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Effect of some herbal extracts on reducing hepatic damage induced by carbon tetrachloride in rats

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Abstract: Cabon tetrachloride (CCl_4) is a well-known hepatotoxic chemical and also induces nephrotoxicity in rats. The study was aimed to investigate the effect of antioxidant activity of coriander, dill and parsley leaf ethanolic extracts (CLE, DLE and PLE) on oxidative stress of carbon tetrachloride (CCl_4) treated in Wistar albino rats. CCl_4 injection induced oxidative stress by a significant rise in the activities of serum aspartate aminotransferase (AST), alanine aminotransferase (ALT) alkaline phosphatase (ALP) and gamma- glutamyl transferase (GGT) as well as the levels of serum creatinine, urea, uric acid and malondialdehyde (MDA) along with the reduction of antioxidant enzymes; glutathione reductase (GR), gutathione-S-transferase (GST), superoxide dismutase (SOD) and catalase (CAT). Oral administration of the herbal leaf ethanolic extracts at dose (500 mg/kg b.w) showed a significant improvement of hepatic and renal biochemical parameters and antioxidant oxidative stress activities compared to CCl_4 treated rats. The activity of DLE showed more amelioration than other leaf ethanolic extracts. Histopathological examinations showed extensive liver and kidney injuries in CCl_4 treated rats and its recovery by three herbal leaf ethanolic extracts treated rats. Based on these results, it was observed that CLE, DLE and PLE protect the liver and kidney from oxidative stress induced by CCl_4.

Key words: coriander, dill, parsley Carbon tetrachloride, hepatoprotective activity, Histopathology.

INTRODUCTION

Oxidative stress is regarded as a mediator of acute and chronic liver injury. The CCl_4-induced hepatotoxicity model was being widely used for the evaluation of the hepatoprotective effects of plant extracts and also induced nephrotoxicity in laboratory animals[1-2]. A number of studies have shown that CCl_4 is metabolized by the P450 enzyme system to yield reactive metabolic products trichloromethyl free radicals, which can initiate the process of lipid peroxidation and ultimately results in the overproduction of reactive oxygen species (ROS) and hepatocyte injuries[3-4]. Additionally, it was reported that hepatic and renal toxicity caused by CCl_4 which was treated by natural products decreased the elevated TBARS and up-regulated the activity of antioxidant enzymes[5-6]. A number of studies also showed that various herbal extracts and plant derived pure chemicals could protect organs against CCl_4-induced oxidative stress by altering the level of antioxidant enzymes[7]. Endogenous antioxidants in medicinal herbs may play an important role in antioxidative defense against oxidative damage, possibly protecting the biological functions of cells[5]. Coriander, both its leaf and seeds are grown as spice group all over the world. The efficiency of coriander was due to presence of several pharmacological agents such as antifertility[8], antihyperglycemic, antihyperlipidemic[9], antiproliferative[10], hypotensive[11] and digestive stimulant[12]. *Anethum graveolens* L. (dill) (Apiaceae) is one of the most popular culinary herbs in the world. There have been records on use of this plant for medicinal and edible purposes dating back to the Greek and Egyptian civilizations[13]. Various pharmacological actions of *A. graveolens* such as antimicrobial, antispasmodic, antidiabetic, antihypercholesteromic, and anti-inflammatory have been reported by Heamalatha *et al*.[14]. Parsley (*Petroselinum crispum*, Apiaceae) is one of the most used medicinal plants to treat arterial hypertension[15]; diabetes, cardiac, renal diseases[16], anti-hyperglycemic[17], anti-hyperlipidimic, anticoagulant[18], anti-oxidant[19] and anti-microbial[20]. The present study was performed to investigate the potential hepatoprotective activity of coriander, dill and parsley leaf ethanolic extracts against hepatic injury produced by carbon tetrachloride in rats.

MATERIALS AND METHODS

Materials:

Chemicals: Carbon tetrachloride was purchased from Sigma Chemical Co., St. Louis, USA. Solvents and chemicals were purchased from Merck (Darmstadt, Germany). Kits used for the estimation of analyzed parameters were purchased from Biosystem, Spain.

Plant material: Fresh leaf of coriander (*Coriandrum sativum* L.), dill (*Anthum graveolens* L.) and parsley (*Petroselinum crispum* L.) were obtained from local market, Cairo, Egypt.

Corn starch, corn oil, cellulose and sucrose: Corn starch and corn oil were purchased from the local market while sucrose and cellulose were purchased from Sigma Chemical Co., St. Louis, USA.

