% inhibition were determined by a linear regression analysis between the inhibition percentages against the extract concentrations by log-probit analysis.
In vivo studies
Acute oral toxicity
Healthy adult male albino rats of Wistar strain weighing 150-200 g were obtained from animal house National Research Centre, Dokki Giza, Egypt. Animals were housed in clean plastic cages in the laboratory animal room (23 ± 2°C) on the standard pellet diet and tap water *ad-libitum*, a minimum relative humidity of 40% and a 12 hr dark/light cycle were maintained. Rats were allowed to acclimate to laboratory condition for seven days prior to dosing. Plant extract was dissolved in distilled water at the following concentration 500, 1000, 1500 mg/kg b.wt. Control group was given distilled water. The experimental protocols and procedure were approved by local Ethics committee No 186 at the National Research Centre.

Biochemical assessments
The extract that recorded higher polyphenolic percentage and antioxidant effect was examined on healthy adult male albino rats of the Wistar strain weighting 150-200g. Plant extracts 500 mg/kg b.wt. was administered orally [11].

Experiential design
Animal were divided into five groups (eight animals each) as follows: control group (Group I) and was given olive oil (0.5 ml/kg b.wt.); CCl₄-treated group (Group II) was intra-peritoneal injected with CCl₄ (1: 9 in olive oil, 0.5 ml/kg b.wt.) twice a weeks for four weeks; dandelion extract group (Group III) was given dandelion extract at 500mg/ kg. b.wt. twice per week for 6 week via oral route; CCl₄-dandelion-treated group (Group IV) was treated with CCl₄ as in group II and administrated dandelion extracts at the same dose in group III and CCl₄ - Legalon drug-treated group (Group V) was treated with CCl₄ as in group II and administrated Legalon drug at dose 100 µg/kg. b.wt.

Samples preparation
Serum sample
Animal were sacrificed under anesthesia and blood samples were withdrawn from the reto-orbital venous plexus in clean and dry test tubes. Blood left 10 min to clot and centrifuged at 4°C 4000 rpm for 20 min, using Hereaus Labofuge 400 R, Kendro Laboratory Products GmbH, Germany.. The supernatant serum was collected and stored at-80°C for further tests required.

Liver homogenate
Liver were excised, trimmed of connective tissues, rinsed with saline to eliminate blood contamination, dried by blotting with filter paper and weighed. The tissues then kept at -80°C until analysis. At experiment time, a portion of liver was weighed and homogenized in saline (0.9 N NaCl) using a glass homogenizer at 4°C. The homogenates were centrifuged at 3000 rpm for 10 minutes at 4°C and the clear supernatant was used for further determination of liver function, antioxidant parameters and hepatic total protein content.

Biochemical analysis
Liver biomarkers such as aspartateaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), hepatic total protein content and bilirubin were determined. Also, hepatic glutathione, malondialdehyde and nitric oxide were determined.

Histopathological Studies
Normal histological procedures were performed on liver tissues and were stained with hematoxylin- Eosin (H&E) followed by microscopic examination for any morphological changes.

Statistical analysis
Data obtained were analyzed by one way analysis of variance (ANOVA) followed by Duncan's test for comparison using SPSS software version 17.0, the P<0.05 was considered as statistically significant.

RESULTS AND DISCUSSION

In vitro studies
Total polyphenols in different extracts of dandelion was determined and presented in table (1) in which the methanolic extract exhibited higher concentration 1.38 µg gallic acid equivalents (GAE)/g. The alcoholic extract was HPLC fractionated to detect its constituents from phenolic acids, which were presented in table (2). The concentration of phenolic compounds (gallic acid equivalents) in methanol, ethyl acetate, methylene chloride and petroleum ether extracts were found to be 1.38 µg, 0.77 µg, 0.94 µg and 0.84 µg GAEs/mg extract of *Taraxacum officinale* (Table 1).
The current results showed that alcoholic extract contains caffeic acid as the major phenolic acid 72.6 mg/g followed by ferulic 28.3 mg/g, the gallic acid amounted to 25.2 mg/g, cinnamic acid came in the fourth order and amounted to 20.8 mg/g.

Chemical investigation of the biologically active extracts

The alcoholic extract of dandelion herb proved to have the highest total polyphenolic content with higher scavenging activity. So, this extract was subjected to chemical investigation. The phytochemical screening showed that, flavonoid is the major chemical group in this plant. So, it was subjected to isolation and identification. Four compounds were purely isolated with workable amounts and were subjected to different spectral analysis to elucidate their structure.

