Hepatoprotective Efficacy of Chicory alone or combined with Dandelion leaves against induced Liver Damage

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Abstract: Several phytomolecules have been reported as potent hepatoprotective agents against different toxicants. Chicory and dandelion water extract was found to have protective effect on acute liver inflammation induced by CCl_4 in rats. In this, study we investigated the efficacy of the hepatoprotective activity of chicory leaves alone or mixed with dandelion leaves water extracts against carbon tetrachloride induced liver intoxication in Wister albino rats. One hundred and twenty rats weighing 150-200 gm were included in the current study. Carbon tetrachloride was used as a hepatotoxic agent while, chicory and chicory/dandelion mixture leaves water extracts were used as a probable hepatoprotective agent. Rats were divided into two main groups. Group A (normal control group) and group B (liver injured group). Various biochemical parameters were studied to evaluate the hepatoprotective activity of chicory alone or chicory/dandelion mixture leaves water extract. The study was also supported by histopathology of liver sections and DNA extraction of the rats' livers to investigate the genomic DNA integrity. Results revealed that the serum biomarkers in carbon tetrachloride treated rats recorded elevated concentration indicating severe hepatic damage by carbon tetrachloride. The results of the serum biomarkers of chicory and its mixture with dandelion treated rats showed significant reduction indicating the effect of the plants leaves extract in restoring the normal functional ability of the hepatocytes.

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1. Introduction

Epidemiological studies have shown that consumption of fruits and vegetables is associated with reduced risk of chronic diseases (Chu *et al.,* 2002). Hepatic dysfunction due to ingestion or inhalation of hepatotoxins such as acetaminophen, cadmium chloride, ethanol, carbon tetrachloride (CCl_4) and allyl alcohols are increasing worldwide (Wolf, 1999). Carbon tetrachloride is metabolized by cytochrome P450 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 (Packer *et al.,* 1978; Recknagel *et al.,* 1989). These free radicals attack microsomal lipids leading to its peroxidation and covalently bind to microsomal lipids and proteins, ultimately initiating a site of secondary biochemical processes (Rao and Recknagel, 1969).

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). 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, investigations into the lead molecules, that may produce better therapeutic effects, is required to overcome the pharmaceutical imbalance between remedies that protect the liver and drugs that induce hepatotoxicity (Anju *et al.,* 2012). The plant *Cichorium intybus Linn* (Family: *Compositae, Asteraceae*) commonly known as Chicory or Kasni is also used as liver tonic, cardiotonic, diuretic, stomachic, cholagogue, depurative, emmenagogue, hepatomegaly, cephalalgia, inflammations, anorexia, dyspepsia, flatulence, colic, jaundice, splenomegaly, amenorrhea dysmenorrhea, and asthma (Sala, 1994). Fresh chicory typically contains 68% inulin, 14% sucrose, 5% cellulose, 6% protein, 4% ash, and 3% other compounds, while dried chicory contains approximately 98% inulin and 2% other compounds (Meehye & Shin, 1996). Chicory leaves are good sources of anthocyanins, vitamins A and C as well as potassium, calcium, and phosphorus (Mulabagal *et al.,* 2009). Moreover, chicory is rich in cichoric acid, which stimulates the immune system as well as prevents inflammation and bacterial infections to a limited extent (Nayeemunnisa, 2009). Chicory has a potent hepatoprotective, antioxidant, hypoglycemic, diuretic, anti-testicular toxicity and

immunomodulatory effects (Jamshidzadeha *et al.,* 2006; Hassan, 2008; Nayeemunnisa, 2009; Mulabagal *et al.,* 2009). Chicory has demonstrated antihepatotoxic potential in animal studies (Ahmed *et al.,* 2008; Hassan & Yousef, 2010; Tabassum *et al.,* 2010).

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. 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).

Cytochrome P450 system is mainly localized in highest amount in the liver than in any other organs such as lung, kidney or intestine (Sheweita, 1999, 2000). The cytochrome P450 system participates in the bioactivation of polycyclic aromatic hydrocarbons (PAHs) and other carcinogens into their reactive intermediates (Sheweita and Mostafa, 1996). Cytochrome P450 activates carbon tetrachloride (CCl_4) into its reactive intermediate, trichloromethyl radical (Recknagel *et al.,* 1989), which further converted to a peroxy radical (Slater, 1987). The metabolites of CCl_4 react with polyunsaturated fatty acids to propagate a chain reaction leading to lipid peroxidation or bind covalently to lipids and proteins, resulting in destruction of cell membranes and also induced liver damage (Slater, 1987; Recknagel *et al.,* 1989).

Glutathione S-transferases (GSTs) (EC 2.5.1.18.) are multifunctional proteins classified into six classes according to their protein sequence homology, active sites residues and gene organization (Moons, 2005). GST genes can be induced by different exogenous factors such as pathogen attack, heavy metals, heat shock, wounding or auxins (Roxas *et al.,* 2000). Exposure of plants to such treatments leads to an increase in the production and accumulation of reactive oxygen species (ROS) in cells. To reduce the adverse effects of ROS, aerobic organisms have evolved defense systems, including antioxidant enzymes, such as superoxide dismutase (SOD), catalases (CAT), ascorbate peroxidase (APX), glutathione peroxidase (GPX) and GST as well as the production of low molecular weight antioxidants like ascorbic acid (AA) and glutathione (GSH) (Rachel Galland *et al.,* 2007). Therefore, the present study was designed to examine the possible effects of either chicory leaves water extract or dandelion leaves water extract and their mixture against carbon tetrachloride induced liver damage in male Wister albino rats.

2. Materials and Methods

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

Preparation of chicory and dandelion leaves water extracts (CLWE, DLWE) for rats

Chicory and dandelion leaves were purchased from the herbal medicine market (Cairo, Egypt). An ecologist in plant department, Faculty of Science, Ain Shams University, identified the dried chicory and dandelion leaves. The dried chicory and dandelion leaves were then homogenized to a

fine powder and stored at room temperature (25 ± 2°C) until use. Briefly, 100 g of powdered chicory and its mixture with dandelion separately were left in boiling water (1:10 w/v) for 4 hours. The water extracts were filtered through Whatman No. 1 filter paper, 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; Al Malki *et al.,* 2013).

Treatment of rats, induction of liver fibrosis and Experimental Design

One hundred and twenty Wister albino rats weighing 150-200 grams were used as the animal model. Rats were housed in well-ventilated polypropylene cages with husk beds. All experiments were performed following "Principles of laboratory animal care" (NIH publication no. 85-23, revised in 1985). The rats were acclimatized to conditions in the laboratory (26 - 28 C°, 60 - 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). On day 1, 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; Al Malki *et al.,* 2013). Carbon tetrachloride was injected three times per week for six consecutive weeks. Liver fibrosis was determined by killing five rats with histopathological examination every two weeks.

Chicory leaves water extract (CLWE) and mixture of chicory and dandelion leaves water extract(DLWE) were 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 described by Dawit *et al.,* (2006) and Al Malki *et al.,* (2013).

Rats were randomly divided into two main groups. Group A (normal control group) and group B (liver injured group) which was sub-classified into:

(i) **Group A**: Twelve rats were left to serve as normal basic control.

(ii) **Group B**: This group included one hundred and eight rats, which were all injected subcutaneously at a dose of 0.2 ml /100 g body weight of previously prepared CCl_4 dissolved in paraffin oil. Group B was divided as follows:

(iii) **Group B1**: Thirty six 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.

(iv) **Group B2**: Twenty five ml/kg body weight of chicory leaves water extract were administrated to thirty six rats once/day throughout the whole study through the mouth using intragastric catheter tube to ensure the proper and secure ingestion of the extracts according to the method described by Dawit *et al.,* (2006) and Al Malki *et al.,* (2013) 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.