Mineral salts and vitamins: Mineral salts and vitamins used for the preparation of the mineral salt and vitamin mixtures were obtained from Sigma Chemical Co., St. Louis, USA and prepared according to **A.O.A.C.[21].**

Animals: Adult male Wistar rats weighing (185 ±5g) were obtained from Faculty of Veterinary Medicine, Cairo University. The experimental animals were carried out according to the ethical

committee and standard regulations by the animal's house of Regional Center for Food and Feed, Agriculture Research Center, Giza, Egypt.

Methods:

Preparation of plant samples: Leaves of three herbal were washed thoroughly 2-3 times with distilled water. The leaves were air dried in a ventilated oven at 40°C for 48 h and ground to a fine powder. The dried leaf powders were stored in polyethylene bags in refrigerator at 4°C until further use.

Preparation of herbal extracts: Powdered of three herbal leaf (200 g) were extracted with 800 ml 80% ethanol by constant shaking at room temperature for 24 h. The mixture filtered through a Whatman No. 1 filter paper for removal of peel particles. The residue was re-extracted twice under the same condition to ensure complete extraction. The combined filtrates were lyophilized using a freeze dryer (Labconco, Model Lyph. Lock 6). The three crude extracts were placed in dark bottles and stored in refrigerator at 4°C for future use. The three crude extracts were suspended in boiling distilled water before administration to the animals.

Carbon tetrachloride induction of hepatic injury: Liver injury was induced by an oral administration of 20% CCl_4 which was suspended in corn oil and then injected orally (1 ml/kg body weight), twice a week, for 4 weeks[22].

Experimental animals and study design: The animals were housed in separated cage and maintained under control laboratory conditions at 25°C ± 2°C on a 12 h light: 12 h dark cycle for one week with free access to standard food and water *ad libitum*. The animals were fed standard diet consisted of casein 20%, sucrose 10%, cellulose 5%, corn oil 10%, salts mixture 4%, vitamin mixture 1% and completed to 100% with corn starch A.O.A.C.[21]. Then, after one week of acclimation, rats were classified into eight groups each containing five animals and received treatment for 28 days as follows.

Group 1: control group.

Group 2: hepatotoxin control group and oral administered with CCl_4 (1ml/kg body weight) twice a week.

Group 3: treated orally by coriander leaf extract (CLE), 500 mg/kg b.wt.

Group 4: treated orally by dill leaf extract (DLE), 500 mg/kg b.wt.

Group 5: treated orally by parsley leaf extract (PLE), 500 mg/kg b.wt.

Group 6: treated orally by coriander leaf extract (CLE), 500 mg/kg b.wt before oral administered by CCl_4 (1ml/kg body weight) twice a week.

Group 7: treated orally by dill leaf extract (DLE), 500 mg/kg b.wt before oral administered by CCl_4 (1ml/kg body weight) twice a week.

Group 8: treated orally with parsley leaf extract (PLE), 500 mg/kg b.wt before oral administered by CCl_4 (1ml/kg body weight) twice a week.

Blood samples were collected from the retro-orbital vein of each animal every two weeks till the end of experiment using a glass capillary tube. Animals were sacrificed and the organs (liver and kidney) from each group were fixed in 10% buffered formalin solution for further examination.

Serum and plasma preparation: The serum and plasma were separated by centrifuging (Hettich, Universal 16, German) at 3000 rpm for 20 min at 4°C, then collected into sterilized tubes and stored at -20°C for biochemical parameters and antioxidant studies. Serum enzymes activities of ALT and AST were assayed according to the methods of Gella *et al.*[23] while ALP and GGT were determined according to the method of Rosalki *et al.*[24] and Persijn and Van der Silk[25], respectively. The levels of creatinine, urea and uric acid were determined according to the method of Fabing and Ertingshausen[26], Tabacco *et al.*[27] and Fossati *et al.*[28], respectively. The level of lipid peroxidation was determined as malondialdehyde (MDA) according to the method reported by Onkawa *et al.*[29].

Plasma biochemical assays: Plasma enzymes activities of GR, GST, SOD and CAT were measured according to the method reported by Goldberg and Spooner[30], Habig *et al.*[31], Nishikimi *et al.*[32] and Aebi[33], respectively.

Histopathological studies: Liver and kidney from each group were fixed in 10% buffered formalin and embedded in paraffin wax. Microtome sections of 3-4 µm thickness were prepared according to the standard procedure and stained with haematoxylin and eosin. Sections were then examined for pathological findings of such as centrilobular necrosis, fatty and lymphocytes infiltration by the light microscope[34].

Statistical analysis: Statistical analysis of the obtained data was done using the least significant difference test (LSD) at the 5% level of probability as outlined by Snedecor and Cochran[35]. Using the Duncan test institute program used a computer in the statistical analysis.