Identification of compound 1 (Luteolin)

**Compound 1** is isolated as a pale yellow. UV (MeOH) nm: λ\text{max} (nm): MeOH (a): 252, 265, 290\text{sh}, 347; (a)+NaOMe: 266\text{sh}, 329\text{sh}, 401; ((a)+NaOAc) (b): 269, 326\text{sh}, 384; (b) + H\text{3}BO\text{3}: 259, 301\text{sh}, 370, 430\text{sh}; ((a)+A1Cl\text{3})(c): 274, 300\text{sh}, 328, 426; (c) +HCl: 266\text{sh}, 275, 294\text{sh}, 355, 385 which are identical with those given for luteolin. Also, the molecular weight of this compound was matched with a luteolin using El-MS [M]\text{+} = m/z 286.1 which is corresponding to the molecular formula C15H10O6 (Mabry et al., 1970). 1H NMR spectral data (500 MHz, DMSO-d\text{6}): δ (ppm): 7.40 (d, J = 2.1 Hz, H-2’), 7.39 (dd, J = 8.5 Hz and J = 2.1 Hz, H-6’), 6.85 (d, J = 8.5 Hz, H-5’), 6.60 (s, H-3), 6.4 (d, J = 2.5 Hz, H-8), 6.15 (d, J = 2.5 Hz, H6). 1H NMR data was in agreement with luteolin reviewed by (Patora and Klimek, 2002).

![Figure 1. Chemical structure of Luteolin](image)

**Identification of compound 2 (Luteolin-7-O-β-D-glucopyranoside)**

**Compound 2** is isolated as a faint yellow amorphous powder, m.p. 252-254°C, which possesses chromatographic properties (dark brown spot on PC, turning bright green when fumed with ammonia vapor, changing yellow with A1Cl\text{3}) UV spectral data of this compound are λ\text{max} (nm): MeOH (a): 253, 265, 346; (a)+NaOMe: 263, 300\text{sh}, 394; ((a)+NaOAc) (b): 259, 266\text{sh}, 405; (b)+H\text{3}BO\text{3}: 259, 372; ((a)+A1Cl\text{3})(c): 274, 298\text{sh}, 329, 432 and (c) +HCl: 273, 294\text{sh}, 358, 387 which are identical with those given for luteolin 7-O-glucoside (Mabry et al., 1970). This suggestion was supported by a molecular weight determination of compound 2 using El-MS (molecular weight 448 m/z, C\text{16}H\text{16}O\text{10}). The 1H-NMR spectrum (DMSO-d\text{6}), showed the structure of this compound to be luteolin 7-O-glucoside (Wang et al., 2003). 1H-NMR revealed signals at δ ppm 7.45 (dd, J = 8.3 Hz, and J = 2.2 Hz, H-6’), 7.42 (d, J = 2.2 Hz, H-2’), 6.9 (d, J = 8.3 Hz, H-5’), 6.8 (d, J = 2.2 Hz, H-8), 6.7 (s, H-3), 6.4 (d, J = 2.2 Hz, H-6) and signal appeared as doublet at δ ppm 5.08 (d, J = 6.6 Hz, H-1” of glucose) assignable for the anomeric proton.
of the sugar moiety. Further confirmation of the structure of compound 2 as luteolin 7-O-glucoside, was achieved through $^{13}$C-NMR spectrum (DMSO-$d_6$). The presence of β-glucopyranoside moiety in the compound confirmed from the anomic carbon resonance at δ 99.82 ppm and from the chemical shift values of the remaining sugar resonances at δ 72.09, 77.14, 69.50, 76.36 and 60.58 ppm which assigned for (C-2'), (C-3'), (C-4'), (C-5') and (C-6') respectively. Resonances of the carbons of the flavonoid moiety were assigned by comparison with the corresponding signals of Luteolin-7-O-β-D-glucopyranoside $^{12}$. Compound 2 was identified as Luteolin-7-O-β-D-glucopyranoside.

