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Effect of Parsley (*Petroselium Crispum*) on Carbon Tetrachloride-Induced Acute Hepatotoxicity in Rats.

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Abstract

Parsley (petroselinum) is natural herbal plant used as food additive. This study aim to investigate the effect of parsley on hepatic toxicity induced by carbon tetra chloride. Thirty male Wister rats were used in this study and divided to three group, one group as normal group, second group as carbon tetrachloride induced hepatotoxicity and third group as carbon tetrachloride induced hepatotoxicity with the treatment with parsley. It was found a significant decrease of AST, ALT and GGT after parsley treatment. Also, superoxide dismutase (SOD), catalase (CAT) were decreased in the group treated with CCl4 plus parsley .The expression of tumor necrosis factor-alpha (TNF-α), was ameliorated in group treated with CCl4 plus parsley which is high expression in group treated with CCL4 without parsley. In addition parsley reduced fatty degeneration, cytoplasmic vascularization and necrosis of liver in CCl4 treated group. In conclusion, this study indicated that parsley has hepatoprotective effect on acute liver injury induced by CCl4.

Keywords: Parsley; Hepatotoxicity; Liver enzymes; Tumor necrosis factor.

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INTRODUCTION

Liver is a primary organ involved in biotransformation of food and drugs hepatic diseases are a major worldwide problem [1]. Liver intoxication has increased as a result of exposure to high levels of environmental toxins. Many hepatotoxic agent such as carbon tetrachloride (CCL4), nitrosamines, and polycyclic aromatic hydrocarbons require metabolic activation, particularly by cytochrome P450 isoenzymes (CYPs), to from reactive toxic metabolites, which cause liver injury in experimental animals and humans [2].

Carbon tetrachloride is widely used for inducing experimental liver injury in animals [3]. CCl4 is metabolized by hepatic microsomal CYPs to the hepatotoxic metabolites trichloromethyl (CCl3) and trichloromethylperoxyl radicals (CCl3OO) [4, 5]. These products are unstable radicals and exhibit strong affinity for binding to protein and lipids of the cell membrane or abstracting a hydrogen atom from an unsaturated lipid, triggering lipid peroxidation and causing liver damage [6].

The oxidative damage caused by reactive oxygen species (ROS) and reactive nitrogen species (RNS) may generate various diseases in the human body [7]. The enhanced production of oxidative stress can be induced by variety of factors, such as ionizing radiation, and exposure to drugs or xenobiotic (e.g. carbon tetrachloride). Liver damage caused by CCl4 is characterized by inflammation in the early stage. Antioxidant action plays an important role by which various natural products protect against CCl4- induced liver damage [8].

Many studies have been shown that the presence of natural antioxidants from various aromatic and medicinal plants is closely related to the reduction of chronic diseases such as DNA damage, mutagenesis, and carcinogenesis [9, 10] .Therefore, there has been a growing interest in research concerning alternative antioxidant active compounds, including plant extracts and essential oils that are relatively less damaging to mammalian health and environment [11].

Parsley (Petroselinum crispum) is a herbs commonly used to flavor the cuisines of china, Mexico, South America, India and South East Asia [12].Parsley is native to Europe and western Asia and cultivated in the united states as an annual for its aromatic and attractive leaves[13]. Components of fresh parsley leaf scavenge superoxide anion in vitro[14], and methanol extracts of parsley scavenge hydroxyl radical in addition to protecting against ascorbic acid induced membrane oxidation [15]. Supplementation of diets with fresh leaf can increase antioxidant capacity of rat plasma [16] and decrease the oxidative stress in humans [17].

MATERIAL AND METHOD

Aqueous extract of parsley

Parsley leave were purchased from (herbs market, kafrelsheikh) and carefully washed with tap water and left to dry in the dark at room temperature. They were stored in well-closed cellophane. The air-dried leaves (100 g) were extracted by adding 1000mL of distilled water and boiled for 30 min. The extract was then filtered, and the filtrates were evaporated, using a rotary evaporator under reduced pressure to dryness. The extract was dissolved in distilled water before the administration.