(vi) **Group B3**: Twenty five ml/kg body weight of equal mixture of CLWE and DLWE were administrated to thirty six rats once/day throughout the whole study through the mouth using intragastric catheter tube to ensure the proper and secure ingestion of the extracts according to the method described by Dawit *et al.,* (2006) and Al Malki *et al.,* (2013) 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 minutes. Serum was then separated by centrifugation at 3000 rpm for 10 minutes using cooling centrifuge (Beckman, CS-15R Centrifuge, California-USA). Serum samples were collected in aliquots for determination of serum alanine transaminase (ALT) (Henry *et al.,* 1960; Henry *et al.,* 1974), serum aspartate transaminase (AST) (Henry *et al.,* 1960; Henry *et al.,* 1974), 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 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 rat was excised immediately after scarifying and the liver was excised and divided into three portions, one portion was immersed immediately into 10% buffered neutral formaldehyde solution to be processed for histopathological examination, the 2nd portion was used for DNA extraction and the 3rd portion was used to prepare liver homogenate

Preparation of the liver homogenate:

Tissues were rinsed in ice-cold PBS (0.02mol/L, pH 7.0-7.2) to remove excess blood thoroughly and weighed before homogenization. The tissues were minced to small pieces and homogenized them in a certain amount of PBS with a glass homogenizer on ice. The resulting

suspensions were subjected to ultrasonication and to two freeze-thaw cycles to further break the cell membranes. After that, the homogenates were centrifuged for 15 minutes at 1500×g. The supernatants were removed and kept in aliquot and stored samples at -80°C until use for determination glutathione-S-transferase (GST) and cytochrome P450 (CYP) in the liver homogenate.

Determination of glutathione-S-transferase (GST) in the liver homogenate of studied rats:

The concentration of glutathione-S-transferase (GST) in the liver homogenate of the studied rats was estimated using Rat Glutathione-S-Transferase (GST) Elisa kit (Catalogue Number: E02G0374, Blue Gene Biotech) applies the competitive enzyme immunoassay technique utilizing a monoclonal anti-GST antibody and a GST-HRP conjugate.

Determination of cytochrome P450 (CYP) in the liver homogenate of studied rats:

The concentration of cytochrome P450 (CYP) in the liver homogenate of the studied rats was estimated using CYP21A ELISA kit applies the competitive enzyme immunoassay technique utilizing a monoclonal anti-CYP21A antibody and CYP21A-HRP conjugate.

Genomic DNA extraction of rat's liver 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 x TAE buffer. The power supply was turned on at 100 volts for 30-45 minutes to allow separation of DNA marker bands.

Histopathological examination

Livers of the sacrificed rats were dissected, removed and one third 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 4-6 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 (SD>50% mean).

3. Results

Serum biochemical parameters of liver functions

Liver profile of untreated control rats and CCl_4 treated control rats

Table (1) shows the 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 2^{nd}, 4^{th} and 6^{th} weeks were significantly increased (P <0.01) compared to that of the untreated control group (negative control), while the mean serum levels of AChE of CCl_4 treated group showed a significant decrease (P <0.01) at the end of the 2^{nd}, 4^{th} and 6^{th} weeks compared to that of the untreated control group. Meanwhile the mean serum levels of urea at the end of the 2^{nd}, 4^{th} and 6^{th} weeks were significantly increased (P <0.01) in CCl_4 treated control group compared to that of the untreated control group (negative control). The mean serum levels of albumin in CCl_4 treated control group were fluctuating between insignificant decrease at the end of the 2^{nd} week and significant decrease (P <0.01) at the end of the 4^{th} week compared to that of the untreated control group. While, at the end of the 6^{th} week, the mean serum level of albumin of CCl_4 treated control group showed significant elevation (P <0.01) compared to that of the untreated control group.

Results of glutathione-S-transferase (GST) and cytochrome P450 (CYP) of untreated control rats and CCl_4 treated control rats

Table (2) shows the GST levels in liver homogenate of untreated control group and CCl_4 treated control group throughout the whole study. The mean GST levels in liver homogenate of CCl_4 treated control group at the end of the 2^{nd}, 4^{th} and 6^{th} weeks were significantly decreased (P <0.01) compared to that of the untreated control group (negative control).

Table (2) also shows the CYP levels in liver homogenate of untreated control group and CCl_4 treated control group throughout the whole study. The mean CYP levels in liver homogenate of CCl_4 treated control group at the end of the 2^{nd}, 4^{th} and 6^{th} weeks were significantly elevated (P <0.01) compared to that of the untreated control group (negative control).

Liver profile of chicory protected rats

Table (1) shows that the mean serum levels of ALT, AST, GGT, ALP and LDH of chicory protected group at the end of the 2^{nd}, 4^{th} and 6^{th} weeks of the study were significantly decreased (P <0.01) compared to that of CCl_4 treated group (+ve control) at the end of the same corresponding weeks of the experiment respectively. While the mean serum levels of AChE of chicory protected group at the end of the 4^{th} and 6^{th} weeks of the study showed a significant elevation (P <0.01)

compared to that of CCl_4 treated group at the end of the same corresponding weeks of the experiment. While, at the end of the 2nd week of the study, the mean serum level of AChE of chicory-protected group displayed an insignificant elevation compared to that of CCl_4 treated group (+ve control).

The mean serum levels of urea in chicory-protected group showed a significant decrease ($P<0.01$) 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 level of albumin in chicory-protected group were fluctuating between significant decrease ($P<0.01$) at the end of the 2nd week, significant increase ($P<0.01$) at the end of the 4th week and, significant decrease ($P<0.01$) the end of the 6th week of the study compared to that of the CCl_4 treated group (+ve control) at the end of the same corresponding week of the study.

Results of glutathione-S-transferase (GST) and cytochrome P450 (CYP) of chicory protected rats

Table (2) shows that the mean GST levels in liver homogenate of chicory protected rats were significantly increased at the end of the 2nd week ($P<0.05$) and at the end of the 4th, 6th weeks ($P<0.01$) compared to that of the CCl_4 treated rats (+ve control) at the end of the same corresponding weeks of the study respectively. In addition, the mean GST levels in liver homogenate of chicory-protected rats at the end of the 6th week of the study were insignificantly lower than that of the untreated control rats (negative control).

Table (2) shows that the mean CYP levels in liver homogenate of chicory-protected rats were insignificantly decreased at the end of the 2nd week and significantly decreased ($P<0.01$) at the end of the 4th and 6th weeks compared to that of the CCl_4 treated rats (+ve control) at the end of the same corresponding weeks of the study respectively. However, the mean CYP levels in liver homogenate of chicory protected rats at the end of the 6th week were significantly higher ($P<0.05$) than that of the untreated control rats (negative control).

Liver profile of dandelion protected rats

Results were not shown as published previously (Al Malki *et al.,* 2013).

Results of glutathione-S-transferase (GST) and cytochrome P450 (CYP) of dandelion protected rats

Table (2) shows that the mean GST levels in liver homogenate of dandelion protected rats were significantly increased at the end of the 2nd week ($P<0.05$) and at the end of the 4th, 6th weeks ($P<0.01$) compared to that of the CCl_4 treated rats (+ve control) at the end of the same corresponding weeks of the study respectively. In addition, the mean GST levels in liver homogenate of dandelion protected rats at the end of the 6th week of the study were insignificantly lower than that of the untreated control rats (negative control).

Table (2) shows that the mean CYP levels in liver homogenate of dandelion protected rats were insignificantly decreased at the end of the 2nd week and significantly decreased at the end of the 4th and 6th weeks ($P<0.01$) compared to that of the CCl_4 treated rats (+ve control) at the end of the same corresponding weeks of the study respectively. However, the mean CYP levels in liver homogenate of dandelion protected rats at the end of the 6th week were significantly higher ($P<0.05$) than that of the untreated control rats (negative control).

Liver profile of chicory/dandelion mixture protected rats

Table (1) showed that the mean serum levels of ALT, AST, GGT, ALP and LDH of chicory/dandelion mixture protected group at the end of the 2nd, 4th and 6th weeks of the study showed significant decrease ($P<0.01$) compared to that of CCl_4 treated rats (+ve control) at the end of the same corresponding weeks of the experiment respectively with insignificant elevation in the serum levels of AST, ALP at the end of the 2nd week of the study 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 chicory/dandelion mixture protected group at the end of the 4th and 6th weeks of the study showed significant elevation ($P<0.01$) compared to that of CCl_4 treated group at the end of the same corresponding weeks of the experiment. While, the serum level of AChE of chicory/dandelion mixture protected group displayed significant decrease ($P<0.01$) at the end of the 2nd week of the study, compared to that of CCl_4 treated group (+ve control) at the end of the same corresponding week of the study.