RESULTS AND DISCUSSION

Body weight and relative organs weights: At the end of experiment, CCl_4 treated group showed a significant decrease in body weight of rats (177.00±1.15 g) as compared to the original body weight (283.67±2.40 g). These results are in agreement with the findings of Eidi *et al.*[36] who demonstrated that the final body weight was significantly decreased as a result of CCl_4 administration compared to the control group. Treatment by three herbal leaf ethanolic extracts (CLE, DLE and PLE) during CCl_4 administration showed a significant increase in the body weights (192.67±1.45, 254.67±5.70 and 208.33±2.03 g, respectively) compared to CCl_4-treated group. However, other groups which treated by three herbal leaf ethanolic extracts only did not show any significant change in their body weight as compared to the control group.

In addition, relative liver and kidney weights were significantly increased in CCl_4-treated group (6.14±0.01 and 0.45±0.03 g, respectively) compared to normal control group (3.04±0.01 and 0.28±0.02 g, respectively). These results run in agreement with the data of Uzma *et al.*[37] who observed that the relative weight of liver was increased in CCl_4 treated rats than those of control group. Also, liver is not the only target organ of CCl_4; it also causes free radical generation in kidneys khan *et al* [38]. In general, the highest decrease in relative liver and kidney weights were noticed in rats treated with DLE and administered CCl_4 (3.47±0.08 and 0.32±0.03 g, respectively) when compared to CLE (4.43±0.022 and 0.43±0.01 g, respectively) and PLE (4.16±0.03 and 0.32±0.03 g, respectively). No significant differences were observed in the relative liver and kidney weights between the rats treated with three herbal leaf ethanolic extracts when compared to normal control group.

Table 1: Effects of three herbal leaf ethanolic extracts on body weight and relative organs weights.

Treatment groups	Body weight (g)		Relative weight	
	2 weeks	4 weeks	Liver	Kidney
Group 1 (Normal control)	242.33±8.41^{a}	283.67±2.40^{a}	3.04±0.01^{a}	0.28±0.02^{a}
Group 2 (CCl_4)	201.67±4.84^{c}	177.00±1.15^{e}	6.14±0.01^{c}	0.45±0.03^{e}
Group 3 (CLE)	240.67±2.96^{a}	283.33±1.45^{a}	3.09±0.01^{a}	0.26±0.01^{a}
Group 4 (DLE)	241.00±1.15^{a}	282.67±2.73^{a}	3.09±0.05^{a}	0.29±0.01^{a}
Group 5 (PLE)	242.67±2.96^{a}	283.00±3.06^{a}	3.01±0.05^{a}	0.28±0.02^{a}
Group 6 (CLE + CCl_4)	207.67±1.76^{c}	192.67±1.45^{d}	4.43±0.022^{c}	0.43±0.01^{d}
Group 7 (DLE + CCl_4)	234.67±3.76^{ab}	254.67±5.70^{b}	3.47±0.08^{ab}	0.32±0.03^{b}
Group 8 (PLE + CCl_4)	223.67±5.78^{b}	208.33±2.03^{c}	4.16±0.03^{b}	0.43±0.004^{c}
LSD P < 0.05	13.5648	8.5236	0.1710	0.0600

Values are mean ± SE of 5 rats. Within the same column, various superscript letters indicate significant differences (Duncan, P <0.05).

Hepatotoxicity markers: The enzymes activity of ALT, AST, ALP and GGT in normal control group, CCl_4 group and treated groups were represented in Tables 2 and 3. In the CCl_4-treated group, the activities of ALT, AST, ALP and GGT enzymes were significantly ($p < 0.05$) increased after the fourth week from 58.67±0.33, 51.33±0.88, 116.67±0.88 and 50.00±0.58 U/l, respectively at the second week to 74.67±0.88, 65.33±0.88, 152.33±1.45 and 55.10±0.56 U/l, respectively compared to normal control group (43.00±0.58, 33.00±0.58, 91.00±0.58 and 33.20±0.31 U/l, respectively at the second week to 44.33±0.88, 34.00±0.58, 92.00±0.58 and 34.33±0.33 U/l, respectively at the fourth week. These results were similar to those shown by Sun *et al.*[39] who found that the activities of ALT, AST and ALP were significantly higher in the CCl_4 group than those in the control group ($P < 0.01$). The elevated activities of AST and ALT in serum are indicative of cellular leakage and loss of functional integrity of cell membranes in liver[7]. On the other hand, the activities of the above enzymes were significantly decreased in groups treated with CLE, DLE and PLE during CCl_4 administration. The activities of these enzymes were decreased in the order of DLE > PLE > CLE during oral administration of rats with CCl_4 compared to CCl_4-treated rats. This observation suggests that the phytochemical content in coriander, dill and parsley leaf ethanolic were reduced the activity of these enzymes. Previous studies have found that total phenolic content of methanol extracts from parsley was greater than from cilantro[40]. Flavonoids, a major group of total phenolic compounds are found in greater concentration in parsley than in cilantro[41]. At the end of experiment, the groups treated with the three herbal leaf ethanolic extracts alone and group treated with DLE + CCl_4 did not show any changes in these enzymes activity as compared to normal control group. These results are in agreement with El-Maghraby *et al.*[42] who found that the treatment by dill plant extract alone did not cause any significant changes in plasma enzyme activities of ALT, AST and ALP.