Animals

The experiment was done on 30 female albino rats, weighting 150-200 gm. Animals maintained in Animal faculty of Kafrelsheikh University under conventional conditions with a regular 12-hour light/dark cycle and temperature controlled at 24^0C animals were fed on a commercial standard animal diet which consist of corn, soybean, water add libtum. Animals were kept with basal diet and water for 2week for acclimation. All procedures described were reviewed and approved by the Institutional Committee for Ethical Use of Animals according to the guidelines laid out by Kafrelsheikh University

Preparation of dose and treatment

The CCl4 was administrated at a dose of 1ml/kg intraperotineal. Water extract of parsley was given by 1ml /150 gm. orally by stomach tube for 4 weeks.

Experimental design

Thirty female albino rats were divided to three groups Group I: is normal group (untreated with CCL4), Group II: is control positive group (treated with CCL4); group III; is treated the hepatotoxicity animal with the parsley extract .all animals kept in the same environmental condition during the period of the experiment.

Chemicals

Liver markers, aspartate aminotransferase (AST), alanine amino transferase (ALT), serum alkaline phosphatase (ALP) and TNF-α primer were purchased from Biodiagnostic campany (Egypt); gamma gltuamyltransferase (γGT) was purchased from Spectrum Company (Egypt). The antioxidants markers, superoxide dismutase (SOD) and catalase were purchased from Biodiagnostic company (Egypt) .carbontetrachloride (CCL4) was get from sigma Aldrich (Egypt)

Blood biochemical assay

The whole blood samples centrifuge at 4000rpm/10 minute for collection of plasma for catalase assay. The erythrocytes was washed four time with 3ml of 0.9%Nacl and centrifuge for 4000rpm/10 minute , the washed centrifuged erythrocytes made up to 2 ml with cold distilled water, well mixed and well left stand at 4^{o}C for 15 minute . This erythrocyte lysate used for SOD assay. Also, serum sample used for biochemical assay of AST: ALT; γGT, ALP.

Histopathology

After sacrificing animal, small pieces of liver tissue was obtained at 4 weeks at the end of the experiment and fixed 10% formalin. The liver specimens were sectioned at 1-µm thickness and stained with hematoxylin and eosin; they were examined for histopathological changes under the microscope (Leitz DMRBE, Germany). Images were taken with a digital camera (Leica DFC 295) at original magnification of x200.

RNA – Extraction, PCR

Liver tissue was snape –frozen in liquid nitrogen, preserved at -80^{o}C till RNA extraction (easy-RED^{TM} Total RNA Extraction Kit, Intronbio Company, Cat.No.17063).

Determination of AST/ ALT

The serum AST was determined as described previously [18].Briefly,100µl of serum was mixed with 500µl AST/ ALT buffer substrate (phosphate buffer PH7.5, Aspartate/ Alanine α-ketoglutarate) after previous incubate the buffer at 37^{o}C for 5 minute , incubate the mixture at 37^{o}C for 60 minute . 500µl of color reagent (2,4 dinitrophenylhydrazine)was added to the previous mixture and incubated at room temperature for 20 minute , 500µl of NaOH 0.4N. Incubate for 5 minute at room temperature, measure the absorbance at 520 nm.

Determination of γGT.

The serum γGT was determine as described previously **[19].**Briefly,50 µl of serum sample was added to the working solution (9/1v/v of reagent 1 : reagent 2), mix well , initial absorbance reading after 30 second and other reading after 1,2,3, minute respectively ,determine the mean absorbance change per minute (ΔA/ min). Absorbance was measured at 405nm.

Determination of ALP

The serum ALP activity was determined as previously described [20].Briefly**,** 25µl of serum sample was added to 500µl of buffer- substrate (buffer PH 10, Phenyl phosphate), incubate at 37^{o}C / 20 minute , added 250µl of enzyme inhibitor (EDTA; 4-Aminophenazone), added 250 µl of color reagent (potassium ferricyanide), and incubate at the room temperature for 5 minute . Measure the absorbance of sample (Δsample), standard (Δstandard),against reagent blank at 510nm . ALP activity can be calculated as this equation

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$$ALP = \frac{A_{sample}}{A_{standard}} X75\ IU/L$$

Determination of SOD

SOD activity was determined as described pervious [21] . Briefly, 100 µl erythrocyte lysate (dilute 30 time) was mixed well with the working solution (phosphate buffer PH8.5: Nitroblue tetrazolium:phenazine methosulphate , 10:1:1 respectively), added 100µl of phenazine methosulphate (diluted to 1000 time),measure the increase of absorbance at 560nm for 5 minute . In which control is the same procedure except we added distill water instead of sample.