**Compound 3 (Chlorogenic acid),** this compound showed a chromatographic properties and color reactions similar to those reported for chlorogenic acid (blue spot on PC under UV light, turning bright yellow when fumed with ammonia vapor, changing to yellow with AlCl$_3$ spray reagents $^{13}$. Mass spectrum showed a molecular ion (M$^+$) at m/z 354, which corresponding to the molecular formula C$_{16}$H$_{18}$O$_5$. The $^1$HNMR spectrum (DMSO-$d_6$, room temperature) showed signals, 6.34 (H-8', d, J=16.0), 6.84 (H-5', d, J=8.4), 7.03 (H-6',dd, J = 8.5, 1.8), 7.13 (H-2', d, J= 1.8), 7.58 (H-7', d, J=16.0). From the above chromatographic and spectroscopic data, compound 3 could be identified as chlorogenic acid $^{14}$.

**Compound 4(cinnamic acid),** it is isolated as a colorless needles. Mass spectrum showed a molecular ion (M$^+$) at m/z 148, which is corresponding to the molecular formula C$_6$H$_5$O$_2$. The $^1$HNMR spectrum (CDCl$_3$,400 MHz) showed signals, 7.56 (1, H,d,J= 16.0 Hz,H-3), 7.58 (2H,m,H-5,9), 7.38 (3H,m,H6,7,8), 6.63 (1H,d,J=16.0, H-2). These spectral data were identical with those of cinnamic acid $^{15}$.

**In vivo studies**

**Body weight**

Table (3) represents the effect of dandelion extract on rat body weight, percentage of change and relative liver weight. The present results showed that CCl$_4$ treatment caused significant decrease in body weights. On the other hand, the body weight gains were improved after supplementation with dandelion extract, but the reduction in body weight was still significant compared to the corresponding control. The reduction in body weight amounted to -27.35 of CCl$_4$ treated group and improved to -7.88 for CCl$_4$+ dandelion. Moreover, liver weight either in the absolute or the relative liver weights showed significant increase and improvement after administration of dandelion extract (Table 3).
Protective effect of dandelion extract on liver biomarkers
As show in Table (4), the activities of serum ALT, AST, ALP and GGT were markedly elevated in CCl₄ intoxicated rats compared to control group, the results indicating liver damage. The activities of ALT, AST, ALP and GGT increased by 187.07, 52.37, 50.58 and 94.59% of control in CCl₄-treated group. Supplementation of T. officinale extract decreased these elevations to 8.50, 32.29, 15.38 and 29.46 for ALT, AST, ALP, and GGT, respectively.

Table 5. Protective effect of dandelion extract on protein, albumin and total bilirubin

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Total protein (g/dl)</th>
<th>% change</th>
<th>Albumin (g/dl)</th>
<th>% change</th>
<th>Bilirubin (g/dl)</th>
<th>% change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>7.12±0.15ᵃ</td>
<td>0.00</td>
<td>5.03±0.23ᵇ</td>
<td>0.00</td>
<td>0.91±0.07ᵇ</td>
<td>0.00</td>
</tr>
<tr>
<td>CCl₄</td>
<td>6.14±0.31ᵃ</td>
<td>-13.76</td>
<td>4.26±0.11ᵇ</td>
<td>-15.31</td>
<td>2.12±0.14ᵇ</td>
<td>132.97</td>
</tr>
<tr>
<td>Dandelion</td>
<td>7.13±0.42ᵃ</td>
<td>0.14</td>
<td>4.64±0.12ᵇ</td>
<td>-7.75</td>
<td>0.90±0.14ᵇ</td>
<td>-1.10</td>
</tr>
<tr>
<td>CCl₄+dandelion</td>
<td>8.44±0.14ᵇ</td>
<td>18.54</td>
<td>4.60±0.41ᵇ</td>
<td>-8.55</td>
<td>1.01±0.06ᵇ</td>
<td>10.99</td>
</tr>
</tbody>
</table>

Each value is a mean of 8 rats ± S.D. a, b, c, d values are not sharing superscripts letters differ significantly at P<0.05. % of change = [(treatment – control)/ control] x 100.

Dealing with hepatic glutathione, malondialdehyde and nitric oxide were assayed; glutathione, malondialdehyde and nitric oxide and their figures due to treatment with dandelion and dandelion + CCl₄ were compared to control. Treatment of intoxicated rats with dandelion showed improvement of 66%. On the other hand significant decrease was detected in both MDA and nitric oxide levels post treated intoxicated rats.