Table 2: Effects of three herbal leaf ethanolic extracts on the activity of ALT and AST.

Treatment groups	Biochemical parameters			
	ALT (U/l)		AST (U/l)	
	2 weeks	4 weeks	2 weeks	4 weeks
Group 1 (Normal control)	43.00±0.58^{d}	44.33±0.88^{d}	33.00±0.58^{d}	34.00±0.58^{d}
Group 2 (CCl_4)	58.67±0.33^{a}	74.67±0.88^{a}	51.33±0.88^{a}	65.33±0.88^{a}
Group 3 (CLE)	43.00±2.08^{d}	44.00±0.58^{d}	33.00±1.53^{d}	34.33±0.88^{d}
Group 4 (DLE)	43.67±1.86^{d}	44.00±1.15^{d}	33.33±1.76^{d}	34.00±1.53^{d}
Group 5 (PLE)	43.00±1.15^{d}	44.67±1.20^{d}	33.33±0.88^{d}	34.50±0.29^{d}
Group 6 (CLE + CCl_4)	54.00±1.15^{b}	60.00±0.58^{b}	44.97±0.58^{b}	47.33±1.20^{b}
Group 7 (DLE + CCl_4)	46.33±0.88^{cd}	44.67±0.88^{d}	36.00±0.58^{d}	36.33±0.88^{d}
Group 8 (PLE + CCl_4)	50.00±0.58^{c}	54.33±0.88^{c}	40.27±0.37^{c}	44.33±0.88^{c}
LSD P < 0.05	3.6717	2.7138	3.239	2.8649

Values are mean ± SE of 5 rats. Within the same column, various superscript letters indicate significant differences (Duncan, P <0.05).

Table 3: Effects of three herbal leaf ethanolic extracts on the activity of ALP and GGT.

Treatment groups	Biochemical parameters			
	ALP (U/l)		GGT(U/l)	
	2 weeks	4 weeks	2 weeks	4 weeks
Group 1 (Normal control)	91.00±0.58^{e}	92.00±0.58^{d}	33.20±0.31^{d}	34.33±0.33^{d}
Group 2 (CCl_4)	116.67±0.88^{a}	152.33±1.45^{a}	50.00±0.58^{a}	55.10±0.56^{a}
Group 3 (CLE)	91.33±1.45^{e}	92.67±1.20^{d}	33.17± 0.38^{d}	34.27±0.82^{d}
Group 4 (DLE)	91.00±1.15^{e}	92.33±0.88^{d}	33.10±0.56^{d}	34.00±0.58^{d}
Group 5 (PLE)	91.67±0.88^{e}	92.67±0.88^{d}	33.13±0.32^{d}	34.60±0.70^{d}
Group 6 (CLE + CCl_4)	111.33±0.88^{b}	120.00±0.58^{b}	47.27±0.37^{b}	49.63±0.47^{b}
Group 7 (DLE + CCl_4)	98.67±0.33^{d}	92.00±0.58^{d}	34.10±0.38^{d}	35.23±0.90^{d}
Group 8 (PLE + CCl_4)	104.67±0.88^{c}	110.33±0.88^{c}	41.17±0.60^{c}	45.50±0.29^{c}
LSD P < 0.05	2.804	2.782	1.3523	1.8464

Values are mean ± SE of 5 rats. Within the same column, various superscript letters indicate significant differences (Duncan, P <0.05).

Nephrotoxicity markers: The result of nephrotoxicity markers including serum creatinine, urea and uric acid are shown in Table 4. Administration of CCl_4 for the fourth week significantly elevated the serum creatinine and urea levels (1.73±0.009 and 54.00±1.15 and mg/dl, respectively) as compared to the control group (0.61±0.015 and 37.47±0.24 mg/dl, respectively). These findings were similar to that recorded by Kang *et al.*[43] who reported that a significant increase in serum blood urea nitrogen and creatinine were confirmed after CCl_4 administration. Treated rats with CLE, DLE and PLE during administration CCl_4 significantly decreased the rise creatinine, urea and uric acid levels. No significant differences were observed in the serum creatinine and urea levels between the rats treated by three herbal leaf ethanolic extracts when compared to the normal control group. Although, during this period, it was no significant differences were found between the eight groups in the serum uric acid levels.