The mean serum levels of urea in chicory/dandelion mixture protected group showed significant decrease ($P<0.01$) 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 chicory/dandelion mixture protected group at the end of the 2nd and the 6th weeks showed significant decrease ($P<0.01$) compared to that of CCl_4 treated group (+ve control**)** at the end of the same corresponding weeks of the study respectively. While, the mean serum levels of albumin in chicory/dandelion mixture protected group at the end of the 4th week of the study showed significant elevation ($P<0.05$) compared to that of the CCl_4 treated group (+ve control**)** at the end of the same corresponding week.

Results of glutathione-S-transferase (GST) and cytochrome P450 (CYP) of chicory/dandelion mixture protected rats

Table (2) shows that the mean GST levels in liver homogenate of chicory/dandelion mixture protected rats were significantly increased at the end of the 2nd week (P <0.05) and at the end of the 4th, 6th weeks (P <0.01) compared to that of the CCl_4 treated rats (+ve control) at the end of the same corresponding weeks of the study respectively. Also, the mean GST levels in liver homogenate of chicory/dandelion mixture protected rats at the end of the 6th week of the study were insignificantly lower than that of the untreated control rats (negative control).

Table (2) shows that the mean CYP levels in liver homogenate of chicory/dandelion mixture protected rats were significantly decreased (P <0.01) at the end of the 2nd, the 4th and the 6th weeks compared to that of the CCl_4 treated group (+ve control) at the end of the same corresponding weeks of the study respectively. Also, the mean CYP levels in liver homogenate of chicory/dandelion mixture protected rats at the end of the 6th week were insignificantly higher than that of the untreated control group (negative control).

DNA Results of chicory

Figure (1) showed the effect of supplementation of chicory leaves water extract (CLWE) on the genomic DNA integrity of different CCl_4 treated rats' livers in addition of the genomic DNA integrity of untreated rat's liver (-ve control) as well as the genomic DNA integrity of CCl_4 treated rat's liver (+ve control) at the end of the 2nd week, where, lane (1) represents DNA marker, lane (2) represents the genomic DNA of untreated rat's liver (-ve control) which is clearly intact, but the genomic DNA of lane (3) is disintegrated as it represents the genomic DNA of CCl_4 treated rat's liver (+ve control) at the end of the 2nd week of the study. The other nine lanes (lanes 4-12) represent the genomic DNA of chicory-protected rats against CCl_4 hepato-intoxication with different responses towards chicory leaves water extract hepatoprotection at the end of studied weeks of the experiment.

DNA Results of dandelion:

Results were not shown as published previously (Al Malki *et al.*, 2013).

DNA Results of chicory/dandelion mixture:

Figure (2) showed the effect of supplementation of chicory/dandelion mixture 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 CCl_4 treated rat liver (+ve control) at the end of the 2nd week of the study and it is obviously disintegrated, while lane (3) represents the genomic DNA of untreated animal liver (-ve control) and it is clearly intact. The other six lanes (lanes 4-9) represent the genomic DNA of dandelion and chicory mixture protected rats against CCl_4 oral administration with different responses towards chicory/dandelion mixture leaves water extract hepatoprotection at the end of studied weeks of the experiment.

Histopathology Results of untreated control group and CCl_4 treated control group:

The results of the biochemical findings and the genomic DNA gel electrophoresis reported in the current study were supported with histopathological examination sections of five rats' livers out of twelve rats, which constitute each subgroup of the study. The histopathological findings of the livers of the normal rats that fed on standard diet (-ve control), showed normal histological picture (Figure-3). Liver examination of CCl_4 treated rats showed insignificant 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 (tables: 3, 4, 5 and 6). While, at the end of the 6th week, portal inflammation, necrosis, fibrosis and steatosis were significantly progressive (P <0.01) in CCl_4 treated rats compared to that of the negative control rats at the end of the same corresponding studied week of the study. Figure (4-C) showed congestion and moderate steatosis of hepatocytes in CCl_4 treated rat's liver at the end of the 6th week.

Histopathology Results of chicory protected group:

The histopathological examination of the livers' sections of chicory protected rats revealed that portal inflammation, necrosis, fibrosis and steatosis at the end of 2nd and 4th weeks of the study were insignificantly 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.(5-A) showed congestion, mild periportal inflammation and mild steatosis in chicory protected rat's liver at the end of the 2nd week, also, fig.(5-B) showed mild periportal inflammation and mild steatosis in chicory protected rat's liver at the end of the 4th week. But, at the end of the 6th week, those histopathological parameters (portal inflammation, necrosis, fibrosis and steatosis) showed significant positive improvement (P <0.01) compared to that of the CCl_4 treated rat's liver (+ve control) at the end of the same corresponding studied week. Figure (5-C) showed congestion, mild periportal inflammation with minimal steatosis in chicory-protected rat liver at the end of the 6th week.

Histopathology Results of dandelion protected group:

Results were not shown as published previously (Al Malki *et al.*, 2013).

Histopathology Results of chicory/dandelion mixture protected group:

The histopathological examination of the livers sections of chicory/dandelion mixture protected rats revealed that the portal inflammation, necrosis, fibrosis and steatosis at the end of 2nd and 4th weeks of the study were insignificantly improved compared to that of the CCl_4 treated rat's liver (+ve control) at the end of the same corresponding studied weeks of the experiment (tables: 3, 4, 5 and 6). Figure (6-A) showed minimal periportal inflammation and minimal steatosis in dandelion and chicory protected rat's liver at the end of the 2nd week, also fig. (6-B) showed minimal periportal inflammation, minimal steatosis and minimal hepatocyte necrosis in chicory/dandelion mixture protected rat's liver at the end of the 4th week. However, at the end of the 6th week, those histopathological parameters (portal inflammation, necrosis, fibrosis and steatosis) showed highly significant positive improvement ($P<0.01$) compared to that of the CCl_4 treated rat's liver (+ve control) at the end of the same corresponding week. Figure (6-C) showed mild steatosis and hepatocytes necrosis in chicory/dandelion mixture protected rat's liver at the end of the 6th week

Table 1: Serum levels of liver profile in CCl_4 treated & untreated, chicory and chicory/dandelion mixture protected rats

	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 wk.	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
P	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	>0.05
Chicory protected rats at the end of the 2nd wk.	122.3±7.3	223.6±21.8	23.8±2.6	218.4±10.7	1255.2±41.9	1873.0±26.4	41.7±2.8	3.2±0.1
P	<0.01	<0.01	<0.01	<0.01	<0.01	>0.05	<0.01	<0.01
Dandelion & chicory mixture protected group at the end of the 2nd wk.	128.4±3.6	281.2±14.0	27.5±2.8	264.4±16.3	847.2±22.99	1669.2±26.2	45.0±3.5	2.75±0.2
P	<0.05	>0.05	<0.01	>0.05	<0.01	<0.01	<0.01	<0.01
CCl_4 treated group at the end of the 4th wk.	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
P	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Chicory protected rats at the end of the 4th wk.	78.5±6.6	163.6±9.9	13.4±1.4	203.0±13.5	861.1±31.6	2051.3±34.7	35.4±1.8	3.9±0.3
P	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Dandelion & chicory mixture protected group at the end of the 4th wk.	74.1±7.2	170.5±16.9	16.2±2.2	219.3±12.4	617.1±15.6	1942.5±28.9	35.8±2.6	3.49±0.2
P	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.05
CCl_4 treated group at the end of the 6th wk.	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
P	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Chicory protected rats at the end of the 6th wk.	60.5±5.2	121.8±10.1	4.8±1.1	169.0±1.4	650.3±25.3	2242.7±34.4	29.7±4.3	4.0±0.4
P	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Dandelion & chicory mixture protected group at the end of the 6th wk.	57.1±11.2	130.5±12.9	15.6±2.0	190.0±10.5	589.8±19.7	2112.8±26.8	34.0±3.1	3.9±0.4
P	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

Table 2: GST and CYP 2E1 levels in liver homogenate of CCl_4 treated & untreated, chicory, dandelion and chicory/dandelion mixture protected rats