Determination of catalase activity

Catalase activity was determined as described previous [22]**.Briefly,** 50µl of serum sample added to 500µl of buffer (phosphate buffer ,PH 7, detergent) ; 100µl of H_2O_2 (diluted 1000 time before use) , incubated at 25^{o}C for 1 minute , added 200µl of chromogen-inhibitor and 500µl of enzyme(peroxidase, 4-aminoantipyrine) , incubate at 37^{o}C for 10 minute ,measure absorbance at 510nm.

Statistical analysis: The mean values and standard errors were calculated for the obtained data, and the significances for all means have been carried out by applying F-test using the SPSS computer program.

RESULTS

Body weight and liver weight

There are significant decrease in CCl4-induced hepatotoxicity rats if compared with the normal rats, parsley treatment rats ameliorate the body weight, significant increase if compared to the CCl4-induced hepatotoxicity rats. Liver weight is significant decrease in parsley treated rat if compared with untreated rats as shown in (fig .1, 2)

Serum AST, ALT, ALP and GGT activities

To evaluate the liver injury, we carried out an analysis of serum AST, ALT, ALP and γGT activities. Our study revealed that, there are significant increase in the AST, ALT, ALP and γGT in the CCL4-induced hepatotoxicity rats .when compared with those of the normal control group. When treated the animals with parsley the four liver enzymes are significant decrease if compared with those in positive control group as shown in (fig. 3, 4, and 5).

Antioxidant enzymes

To evaluate the antioxidant effect of parsley .we carried analysis of SOD, Catalase. Our study revealed that, in positive control animal there are significant decrease in SOD, catalase if compared with those in normal animals. Parsley treatment animal showing a significant increase in SOD and catalase activity similar to the normal animal as shown in (fig.6, 7). From this, we documented that, parsley administration to rats has ability to ameliorate the liver enzymes and oxidative stress associated with the liver injury.

Histopathological findings

Liver damage in rats was evaluated by histological examination after staining with H&E; liver tissue from each group was examined. There are normal architecture with central vein in the normal animal (fig.8 a). There are marked fatty degeneration, portal inflammation and necrosis, hepatocyte loosening in hepatotoxicity animal (fig.8 b). In parsley treatment animal, all the pathological changes are relieved as shown (fig 8 C).

mRNA expression

TNF-α mRNA expression was significant high expressed in carbon tetrachloride-group in relation to both of control and parsley treated rat normal animals as shown in (fig.9).

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DISCUSSION

Oxidative stress plays a great role in the development of CCl4-induced hepatotoxicity and a relation between oxidative stress and lipid peroxidation has been reported [23] . Many studies have reported that CCl4 is widely used to induce liver damage because it is metabolized in hepatocytes by cytochrome P450, generating a highly reactive carbon-centered trichoromethyl radicals , leading to initiating a chain of lipid peroxidation and so causing liver damage [24]. CCl4 –induced hepatotoxicity model was widely reported in studies on therapies against various hepatic diseases in that CCl4- induced liver damage shares mechanism with viral hepatitis [25]. The medicinal properties of plants have been investigated in the recent scientific developments throughout the world, due to their potent biological activities, low toxicity and economic viability [26]. So in this study we focused on the parsley , many studies showed that parsley including flavonoid[27], carotenoids [28], ascorbic acid [29].Tocopherol and coumarines [27]. These phytochemicals improve total antioxidant capacity, destructive oxygen free radicals and prevents oxidative damage [30]. In this study we investigated the antioxidant activity of parsley and its effect on hepatotoxicity by using CCl4- induced hepatotoxicity model rat. And try to found a new strategy, new medicine plant to be effective in treatment of liver injury that is a big problem and great threat human health. Our result reported that the parsley treatment improves the body weight and decrease the liver index if compared with control group. Many studies have confirmed that the reduction in body weight was related to the toxicity.