Table 4: Effects of three herbal leaf ethanolic extracts on the levels of creatinine, urea and uric acid.

Treatment groups	Biochemical parameters					
	Creatinine (mg/dl)		Urea (mg/dl)		Uric acid (mg/dl)	
	2 weeks	4 weeks	2 weeks	4 weeks	2 weeks	4 weeks
Group 1 (Normal control)	0.60±0.054^{d}	0.61±0.015^{d}	36.00±0.58^{d}	37.47±0.24^{d}	3.63±0.01^{a}	3.69±0.00^{a}
Group 2 (CCl_4)	1.53±0.035^{a}	1.73±0.009^{a}	48.80±0.15^{a}	54.00±1.15^{a}	3.64±0.02^{a}	3.68±0.01^{a}
Group 3 (CLE)	0.60±0.009^{d}	0.61±0.012^{d}	36.17±0.44^{d}	37.10±0.46^{d}	3.64±0.01^{a}	3.68±0.00^{a}
Group 4 (DLE)	0.60±0.009^{d}	0.61±0.018^{d}	36.17±0.58^{d}	37.30±0.35^{d}	3.64±0.01^{a}	3.68±0.01^{a}
Group 5 (PLE)	0.60±0.006^{d}	0.61±0.006^{d}	36.10±0.38^{d}	37.00±0.58^{d}	3.66±0.00^{a}	3.67±0.01^{a}
Group 6 (CLE + CCl_4)	1.18±0.044^{b}	1.49±0.006^{b}	43.00±0.58^{b}	47.33±1.20^{b}	3.64±0.02^{a}	3.68±0.01^{a}
Group 7 (DLE + CCl_4)	0.61±0.006^{d}	0.61±0.012^{d}	37.00±0.58^{d}	37.27± 0.37^{d}	3.66±0.01^{a}	3.67±0.01^{a}
Group 8 (PLE + CCl_4)	0.91±0.009^{c}	1.20±0.032^{c}	39.50±0.55^{c}	44.00±0.58^{c}	3.65±0.01^{a}	3.67±0.01^{a}
LSD P < 0.05	0.08450	0.0470	1.3010	2.1128	0.0356	0.0223

Values are mean ± SE of 5 rats. Within the same column, various superscript letters indicate significant differences (Duncan, P <0.05).

Oxidative stress markers: Effects of coriander, dill and parsley leaf ethanolic extracts on oxidative stress markers; GR, GST, SOD, CAT and MDA are shown in Table 5 and 6. It was found that CCl_4 administration in rats significantly depleted the activity of antioxidant enzymes (GR, GST, SOD, CAT) as well as a significant increase MDA level when compared to the normal control group. These results confirm those demonstrated by Adetoro *et al.*[44] who showed that there was a significant (P<0.05) increase in the level of Thiobarbituric acid-reactive substances (TBARS) and a significant (P<0.05) decrease in the level of catalase (CAT) and SOD of the CCl_4 induced liver damage of rats compared to the normal control. In the present study, the administration of CCl_4 resulted in a significant increase in the hepatic

MDA concentration, indicating increased lipid peroxidation. Lipid peroxidation of hepatocyte membranes is one of the principal causes of CCl_4-induced hepatotoxicity, and is mediated by the production of free radical derivatives of CCl_4[45]. In contrast, treated rats with CLE, DLE and PLE during administration CCl_4 significant increase the activities of GR, GST, SOD and CAT enzymes while decrease the MDA level as compared to the CCl_4-treated group. Furthermore, treated rats with DLE during administration CCl_4 brought back these antioxidant enzymes activities and MDA level to near control groups. The green leaf ethanol extracts of *A. graveolens* was higher in antioxidant activity assays[46] found that. The antioxidant property of *A. graveolens* is related to the large amounts of phenolic acid[47], which act through different mechanisms. Meanwhile, no significant differences were observed in the antioxidant enzymes activities (GR, SOD and CAT) and MDA level between the rats treated with CLE, DLE and PLE only when compared to normal control group.

Table 5: Effects of three herbal leaf ethanolic extracts on the activities of GR, GST, SOD enzymes.