	*GST (ng/ml)	*CYP (ng/ml)
Untreated Control (- ve)	26.02 ± 1.88	0.170 ± 0.049
CCL_4 treated rats at the end of 2nd week	21.42 ± 1.57	0.250 ± 0.039
P	<0.01	<0.05
Chicory protected rats at the end of 2nd week	24.78 ± 1.98	0.230 ± 0.025
P	<0.05	>0.05
Dandelion protected rats at the end of 2nd week	24.36 ± 1.57	0.226 ± 0.036
P	<0.05	>0.05
Chicory & Dandelion Mixture protected rats at the end of 2nd week	24.44 ± 1.60	0.200 ± 0.015
P	<0.05	<0.01
CCL_4 treated rats at the end of 4th week	15.32 ± 1.72	0.384 ± 0.035
P	<0.01	<0.01
Chicory protected rats at the end of 4th week	21.32 ± 1.45	0.312 ± 0.023
P	<0.01	<0.01
Dandelion protected rats at the end of 4th week	20.14 ± 1.49	0.308 ± 0.025
P	<0.01	<0.01
Chicory & Dandelion Mixture protected rats at the end of 4th week	22.32 ± 1.59	0.258 ± 0.016
P	<0.01	<0.01
CCL_4 treated rats at the end of 6th week	8.82 ± 1.49	0.664 ± 0.071
P	<0.01	<0.01
Chicory protected rats at the end of 6th week	25.26 ± 1.64	0.236 ± 0.024
P	<0.01	<0.01
Dandelion protected rats at the end of 6th week	24.88 ± 1.73	0.240 ± 0.025
P	<0.01	<0.01
Chicory & Dandelion Mixture protected rats at the end of 6th week	25.70 ± 1.77	0.194 ± 0.020
P	<0.01	<0.01
Untreated Control (- ve)	26.02 ± 1.88	0.170 ± 0.049
chicory protected rats at the end of 6th week	25.26 ± 1.64	0.236 ± 0.024
P	>0.05	<0.05
Dandelion protected rats at the end of 6th week	24.88 ± 1.73	0.240 ± 0.025
P	>0.05	<0.05
Chicory & Dandelion Mixture protected rats at the end of 6th week	25.70 ± 1.77	0.194 ± 0.020
P	>0.05	>0.05

*GST: Glutathione –S-Transferase *CYP: Cytochrome P 450 (2E1)

Table 3: Portal Inflammation grades of CCl_4 untreated & treated, chicory and chicory/dandelion mixture protected rats throughout the whole study.

Grades of Portal inflammation / Groups	0	1	2	3	4
Number of Untreated Control (Negative Control)	2	3	-----	-----	-----
Number of CCl_4 treated rats (+ve control) at the end of 2nd week	2	3	-----	-----	-----
P	N.S.				
Number of chicory protected rats at the end of the 2nd week	2	3	-----	-----	-----
P	N.S.				
Number of chicory and dandelion mixture protected rats at the end of the 2nd week	2	3	-----	-----	-----
P	N.S.				
Number of CCl_4 treated rats (+ve control) at the end of 4th week	-----	4	1	-----	-----
P	N.S.				
Number of chicory protected rats at the end of the 4th week	1	4	-----	-----	-----
P	N.S.				
Number of chicory and dandelion mixture protected rats at the end of the 4th week	1	4	-----	-----	-----
P	N.S.				
Number of CCl_4 treated rats (+ve control) at the end of the 6th week	-----	-----	1	4	-----
P	H.S.				
Number of chicory protected rats at the end of the 6th week	2	3	-----	-----	-----
P	H.S.				
Number of chicory and dandelion mixture protected rats 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 4: Necrosis grades of CCl_4 untreated & treated, chicory and chicory/dandelion mixture protected rats throughout the whole study.

Grades of Necrosis / Groups	0	1	2	3	4	5	6
Number of Untreated Control (Negative Control)	2	3	----	----	----	----	----
Number of CCl_4 treated rats (+ve control) at the end of 2nd week	2	1	2	----	----	----	----
P	N.S.						
Number of chicory protected rats at the end of the 2nd week	2	2	1	-----	-----	-----	-----
P	N.S.						
Number of chicory and dandelion mixture protected rats at the end of the 2nd week	1	4	-----	-----	-----	-----	-----
P	N.S.						
Number of CCl_4 treated rats (+ve control) at the end of 4th week	2	2	-----	-----	1	-----	-----
P	N.S.						
Number of chicory protected rats at the end of the 4th week	1	3	1	-----	-----	-----	-----
P	N.S.						
Number of chicory and dandelion mixture protected rats at the end of 4th week	-----	5	-----	-----	-----	-----	-----
P	N.S.						
Number of CCl_4 treated rats (+ve control) at the end of 6th week	-----	-----	-----	2	3	-----	-----
P	H.S.						
Number of chicory protected rats at the end of the 6th week	3	2	-----	-----	-----	-----	-----
P	H.S.						
Number of chicory and dandelion mixture protected rats 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-Cirrhosis stages of CCl_4 untreated & treated, chicory and chicory/dandelion mixture protected rats throughout the whole study.

Groups \ Stage of Fibrosis/Cirrhosis	0	1	2	3	4	5	6
Number of Untreated Control (Negative Control)	3	2	----	----	----	----	----
Number of CCl_4 treated rats (+ve control) at the end of 2nd week	3	1	-----	1	-----	-----	-----
P	N.S.						
Number of chicory protected rats at the end of the 2nd week	4	1	-----	-----	-----	-----	-----
P	N.S.						
Number of chicory and dandelion mixture protected rats at the end of the 2nd week	3	2	-----	-----	-----	-----	-----
P	N.S.						
Number of CCl_4 treated rats (+ve control) at the end of 4th week	1	4	-----	-----	-----	-----	-----
P	N.S.						
Number of chicory protected rats at the end of the 4th week	3	2	-----	-----	-----	-----	-----
P	N.S.						
Number of chicory and dandelion mixture protected rats at the end of the 4th week	2	2	1	-----	-----	-----	-----
P	N.S.						
Number of CCl_4 treated rats (+ve control) at the end of 6th week	-----	-----	2	-----	3	-----	-----
P	H.S.						
Number of chicory protected rats at the end of the 6th week	4	1	-----	-----	-----	-----	-----
P	H.S.						
Number of chicory and dandelion mixture protected rats 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).

Table 6: Steatosis grades of CCl_4 untreated & treated, chicory and chicory/dandelion mixture protected rats throughout the whole study.

Groups \ Grades of Steatosis	0	1	2	3
Number of Untreated Control (Negative Control)	4	1	-----	-----
Number of CCl_4 treated rats (+ve control) at the end of 2nd week	4	1	-----	-----
P	N.S.			
Number of chicory protected rats at the end of the 2nd week	4	1	-----	-----
P	N.S.			
Number of chicory and dandelion mixture protected rats at the end of the 2nd week	4	1	-----	-----
P	N.S.			
Number of CCl_4 treated rats (+ve control) at the end of 4th week	1	3	1	-----
P	N.S.			
Number of chicory protected rats at the end of the 4th week	4	1	-----	-----
P	N.S.			
Number of chicory and dandelion mixture protected rats at the end of the 4th week	2	3	-----	-----
P	N.S.			
Number of CCl_4 treated rats (+ve control) at the end of the 6th week	-----	-----	3	2
P	H.S.			
Number of chicory protected rats at the end of the 6th week	5	-----	-----	-----
P	H.S.			
Number of chicory and dandelion mixture protected rats 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) Chicory