Hepatic cells contain higher concentrations of AST and ALT in the cytoplasm, and AST, particularly exists in the mitochondria. Due to damage caused to hepatic cells, the leakage of cytosol will cause increased levels of hepatotospecific enzymes in the serum. The elevated serum enzyme levels such as AST and ALT are indicative of cellular leakage and functional integrity of cell membrane in the liver [31].ALP is an ectoenzyme of the hepatocyte plasma membrane, its increase is related to damage to the liver cell membrane [32]. GGT is an enzyme in the metabolism of GSH is another sensitive marker of alcoholic liver injury. Increases in plasma GGT can lead to an increase in the production of free radicals, particularly in the presences of iron [33]. The increased level of AST, ALT, ALP, GGT in the serum consider marker of liver injury. In this study, the parsley treatment significant decrease the serum level of AST, ALT, ALP, and GGT if compared with the hepatic toxicity animal. And this result indicated that parsley has ability to liver regeneration after liver cell damage. TNF-α is a major endogenous mediator of hepatotoxicity in several experimental liver injuries [34],this study confirmed a significant increase in the TNF-α mRNA expression in the liver after CCl4 administration; these increases were attenuated by treatment with Parsley. These results suggest that the Parsley modulates the expressions of TNF-α.

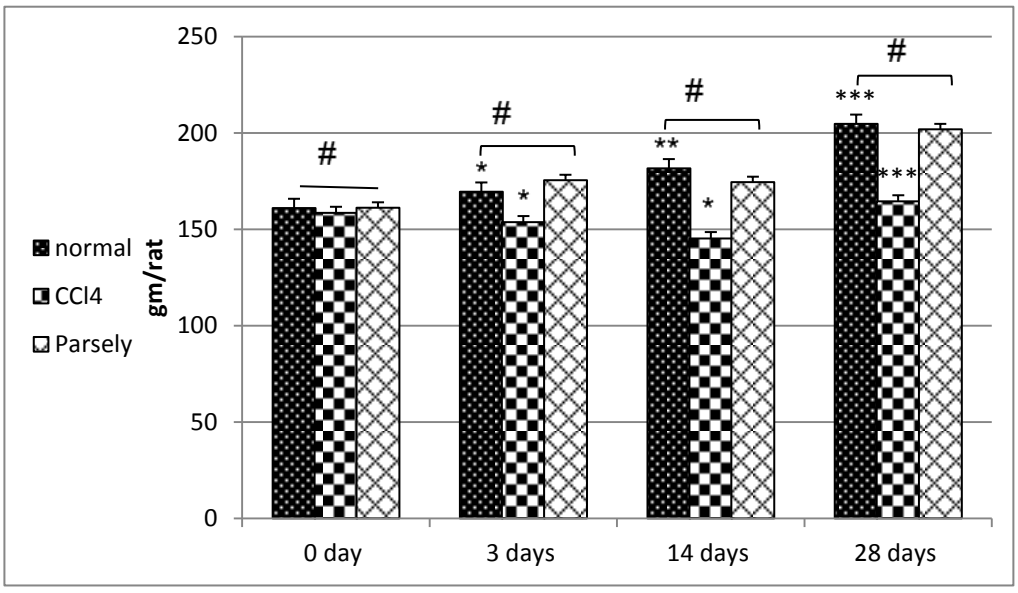

Figure (1) Mean levels of body weight at 0, 3, 14, 28 days of age in albino rat. Data are expressed as Mean ±SE