Treatment groups	Biochemical parameters					
	GR (U/ml)		GST (U/ml)		SOD (U/ml)	
	2 weeks	4 weeks	2 weeks	4 weeks	2 weeks	4 weeks
Group 1 (Normal control)	18.00±0.58^a	19.00±0.58^a	26.00±0.58^a	26.47±0.24^a	345.17±0.44^a	346.33±0.88^a
Group 2 (CCl_4)	13.83±0.44^c	11.00±0.58^c	18.80±0.44^c	15.87±0.49^e	331.33±0.88^d	325.00±1.15^d
Group 3 (CLE)	18.17± 0.44^a	19.30±0.89^a	26.63±0.32^a	26.73±0.12^a	345.00±1.73^a	346.30±0.89^a
Group 4 (DLE)	18.27±0.37^a	19.13±0.41^a	26.27±0.37^a	26.67±0.09^a	345.13±0.58^a	346.17±0.93^a
Group 5 (PLE)	18.00±1.00^a	19.17±0.44^a	26.30±0.35^a	26.57±0.12^a	345.33±1.67^a	346.23±0.39^a
Group 6 (CLE + CCl_4)	14.30±0.35^c	11.87±0.09^c	18.63±0.32^c	18.13±0.19^d	335.00±1.15^c	331.30±0.35^c
Group 7 (DLE + CCl_4)	18.23±0.39^a	18.43±0.35^a	25.63±0.32^a	25.80±0.06^b	344.17±0.44^a	345.00±0.58^a
Group 8 (PLE + CCl_4)	16.00±0.58^b	14.27±0.32^b	22.47±0.26^b	21.87±0.09^c	339.33±0.33^b	335.30±0.91^b
LSD P < 0.05	1.6662	1.5172	1.1394	0.6543	3.1306	2.4162

Values are mean ± SE of 5 rats. Within the same column, various superscript letters indicate significant differences (Duncan, P <0.05).

Table 6: Effects of three herbal leaf ethanolic extracts on the CAT enzymes activity and MDA level.

Treatment groups	Biochemical parameters			
	CAT (U/ml)		MDA (nmol/ml)	
	2 weeks	4 weeks	2 weeks	4 weeks
Group 1 (Normal control)	43.60±0.70^{a}	44.63±0.32^{a}	11.23±0.39^{d}	11.63±0.19^{d}
Group 2 (CCl_4)	37.60±0.60^{c}	33.60±0.31^{e}	17.50±0.29^{a}	21.67±0.88^{a}
Group 3 (CLE)	43.53±0.74^{a}	44.30±0.65^{ab}	11.33±0.33^{d}	11.53±0.29^{d}
Group 4 (DLE)	43.57±0.72^{a}	44.87±0.09^{a}	11.47±0.29^{d}	11.67±0.44^{d}
Group 5 (PLE)	43.17±0.44^{a}	44.73±0.99^{a}	11.30±0.35^{d}	11.50±0.29^{d}
Group 6 (CLE + CCl_4)	38.23±0.34^{bc}	38.17±0.27^{d}	15.63±0.32^{b}	19.33±0.34^{b}
Group 7 (DLE + CCl_4)	42.30±0.35^{a}	43.00±0.58^{bc}	11.77±0.15^{d}	12.20±0.25^{d}
Group 8 (PLE + CCl_4)	40.10±0.38^{b}	41.63±0.32^{c}	13.57±0.30^{c}	16.30±0.35^{c}
LSD P < 0.05	1.7775	1.5441	0.9280	1.2875

Values are mean ± SE of 5 rats. Within the same column, various superscript letters indicate significant differences (Duncan, P <0.05).

Hepato-renal histopathological examinations:

Liver: Histopathological alterations in liver specimens from different treatment groups are shown in **figure 1**. Liver specimens from control rats showed normal histological structure of the hepatic lobule (Fig.1 (a)). Similarly, liver specimens from rats treated with CLE, DLE and PLE only showed normal histological structure similar to normal control group (Fig.1 (a)). However, treatment of rats with CCl_4 revealed cytoplasmic vacuolization of hepatocytes and oval cells proliferation (fig.1 b). Generally, treatment of rats with CLE, DLE and PLE during CCl_4 administration ameliorated the pathological changes induced by CCl_4 administration. Liver specimens from rats treated with CLE during CCl_4 administration showed congestion of central vein and hepatic sinusoids (fig.1 c). No histopathological changes were detected in liver specimens from rats treated with DLE during CCl_4 administration (fig.1 d). Regarding liver specimens from rats treated with PLE during CCl_4 administration showed slight cytoplasmic vacuolization of hepatocytes (fig.1 e).