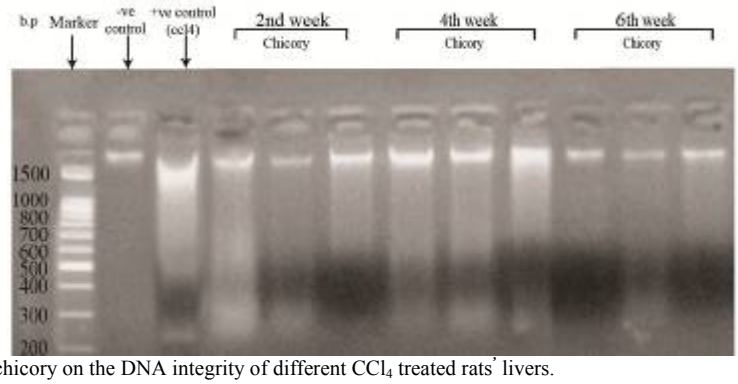

Fig. 1: Effect of supplementation of chicory on the DNA integrity of different CCl_4 treated rats' livers.
Lane 1: DNA Marker.
Lane 2: genomic DNA of untreated rat's liver (-ve control).
Lane 3: genomic DNA of CCl_4 treated rat's liver (+ve control) at the end of the 2nd week.
Lane 4: genomic DNA of chicory-protected rat's liver against CCl_4 administration at the end of the 2nd week.
Lane 5: genomic DNA of chicory-protected rat's liver against CCl_4 administration at the end of the 2nd week.
Lane 6: genomic DNA of chicory-protected rat's liver against CCl_4 administration at the end of the 2nd week.
Lane 7: genomic DNA of chicory-protected rat's liver against CCl_4 administration at the end of the 4th week.
Lane 8: genomic DNA of chicory-protected rat's liver against CCl_4 administration at the end of the 4th week.
Lane 9: genomic DNA of chicory-protected rat's liver against CCl_4 administration at the end of the 4th week.
Lane 10: genomic DNA of chicory-protected rat's liver against CCl_4 administration at the end of the 6th week.
Lane 11: genomic DNA of chicory-protected rat's liver against CCl_4 administration at the end of the 6th week.
Lane 12: genomic DNA of chicory-protected rat's liver against CCl_4 administration at the end of the 6th week.

Figure (2) Chicory/ Dandelion mixture

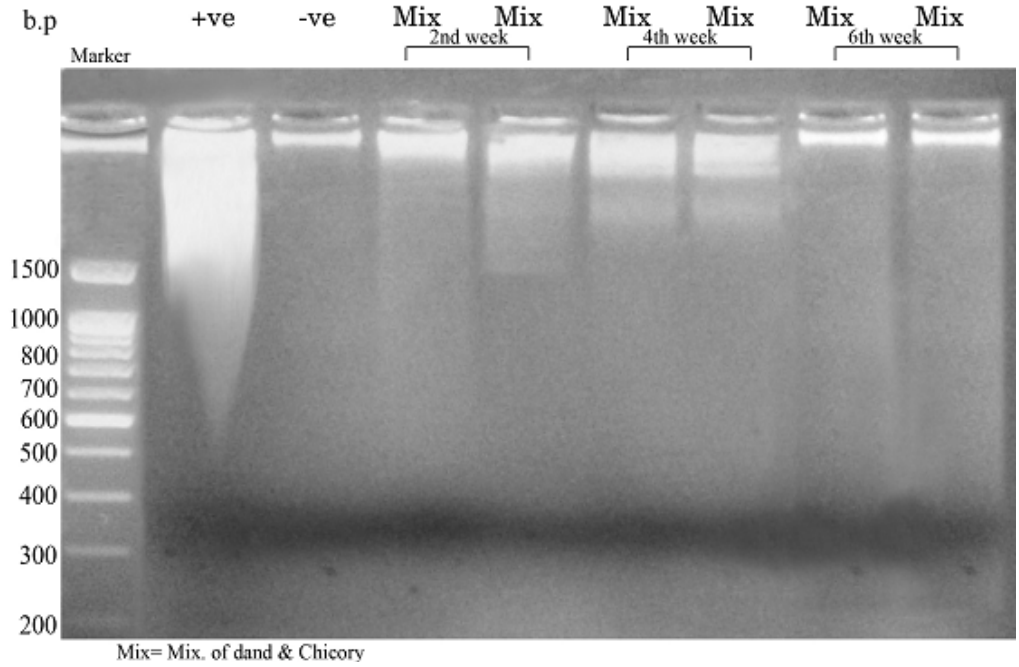

Fig. 2: Effect of supplementation of chicory and dandelion leaves water extracts mixture on the DNA integrity of different CCl_4 treated rats' livers.
Lane 1: DNA Marker.
Lane 2: genomic DNA of CCl_4 treated rat's liver (+ve control) at the end of the 2nd week.
Lane 3: genomic DNA of untreated rat's liver (-ve control).
Lane 4: genomic DNA of chicory and dandelion mixture protected rat's liver against CCl_4 administration at the end of the 2nd week.
Lane 5: genomic DNA of chicory and dandelion mixture protected rat's liver against CCl_4 administration at the end of the 2nd week.
Lane 6: genomic DNA of chicory and dandelion mixture protected rat's liver against CCl_4 administration at the end of the 4th week.
Lane 7: genomic DNA of chicory and dandelion mixture protected rat's liver against CCl_4 administration at the end of the 4th week.
Lane 8: genomic DNA of chicory and dandelion mixture protected rat's liver against CCl_4 administration at the end of the 6th week.
Lane 9: genomic DNA of chicory and dandelion mixture protected rat's liver against CCl_4 administration at the end of the 6th week.

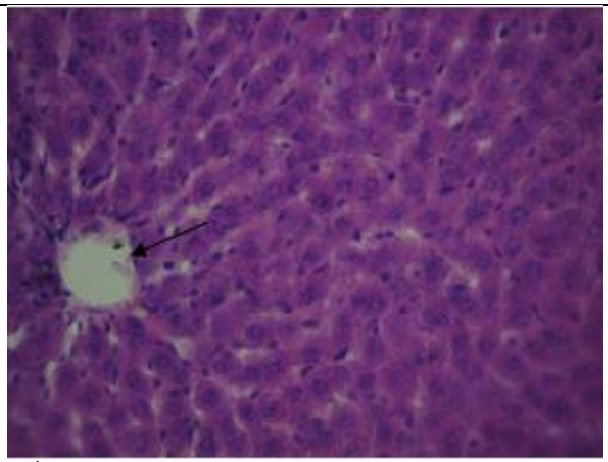

Figure 3: Untreated rat's liver (negative control group) with normal hepatocytes and central vein (One arrow).

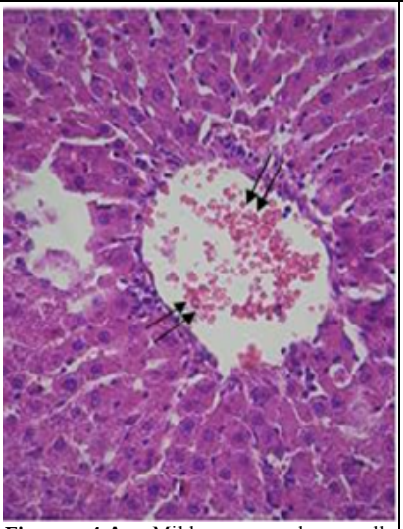

Figure 4-A. Mild mononuclear cell infiltration, congestion (Two arrows) and vacuolated hepatocytes in CCl_4 treated rat's liver at the end of the 2nd week.

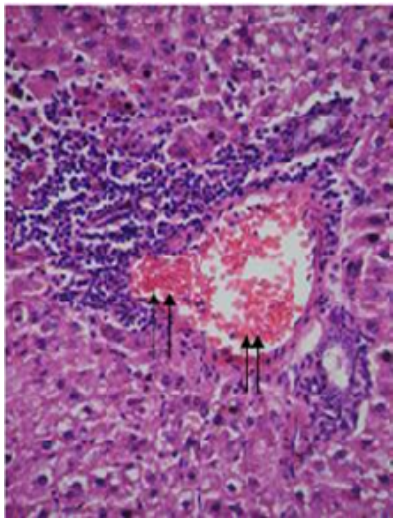

Figure 4-B. Moderate mononuclear cell infiltration, congestion (Two arrows) and vacuolated hepatocytes in CCl_4 treated rat's liver at the end of the 4th week.

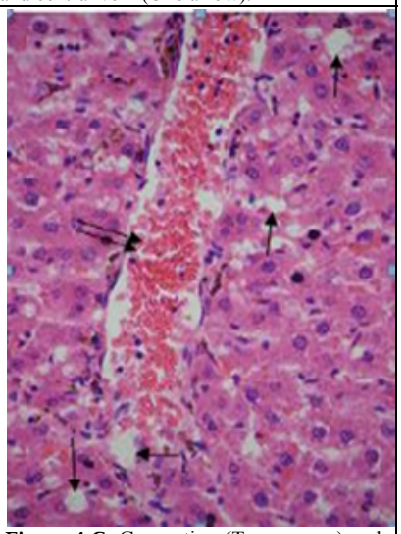

Figure 4-C. Congestion (Two arrows) and moderate steatosis of hepatocytes in CCl_4 treated rat's liver at the end of the 6th week (fat vacuoles, one arrow).