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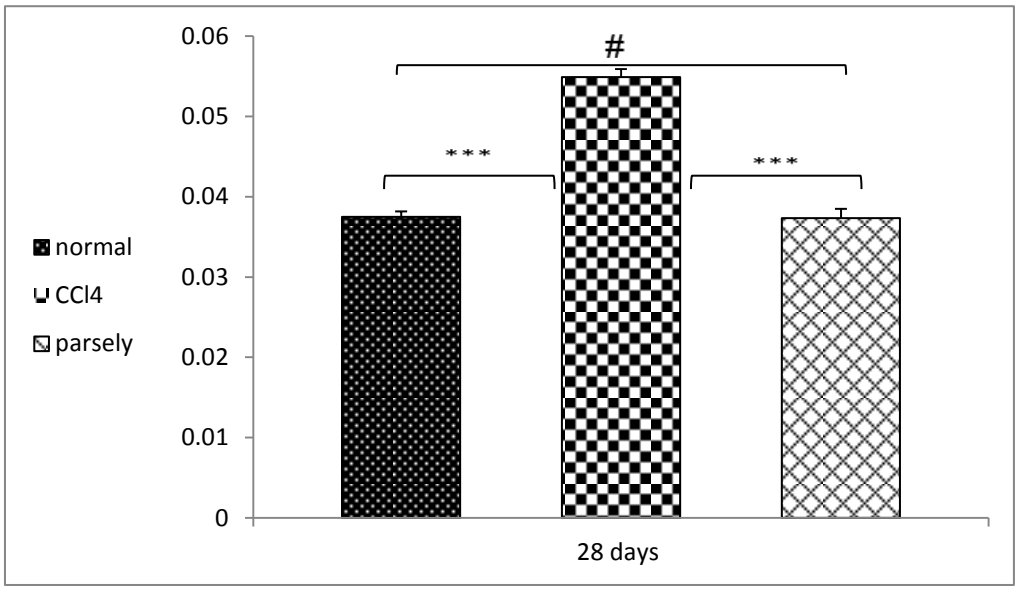

Figure (2) Mean level of liver weight/ body weight at28 days of age in albino rat. Data are expressed as Mean ±SE

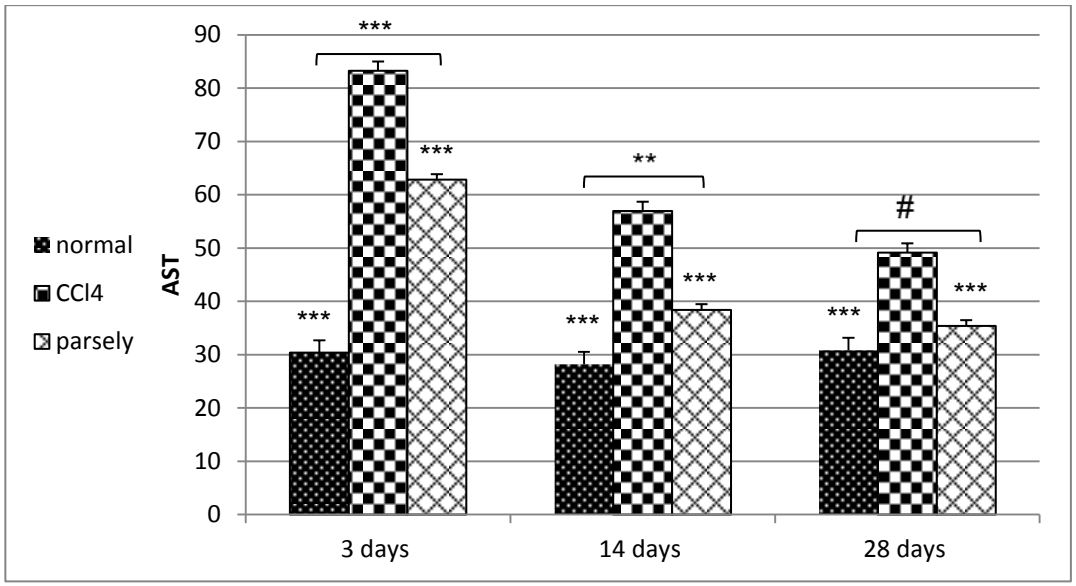

Figure (3) Mean level of aspartate aminotransferase (AST) at3, 14, 28 days of age in albino rat. Data are expressed as Mean ±SE