Histopathological investigation
Figure 5 shows liver section of control rats with normal hepatic lobular architecture. Liver injured with CCl₄ revealed portal loss of hepatic lobular architecture, ballooning of hepatocytes, deformed cord arrangement and disturbed sinusoids. The hepatocytes showed marked degree of hydropic and steatotic changes as well as massive hepatic necrosis. Portal tracts were extended with marked number of chronic inflammatory cells and fibrous tissue. There was porto-portal and Porto-central fibrosis. Photomicrograph of liver section of animal treated with dandelion after CCl₄ intoxication showed dilated central vein with some degenerative changes in hepatocytes.
Figure 5. Photomicrograph of hematoxylin and eosin (H&E) stained section (100 x) of control (A) shown normal liver with well arranged hepatocytes (arrow). CCl₄ group (B) shown fibrosis (arrow), lymphocytes inflammations and infiltrations, steatotic changes and foamy appearance of hepatocytes (small arrows). CC14 + dandelion group (C) had shown dilated central view with the some degenerative changes in the hepatocytes. CC14 + silymarin (D) shown fibrosis, inflammations and infiltrations (arrow), hepatocytes swelling, foamy appearance and a stage of steatosis (small arrows)

Dandelion (T. officinale) has been used in folkloric and Traditional Chinese Medicine in the treatment of inflammation and severe women's diseases, such as breast and uterine cancers[16] and it is also acclaimed as a non – toxic medicinal herb have a great value for its chloretic, and dichloretic[17] antirheumtic and anti-inflammatory properties[18]. Plants produce diverse arrays of phytochemicals which are useful in the development of new drugs. These phytochemicals are mostly secondary metabolites constantly synthesized by the plant for defensive purposes[19]. For instance, antioxidants are biologically produced as defensive mechanism to prevent tissues destruction caused on highly reactive chemical species, which are formed from various biochemical reactions. In fact, phenolic is one of the major classes of natural antioxidants found in plants that remove such free radicals. Polyphenols are able to neutralize free radicals, scavenge singlet and triplet oxygen, and to break down peroxides.

As known CC14 is used as hepatotoxic agent that enhances creation of free radicals through their metabolism leading to lipid peroxidation of cellular and organelle membranes as primary pathogenic step[20]. The elevated levels liver enzymes in CCl₄ treated rats could be due to the leakage of enzymes into the serum[21]. Subsequent recoveries towards normalization of liver enzymes as shown from the results in table (4) strongly suggest the possibility of dandelion herb extract being able to protect liver from hepatotoxic effect of CCl₄. Al-Malki et al.[22] in their study on dandelion and chicory came to a conclusion that the mixture used reached its maximum peak at the end of six weeks of their study. This maximum peak of effect could be an indication that dandelion extract is able to repair the probable hepatic injury and /or restore the cellular permeability, thus reducing the toxic effect of CCl₄ in liver tissue which is in agreement with the results obtained in the present investigation.

The chemical investigation of dandelion extract revealed the presence of letulein as aglycon, and as glycoside in addition to chlorogenic acid and cinnamic acid. These compounds are polyphenolic in nature and are responsible for its antioxidant defence system in the plant moreover these compounds may confirm the role of dandelion extracts as inducing the hepatoprotective activity. The anti-inflammatory activity of luteolin has been shown to be active via NF-kB and activator protein-1 modulation in LPS-stimulated RAW 264.7 cells[23]. They also added that chicoric and a chlorogenic acid has the most powerful antioxidant capacity among reference compounds such as echinacoside, caffeic and rosmarenic acids[24]. These finding of the previous researchers concerning the isolated compounds from dandelion came in agreement with our findings also, subsequent recovery towards normalization of the enzymatic parameters studied suggest that dandelion extract is capable to condition the hepatic cell with subsequent
acceleration of parenchymal cells, regeneration and in turn protecting against membrane fragility and minimizing the leakage of liver enzymes into the blood circulation. In the meantime, it has been reported that dandelion supplements has antioxidant activity which coincide with the results postulated from the amount of polyphenolic content and its constituents and their role as scavenging the free radicals. Also, Sooyeul et al. [25] stated that dandelion supplementation could be beneficial for improvement lipid metabolism. 

CONCLUSION

In the present study, it can be concluded that *Taraxacum* extract has a protective effect against CCl₄ induced hepatotoxicity in albino rats. The restoration of transaminase enzymes to normal level in the CCl₄ treated group is clear evidence that dandelion has a marked role as hepatoprotective agent.

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