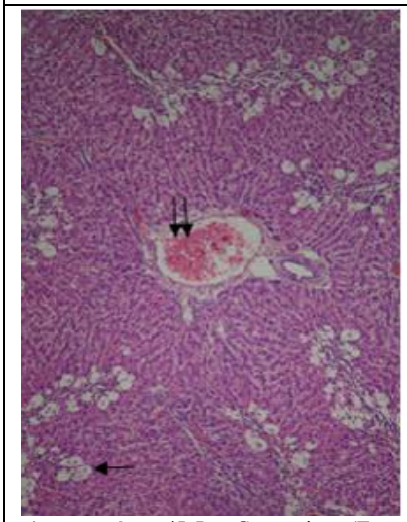

Figure 5-A. *L.P., Congestion (Two arrows), mild periportal inflammation and mild steatosis (fat vacuoles, one arrow) in chicory-protected rat's liver at the end of the 2nd week.

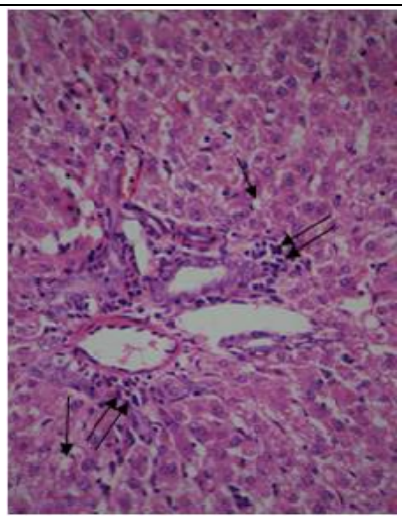

Figure 5-B. Mild periportal inflammation (lymphocytes, two arrows) and mild steatosis (fat vacuoles, one arrow) in chicory-protected rat's liver at the end of the 4th week.

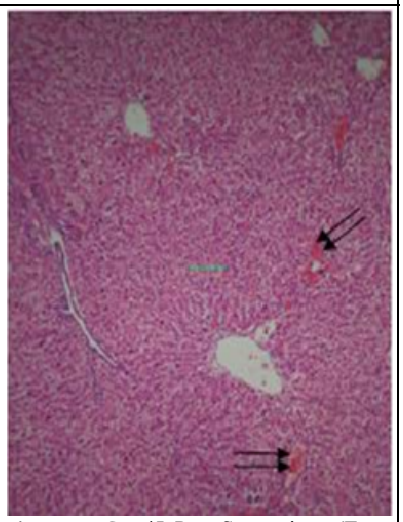

Figure 5-C. *L.P., Congestion (Two arrows), mild periportal inflammation and minimal steatosis in chicory protected rat's liver at the end of the 6th week.

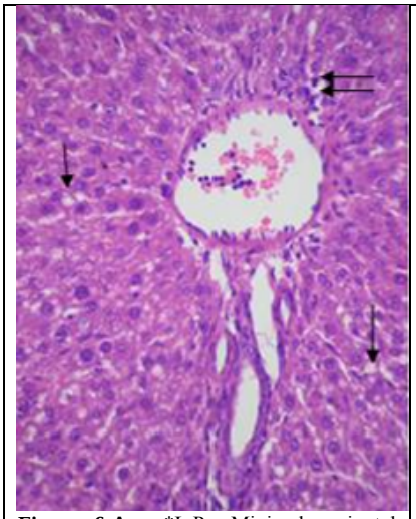

Figure 6-A. *L.P., Minimal peripotal inflammation (lymphocytes, two arrows) and minimal steatosis (fat vacuoles, one arrow) in chicory/dandelion mixture protected rat liver at the end of the 2nd week.

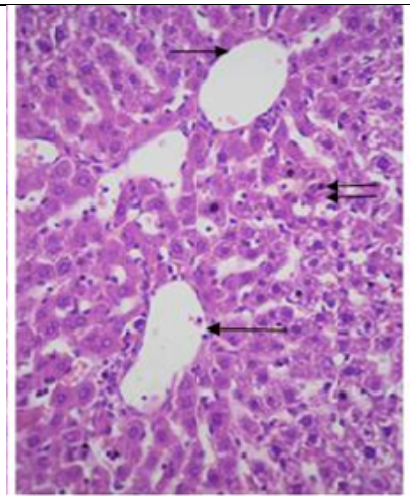

Figure 6-B. Minimal peripotal inflammation (lymphocytes, two arrows), minimal steatosis (fat vacuoles, one arrow) and minimal hepatocyte necrosis in chicory/dandelion mixture protected rat liver at the end of the 4thweek.

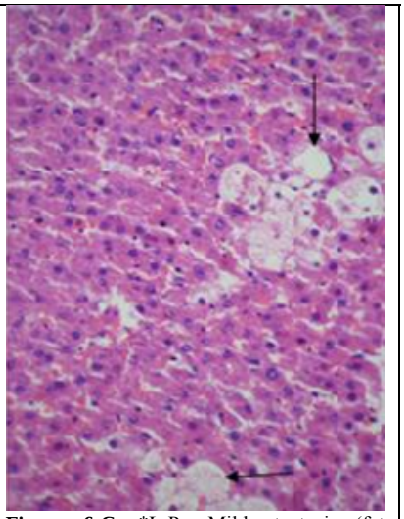

Figure 6-C. *L.P., Mild steatosis (fat vacuoles, one arrow) and hepatocytes necrosis in chicory/dandelion mixture protected rat liver at the end of the 6th week
*L.P.: Low power

4. Discussion

Carbon tetrachloride is used as hepatotoxic agent that enhances creation of free radicals through their metabolism leading to lipid peroxidation of cellular and organelle membranes as a primary pathogenic step (Ming *et al.,* 2006). It has been shown that CCl_4 is metabolized to trichloromethyl radical ($\cdot CCl_3$) by liver microsomal cytochrome P450 isozymes in the endoplasmic reticulum. This radical can interact with critical target molecules (nucleic acids, proteins, lipids and fatty acids) and impair cellular processes such as lipid metabolism (Rechnagel and Glende, 1973).

The results of the present study revealed that the serum levels of ALT, AST, GGT, ALP, LDH and urea significantly elevated (P <0.01) at the end of the 2nd, 4th and 6th weeks compared to that of the untreated (negative control) group at the end of the same corresponding studied weeks with marked significant decrease (P <0.01) in the serum level of AChE post CCl_4 subcutaneous injection at the end of the 2nd, 4th and 6th weeks compared to that of the untreated (negative control) group at the end of the same corresponding weeks of the study. The elevated levels of these enzymes in CCl_4 treated rats could be due to the leakage of enzymes into the serum (Desai *et al.,* 2010). Similar studies indicating the elevation of serum ALT, AST, LDH and ALP after CCl_4 treatment of experimental rat (Anupam *et al.,* 1995; Mansour 2000; Teocharis *et al.,* 2001; Bahar *et al.,* 2003) that leads to a damage to the structural integrity of the liver resulting in an increase in the level of serum transaminases as these are located in the cytoplasm and released into the circulation after cellular damage (Sallie *et al.,* 1991). A similar elevation in the levels of ALT, AST, ALP and LDH after injecting rats subcutaneously with CCl_4 three times per week to induce liver injury was recorded in previous study (Dwivedi *et al.,* 1990; Braide, 1991).

The mean serum values of albumin after administration of CCl_4 were fluctuated between insignificant decrease at the end of the 2nd week, significant decrease (P <0.01) at the end of the 4th week and significant elevation (P <0.01) at the end of the 6th week compared to that of the untreated (negative control) group at the end of the same corresponding studied weeks and this fluctuation of albumin values could be due to interaction of trichloromethyl radical (CCl_3) with protein molecules resulting in an impairment of cellular processes (Chung *et al.,* 2010).