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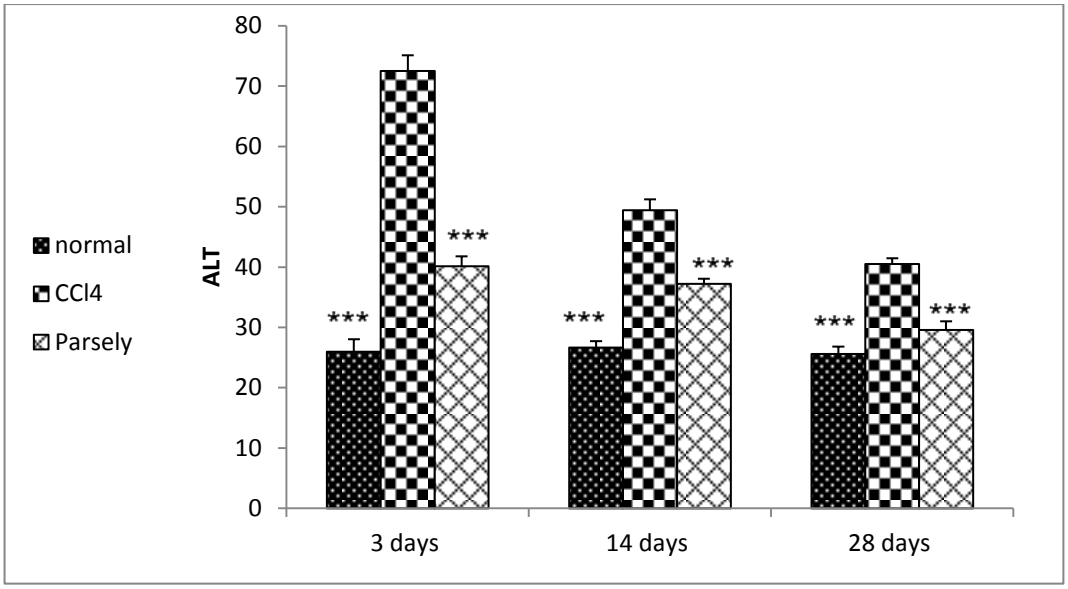

Figure (4) Mean level of Alanine aminotransferase (ALT) at3, 14, 28 days of age in albino rat. Data are expressed as Mean ±SE

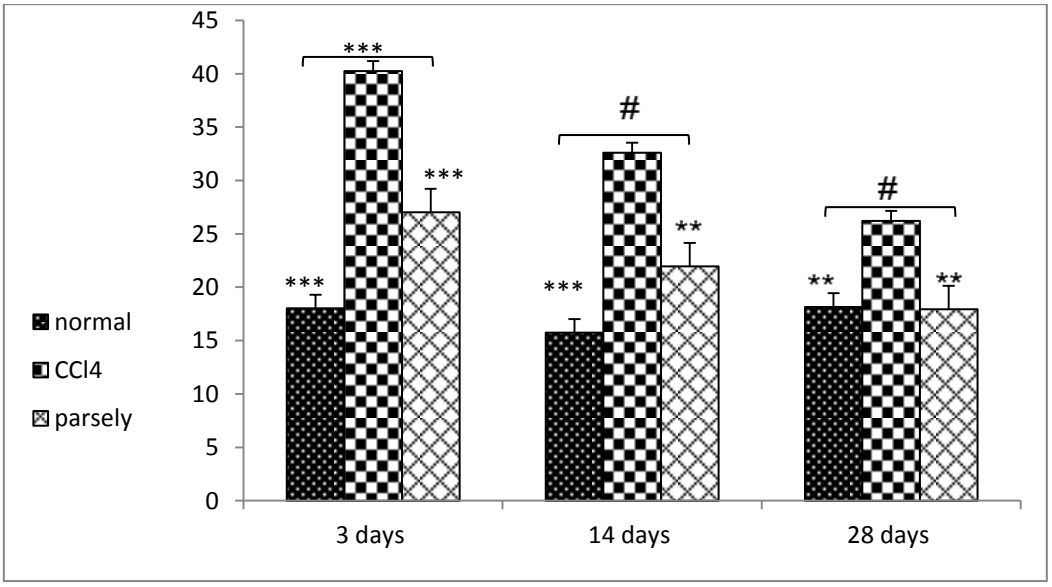

Figure (5) Mean level of γglutamyltransferase (γ GT) at3, 14, 28 days of age in albino rat. Data are expressed as Mean ±SE