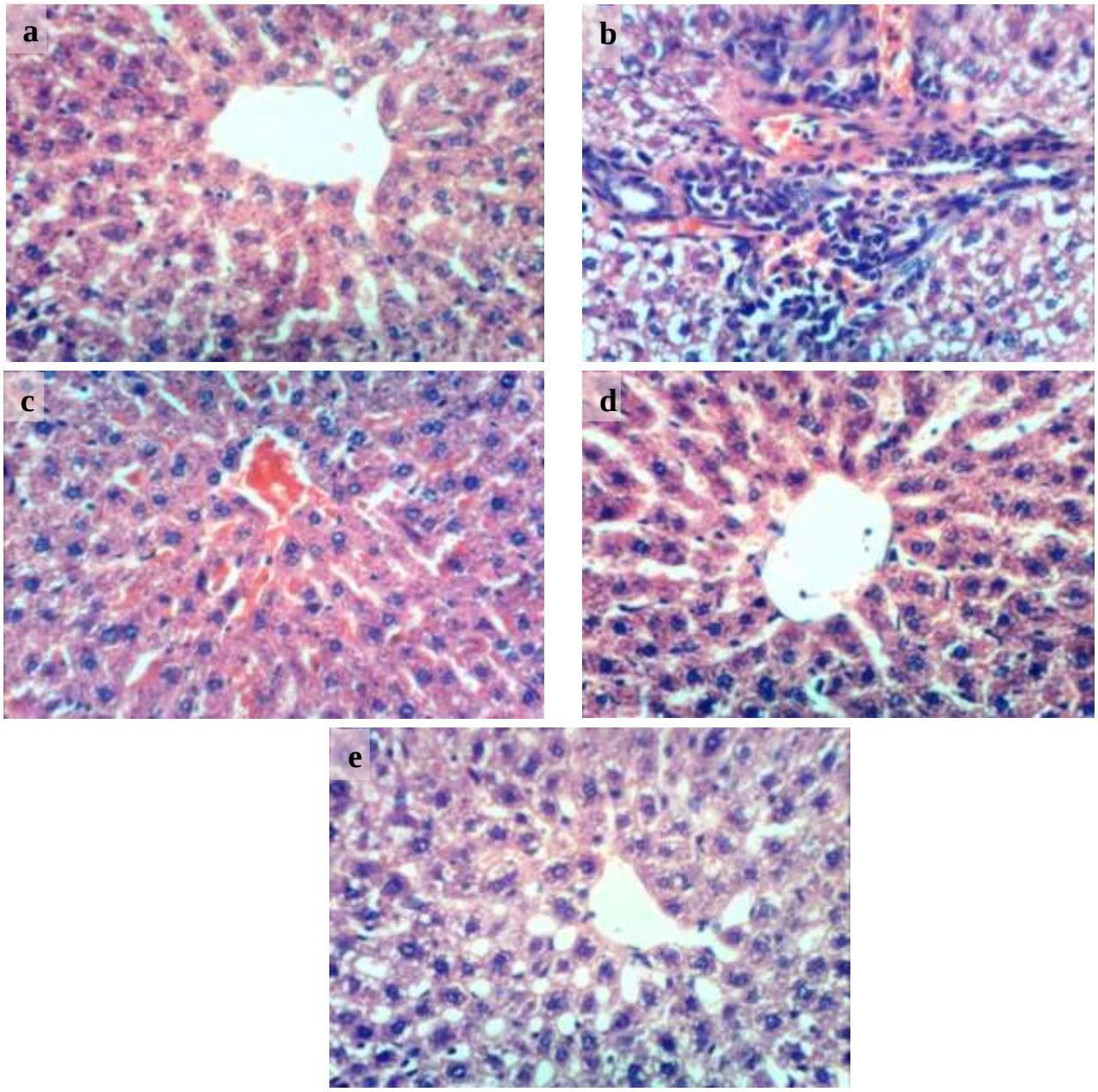

Figure1: Representative photomicro graphs of liver sections (H & E X 400). (a) Control; CLE, DLE and PLE groups; (b) and (c) CCl_4 group; (d) CLE + CCl_4 group; (e) DLE + CCl_4 group; (f) PLE + CCl_4 group.

Kidney: Histopathological alterations in kidney specimens from different treatment groups are shown in **Figure 2**. Kidney specimens from CCl_4-intoxicated rat showed vacuolization of epithelial lining renal tubules and congestion with vacuolation of glumerular tuft (fig.2 b) and focal interstitial nephritis (fig.2 c) as compared to normal control rats (Fig.2 (a)). Meanwhile, kidney specimens from rats treated with CLE, DLE and PLE only showed normal histological structure similar to normal control group (Fig.2 (a)). Kidney specimens of CCl_4 intoxicated rat treated with CLE showed vacuolization of epithelial lining renal tubules (fig.2 d). Kidney specimens of CCl_4 intoxicated rat treated with DLE showed no histopathological changes (fig.2 e). Kidney specimens of CCl_4 intoxicated rat treated with PLE showed apparent normal histological structure of renal tubules (fig.2 f).