The serum ALT and AST results of the present study could be correlated with that of Rasheeduz and S. Mujahid (1998), who proved that root callus extracts of *Cichorium intybus* could efficiently reduce the serum ALT and AST in rats with significant hepatic damage and elevated ALT and AST as a result of CCl_4 treatment. Also, these results came in agreement with that of Gadgoli and Mishra (1995) who mentioned that aqueous extracts of *Cichorium intybus* seeds could successfully improve the serum levels of ALT and AST in rats with CCl_4-Paracetamol hepatotoxicity. The results of Bahar Ahmed et al., (2003) agreed with our present results, as they reported that different fractions of alcoholic extract and one phenolic compound AB-IV of *Cichorium intybus* seeds could decrease the serum levels of ALT, AST and ALP in Wistar albino rats suffering from hepatotoxicity resulted from carbon tetrachloride oral administration. The results of another study (Hanaa and Mokhtar, 2010) also agreed with that of the present study, as they reported that chicory supplements in diets could positively modulate nitrosamnine precursors-induced hepatotoxicity in male Sprague Dawely rats and achieve a significant decrease in the serum levels of ALT, AST, GGT, ALP & LDH of chicory protected rats as well as a significant improvement in albumin serum level of chicory protected rats compared to nitrosamine precursors-treated rats. Meanwhile, the serum level of AChE of chicory protected group showed significant elevation (P <0.01) at the end of the 4th and 6th weeks

and insignificant elevation at the end of the 2nd week compared to that of the CCl_4 treated control group at the end of the same corresponding weeks of the study. The elevation of the serum level of AChE of chicory-protected group throughout the study is matched with the improvement in levels of the rest of the liver enzymes in chicory-protected group. This mirrors the hepatoprotective effect of chicory leaves water extract against CCl_4 hepato-intoxication. This indicates that plant extract was able to repair the probable hepatic injury and/or restore the cellular permeability; thus reducing the toxic effect of CCl_4 in the liver tissue.

Serum levels of urea in chicory protected group showed a significant decrease (P <0.01) at the end of the 2nd, 4th and 6th weeks compared to that of the CCl_4 treated group at the end of the same corresponding studied weeks of the experiment. This clearly indicates that the functional status of liver cells has been improved as a result of chicory oral administration.

The present study showed that serum levels of ALT, AST, GGT, ALP and LDH of chicory/ dandelion leaves water extracts mixture protected group at the end of the 2nd, 4th and 6th weeks of the study showed highly significant decrease (P <0.01) compared to that of CCl_4 treated rats (+ve control) at the end of the same corresponding weeks of the experiment respectively with insignificant elevation of serum AST and ALP levels at the end of the 2nd week compared to that of CCl_4 treated group (+ve control) at the end of the same corresponding week of the study. A subsequent recovery towards normalization of these enzymes strongly suggests the possibility of chicory/ dandelion leaves water extracts mixture being able to protect the liver from the hepatotoxic effect of carbon tetrachloride (CCl_4). The serum levels of AChE of chicory/ dandelion leaves water extracts mixture protected group at the end of the 4th and 6th weeks displayed significant elevation (P <0.01) compared to that of CCl_4 treated group at the end of the same corresponding weeks of the experiment. The increase in the mean serum levels of AChE of chicory/dandelion mixture protected animals reached its maximum peak at the end of the 6th week of the current study. This could be an indication that dandelion and chicory leaves water extracts mixture had an efficient hepatoprotective activity by strengthening the antioxidant defense system against carbon tetrachloride (CCl_4) hepatotoxic effect.

GST plays an important role in scavenging toxic intermediates of ROS. During hepatotoxicity, this enzyme is structurally and functionally impaired by free radicals resulting in liver damage. GST is the important detoxification enzyme involved in catalyzing the conjugation of a wide variety of electrophilic substrates to reduced glutathione and thus protects the cell from chemically induced damages in hepatic and extra hepatic tissues (Siddiqui, 1990). The results of the present study revealed that the Glutathione-S-transferase (GST) levels in liver homogenate of CCl_4 treated control group at the end of the 2nd, 4th and 6th weeks were significantly decreased (P <0.01) compared to that of the untreated control group (negative control). In agreement with the present results, significant reduction of hepatic glutathione-S-transferase in CCl_4 administrated rats was reported by Cabre *et al.,* (2000). Results of another study conducted by Carmel and Rajasekaran (2011) agree with the current results indicating that intraperitoneal administration of CCl_4 for five days caused a significant decrease of GST levels in CCl_4 treated rats compared to the untreated negative control rats.

The cytochrome (CYP) levels in liver homogenate of CCl_4 treated control group at the end of the 2nd, 4th and 6th weeks were significantly elevated (P <0.01) compared to that of the untreated control group (negative control). Hye and Hyeon (1998) reported that carbon tetrachloride requires P450 2E1-associated bioactivation to produce liver injury. Supporting the present results, It has been reported that CCl_4 is basically metabolized by cytochrome P450 system to trichloromethyl radical, which reacts with lipids and proteins leading to the deleterious effects of CCl_4 (Poyer *et al.,* 1978; Sipes and Gardolfi, 1982; Recknagel, 1983). In response to hepatocellular injury initiated by the biotransformation of CCl_4 into reactive radicals, "activated" Kupffer cells respond by releasing an increased amount of active oxygen species and other bioactive agents (Yam *et al.,* 2007).

The GST levels in liver homogenate of chicory protected rats were significantly elevated at the end of the 2nd, 4th (P <0.05) and 6th (P <0.01) weeks compared to that of the CCl_4 treated group (+ve control) at the end of the same corresponding weeks of the study respectively. Chicory supplemented diet is reported to have free radical scavenging and antioxidant properties by restoring glutathione, GSP, SOD and catalase levels. These findings may be due to the presence of antioxidant compounds such as anthocyanins, flavonoids, polyphenols and vitamin C that could contribute to protection against free radicals generation and carcinogenic effects of nitrosamines (Mulabagal, 2009; Ilaiyaraja, 2010).

The results show that the CYP levels in liver homogenate of chicory protected rats were significantly decreased at the end of the 4th (P <0.01) and 6th (P <0.01) weeks compared to that of the CCl_4 treated group (+ve control) at the end of the same corresponding weeks of the study respectively. Inhibition of cytochrome P450 system was found to be effective in protecting the liver against the toxicity of a wide variety of toxic agents (Jorquera *et al.,* 1996).

The results of the current study show that the GST levels in liver homogenate of dandelion leaf water extract protected rats were significantly elevated at the end of the 2nd (P <0.05), 4th (P <0.01) and 6th (P <0.01) weeks compared to that of the CCl_4 treated group (+ve control) at the end of the same corresponding weeks of the study respectively. Ung-Kyu *et al.* (2010) reported that supplementation with dandelion leaf insignificantly decreased the GST activities compared to the control group while, supplementation with dandelion root significantly decreased (P < 0.05) the GST activities compared to the control group.

You *et al.,* 2010 reported that, the mice receiving ethanol plus dandelion water extract exhibited significant increases in hepatic antioxidant activity of

glutathione-S-transferase. Furthermore, the amelioration of malondialdehyde levels indicated dandelion water extract's protective effects against liver damage mediated by alcohol in vivo. These results suggest that the aqueous extract of dandelion root has protective action against alcohol-induced toxicity in the liver by elevating antioxidative potentials and decreasing lipid peroxidation.

The results of the present study show that the mean CYP levels in liver homogenate of dandelion leaf water extract protected rats were significantly decreased at the end of the 4th (P <0.01) and 6th (P <0.01) weeks compared to that of the CCl_4 treated group (+ve control) at the end of the same corresponding weeks of the study respectively. Our results agreed with that of Maliakal and Wanwimolruk (2001) who investigated the effect of dandelion tea and the activity of hepatic phase I and phase II metabolizing enzymes using rat liver microsomes. After 4 weeks of treatment, the activity of cytochrome P450 (CYP) isoforms in the liver microsomes or cytosol was significantly decreased. There was also a dramatic increase (244% of control) in the activity of phase II detoxifying enzyme UDP-glucuronosyl transferase. Park et al. (2007) reported that the oral administration dandelion watery extract (500 mg/kg) daily for 7 days to rats suffering from liver injury induced by CCl_4 significantly decreased protein expression levels of cytochrome P450 2E1 in the dandelion treated group, indicating that DWE has a protective effect on acute liver inflammation induced by CCl_4. Activity of CYP1A2 in the liver microsomes of rats receiving dandelion tea was significantly decreased (P < 0.05) to 15 of the control value and activity of CYP2E in rats receiving dandelion tea was significantly lower than in the control group, 48 % of the control (Pius and Sompon 2001).