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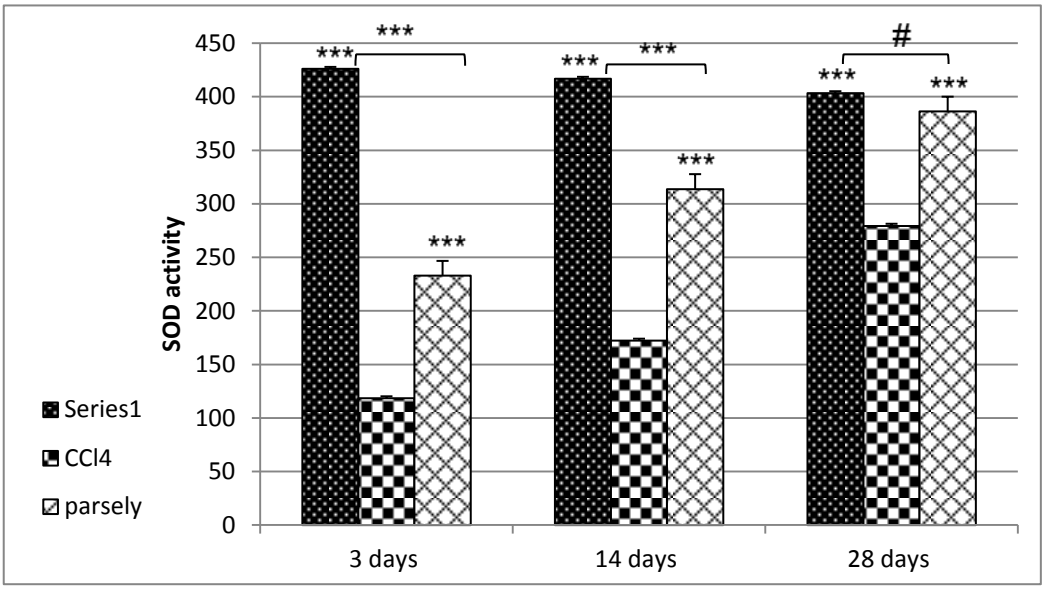

Figure (6) Mean level ofsuperoxidedismutase (SOD) at3, 14, 28 days of age in albino rat. Data are expressed as as Mean ±SE