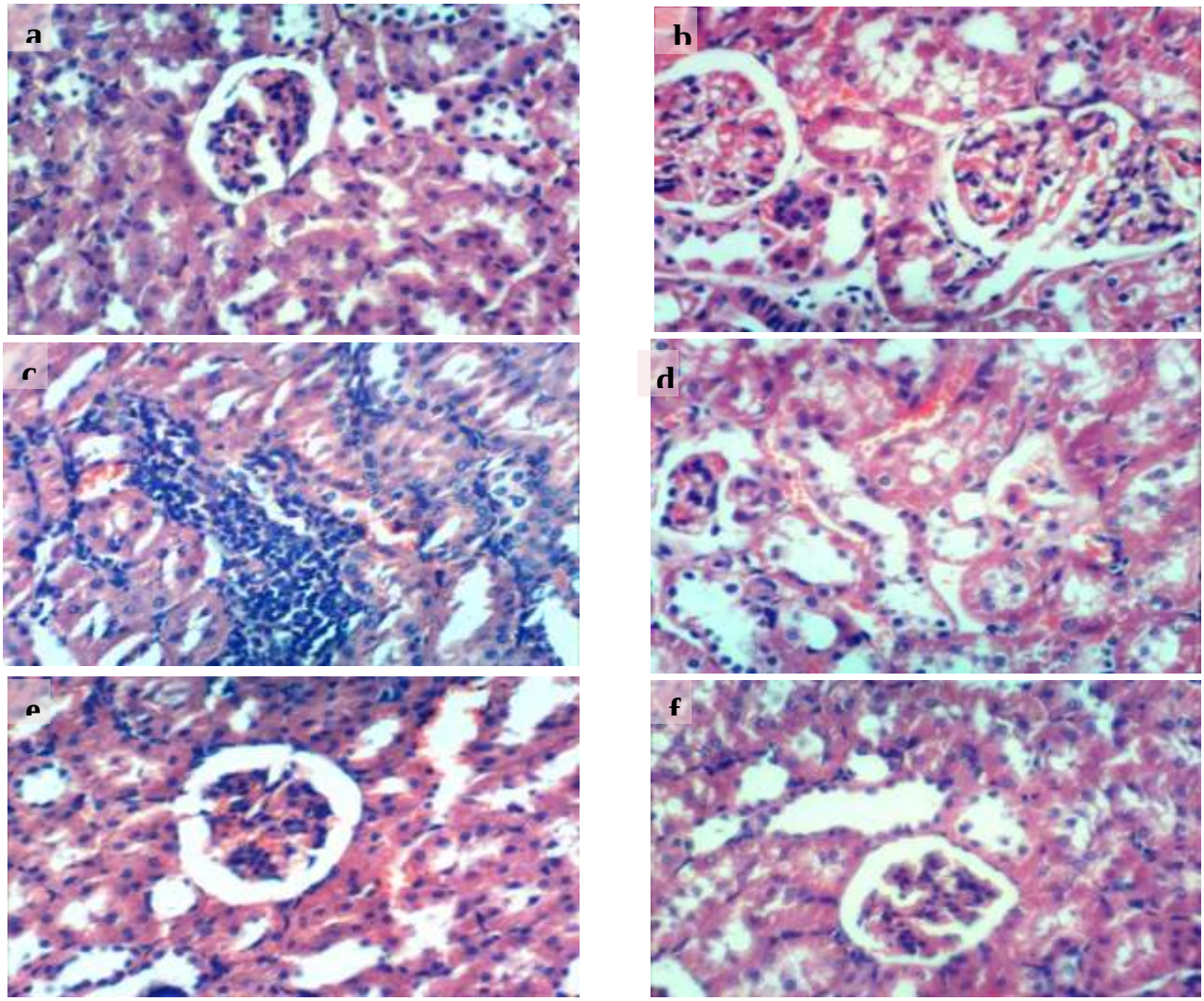

Figure 2: Representative photomicrographs of kidney sections (H & E X 400). (a) Control; CLE, DLE and PLE groups; (b) and (c) CCl_4 group; (d) CLE + CCl_4 group; (e) DLE + CCl_4 group; (f) PLE + CCl_4 group.

CONCLUSIONS

The results of the present study revealed that the changes in hepatic and renal dysfunction due to CCl_4 were rectified by three herbal leaf ethanolic extracts. The leaf ethanolic extract of dill showed better protection than coriander and parsley. The histological observations basically supported the results obtained from serum enzyme assays. Protective effects of plant extracts under study may be due to the presence of some phenolic components that have membrane stabilizing effects. These results suggests that the compound present in the plant extracts efficiently works on the liver and kidney to keep them normally functioning and minimizing cell membrane disturbances.

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