The present results of detoxifying enzymes revealed that the GST levels in liver homogenate of chicory/dandelion leaves water extracts mixture protected rats were significantly elevated at the end of the 2nd (P <0.05) and at the end of the of the 4th and 6th (P <0.01) weeks compared to that of the CCl_4 treated group (+ve control) at the end of the same corresponding weeks of the study respectively. The GST levels of chicory, dandelion and chicory/dandelion leaves water extracts mixture protected rats at the end of the 6th week (end of the study) were insignificantly lower than that of the untreated control group (negative control) particularly in chicory/dandelion mixture protected rats that reaching almost to the GST level of untreated negative control. This is an indication that the protective effect of chicory/dandelion leaves water extracts mixture is stronger than chicory or dandelion leaves water extracts independently.

Also the results of the current study show that the CYP levels in liver homogenate of chicory/dandelion leaves water extracts mixture protected rats were significantly decreased (P <0.01) at the end of the 2nd, 4th and 6th weeks compared to that of the CCl_4 treated group (+ve control) at the end of the same corresponding weeks of the study respectively. The CYP levels of chicory leaves water extract and dandelion leaves water extract protected rats at the end of the 6th week (end of the study) were significantly elevated (P <0.05) than that of the untreated control group (negative control) while, The CYP levels of chicory/dandelion mixture protected rats at the end of the 6th week (end of the study) were insignificantly increased than that of the untreated control group (negative control) that reaching almost to the CYP level of untreated negative control. This finding suggests that both chicory and dandelion potentiate each other to oppose the CCl_4-induced hepatotoxicity. The results obtained in this study suggest the protective effects of chicory/dandelion mixture extracts against CCl_4-induced oxidative stress, could be attributed to its high levels of polyphenols and other antioxidants like flavonoids (Mulabagal *et al.,* 2009; Ilaiyaraja and Farhath 2010). These compounds could scavenge the free radicals of CCl_4 generated through cytochrome P450 enzyme system thereby diminished the oxidative injuries (Ahmed and Kamal, 2012).

The results of the current study elicited that the genomic DNA of CCl_4 treated group (+ve control) (Figure-1, lane-3) showed a massive degradation compared to the untreated group (-ve control) (Figure-1, lane-2). This dramatic picture is a clear indication that CCl_4 caused DNA damage to this group. The genomic DNAs of chicory protected rats' livers against CCl_4 hepatotoxicity at the end of the 2nd week (lanes: 4, 5 and 6) appear to be partially degraded and seem to be less disintegrated compared to that of CCl_4 treated group (lane: 3). While, excess degradation occurred in the genomic DNAs of chicory protected rats' livers against CCl_4 hepato-intoxication at the end of the 4th week (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, the genomic DNAs of chicory protected rats' livers against CCl_4 intoxication (lanes 10, 11 and 12) were clearly improved and became more intact compared to the genomic DNAs of chicory protected rats' livers at the end of both 2nd and 4th weeks of the study. This DNA repair could be due to the cumulative protective effect of chicory throughout the whole study and an indication that chicory had time-dependent and cummulative hepatoprotective effect against CCl_4 hepatotoxicity. Hanaa and Mokhtar (2010) stated that chicory has antioxidant and free radical scavenging properties by restoring GSH, GSH-Rx, SOD and catalase levels as well as attenuating TBARS levels. These finding could be due to the presence of antioxidant compounds such as flavonoids, anthocyanins, polyphenols and vitamin C, which could participate in protection against free radicals production, and carcinogenic effects of nitrosamines (Kocsis *et al.,* 2003 and Mulabagal *et al.,* 2009). Furthermore, Nayeemunnisa (2009) reported that chicory supplemented diets can play a role in reduction of oxidative stress , GSH levels restorement and induction of gene expression resulting in overexpression of activity of the violent antioxidant enzyme CAT causing up-regulating the endogenous antioxidant defense system. The present findings recommended that CLWE as an antioxidant therapy

could be beneficial for opposing the hepatic intoxication and preventing the complications resulting from exposure to carbon tetrachloride.

The results of the current study elicited that the genomic DNA of CCl_4 treated group (+ve control) (Figure-2, lane-2) showed a massive degradation compared to the untreated group (-ve control) (Figure-2, lane-3). This dramatic picture is a clear indication that CCl_4 caused damage to the genomic DNA of rats' livers of this group. Supplementation of chicory/dandelion leaves water extracts mixture on the genomic DNA integrity of different CCl_4 treated rats' livers is clear through the different responses towards the plants extract mixture on DNA gel electrophoresis. This reveals that pretreatment of chicory/dandelion water extracts mixture could efficiently keep the integrity of DNA of chicory/dandelion leaves water extracts mixture-protected rats' livers at the end of 6th week of the study. This might be an indication that chicory/dandelion leaves water extract mixture have a cumulative antioxidant and restorative activity mixture is beneficial in preventing and opposing the deleterious effects of CCl_4 hepatotoxicity.

The histopathological examination of the normal rats' livers fed on standard diet (-ve control), showed normal histological picture. The histopathological examination of the livers of CCl_4 treated rats showed significant (P <0.01) degrees of portal inflammation, necrosis, fibrosis and steatosis only at the end of the 6th week of the study compared to that of the negative control rats. While, at the end of 2nd and 4th weeks of the study, those histopathological parameters (portal inflammation, necrosis, fibrosis and steatosis) were insignificantly progressive compared to that of the negative control rats. These histopathological results came in agreement with that of Bahar *et al.* (2003) who reported that necrosis and swelling in hepatocytes in CCl_4-treated rats compared to normal control rats were observed. Also, Al-Shabanah et al., (2000) reported that CCl_4 administration to rats causes necrosis, mononuclear cell infiltration, steatosis foamy degeneration of hepatocytes cirrhosis (Natusme *et al.,* 1999; Naziroglu *et al.,* 1999). Supporting the present histopathological results of necrosis Weber *et al.* (2003) 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.

While, the histopathological examination of the livers' sections of chicory alone and chicory/dandelion leaves water extracts mixture protected rats revealed that portal inflammation, necrosis, fibrosis and steatosis at the end of 6th week of the study showed significant positive improvement (P <0.01) compared to that of the CCl_4 treated rats (+ve control) at the end of the same studied week. These histopathological findings agreed with that of Bahar *et al.* (2003) who reported that administration of different chicory extracts exhibited a significant remarkable recovery of hepatocytes in different liver sections of their study. Bahar *et al.* (2003) also elicited that chicory extract could efficiently keep the normalization of the hepatic tissue as neither necrosis nor fatty accumulation were observed and the central vein was clearly appeared which. Cichorium root extract therapy leads to normalization of some morphofunctional liver features (decreases glycogen content and cell of necrosis and increases the number of cells with pronounced protein synthesis activity) in rats with CCl_4-induced hepatitis (Krylova *et al.*,, 2006). This could be an indication of chicory's potent hepatoprotective effect.

In a previous study conducted by us, the histopathological results indicated that supplementation of dandelion leaves water extract could improve significantly (P <0.01) the portal inflammation, necrosis, fibrosis and steatosis at the end of 6th week of the study compared to that of the CCl_4 treated rats (+ve control) at the end of the same studied week. Chung *et al.* (2010) 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.

The current findings suggested that a CLWE and DLWE supplement was associated with beneficial effects on the improvement of increased liver enzyme activities produced by CCl_4 administration.

In conclusion, chicory, dandelion and chicory/ dandelion leaves water extracts mixture showed a remarkable anti-hepatotoxic activity against carbon tetrachloride induced hepatic damage. Also, the present results suggest that both chicory and dandelion in the form of mixture potentiate each other opposing the CCl_4-induced hepatotoxicity. However, more elaborate work is recommended to establish the efficacy of chicory and dandelion leaves water extract as potent anti-hepatotoxic agents. Further studies are required to isolate and identify the active ingredients present in chicory and dandelion leaves, which are responsible for the anti-hepatotoxic activity and determine the precise molecular mechanism of action of both chicory and dandelion leaves.

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