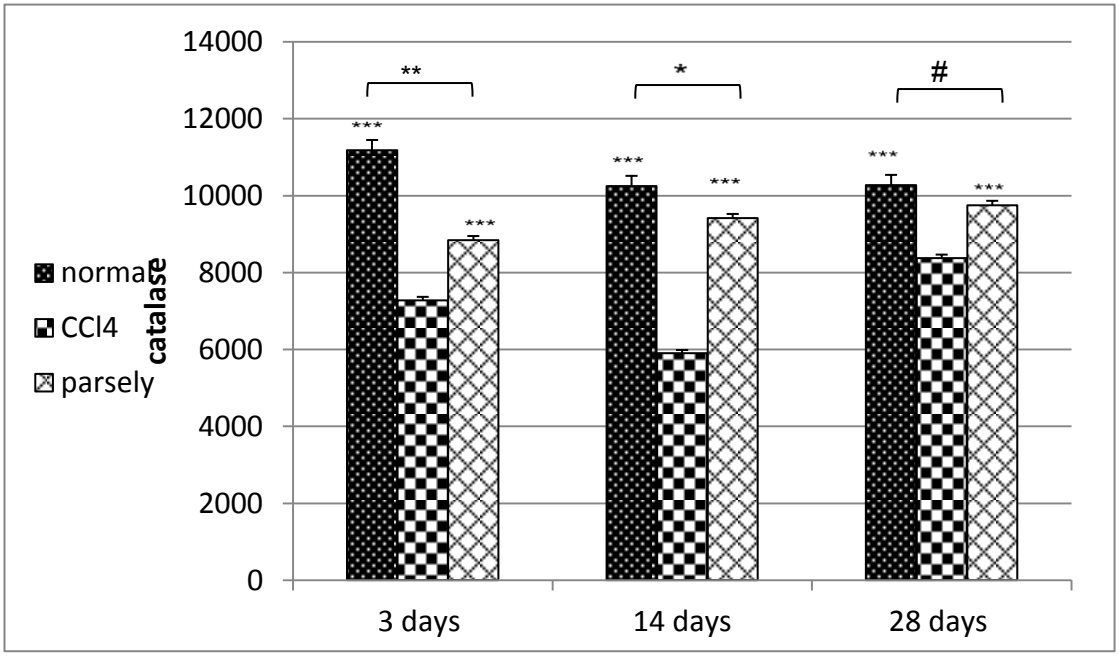

Figure (7) Mean level of catalase at3, 14, 28 days of age in albino rat. Data are expressed as Mean ±SE

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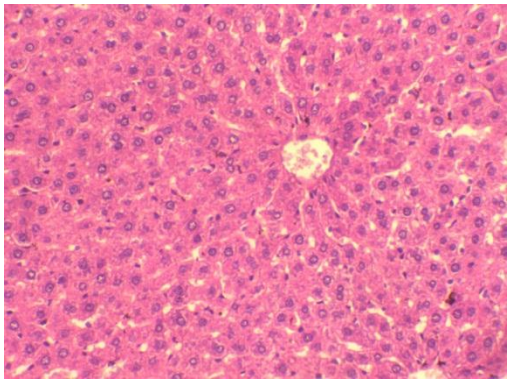
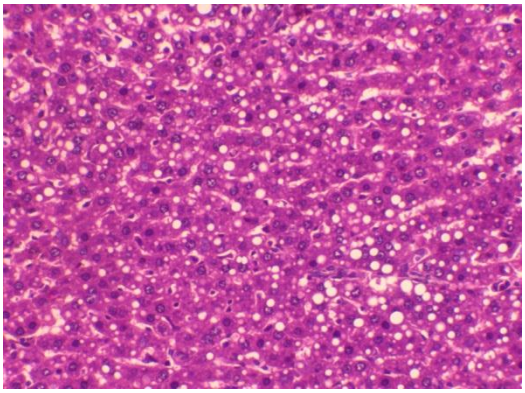
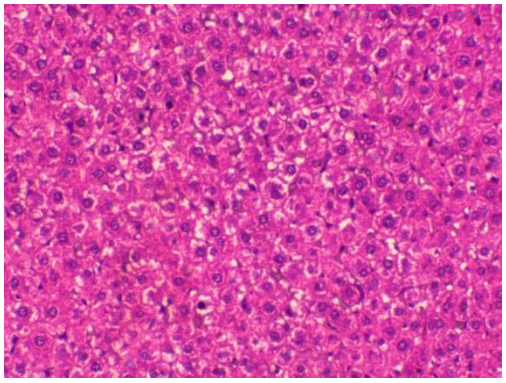


(A)

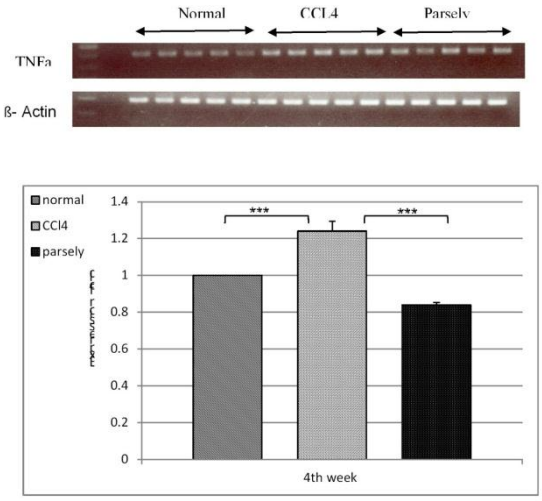
(B)





(C)

Figure (8) H&E staining for liver tissue of albino rat. (A) Normal ;(B) CCl4 treated albino rat; (C) parsley treated albino rat.



Figure(9) mRNA expression of TNFα in normal, CCL4 , parsley treated albino rats.

In conclusion, the hepatoprotective of Parsley could be attributed to its mechanistic intervention in several cellular events, providing beneficial effects, by attenuating oxidative stress and normalized the TNF-α; this study provides evidence that Parsley could be used to prevent hepatocellular damage.

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