

Original Research

Effect of *Camellia sinensis*, *Hypericum perforatum* and *Urtica dioica* on kidney and liver injury induced by carbon tetrachloride in rats

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Abstract: This study is aimed to investigate the effects of *Camellia sinensis* (CS), *Hypericum perforatum* (HP) and *Urtica dioica* (UD) in kidney and liver injury induced by carbon tetrachloride (CCl₄) in rats. Highly toxic CCl₄ which is used as a solvent in industry comprises experimental toxicity in rats and is widely used in hepatotoxicity and other tissue injury models. The purpose of this investigation is to monitor blood and various tissues by biochemical and histopathological analysis for preventive effects of CS, HP and UD on oxidative stress induced by administration of CCl₄ and to enlighten the probable mechanism. Fifty eight rats were divided into five groups; sham group (Group 1, untreated animals), control CCl₄ treated group (Group 2), HP extract-treated group (Group 3), UD extract-treated group (Group 4), CS extract-treated group (Group 5). All rats were anaesthetized at the end of the experiment and the blood was collected from each rat. Afterwards, tissue specimens were obtained. The tissue specimens were immersed in 10% formaldehyde for 24 hours. After routine tissue processing, the liver, kidney and stomach were sectioned in 5µm thickness, stained in hematoxylin and eosin. The histological study was performed by using light microscope. The serum marker enzymes were found to be significantly increased in CCl₄-induced liver and kidney damage when compared with the sham group ($p < 0.05$). However, treatment with CS, HP, and UD extracts resulted in decreased activity of serum enzymes. Malondialdehyde (MDA) levels were decreased by 20.51±0.95, 27.98±1.58, and 32.39±3.1 nmol/g wet weight protein in kidney homogenates and 16.65±1.75, 17.22±0.71 and 18.92±71 nmol/g wet weight protein in liver homogenates in CS, HP and UD treated groups, respectively. Our results have shown that additive antioxidants like CS, HP and UD will aid in diminishing these deviations in cases of liver and kidney dysfunction.

Key words: *Hypericum perforatum*; *Urtica dioica*; Carbon tetrachloride; Malondialdehyde; Catalase; Glutathione; Superoxide dismutase; Glutathione peroxidase.

Introduction

Carbon tetrachloride is one of the highly toxic halogenated alkanes utilized as a solvent for many industrial purposes (1). The experimental intoxication induced by CCl₄ is widely used for modeling hepatotoxicity and other tissue injuries in rats (2, 3). Tissue injury caused by CCl₄ is related to severe impairment of cell protection mechanisms. CCl₄-induced overproduction of reactive free radicals initiate cell damage through two different mechanisms, covalent binding to the membrane enzymes and MDA generation (4). Thus, many researchers have shown CCl₄ to perform liver and kidney injury. CCl₄ acts as a virulent inhibitor of cytochrome P-450 enzyme as toxic to the enzyme of iron needed for its formation. Therefore, the protective effect of CCl₄ against a chemical-induced hepatic injury is attributed to the reduction of the hepatic dependent metabolizing capacity (5).

Xenobiotic-metabolizing enzyme activities are widely distributed throughout the body. Kidney has a relatively high level of enzyme activities, and the vital role of this organ in converting xenobiotics and endogenous substances into excretory forms is considerable (6). The

experimental intoxication, induced by oral administration of CCl₄ to rats results in liver necrosis and acute tubular or glomerular injury in kidney (7). CCl₄ gives rise to oxidative stress in many cases and it also inhibits the activity of antioxidant enzymes in renal tissue. Overproduction of free radicals causing oxidative stress can lead to peroxidation of lipid membranes resulting in tissue injury (8). For protection against oxidative injuries, tissues possess an antioxidative defense system including Glutathione (GSH), Superoxide dismutase (SOD), Catalase (CAT), Glutathione -S-transferase (GST) and Glutathione peroxidase (GSH-Px) (9). It has been reported that various antioxidant compounds such as Vitamin E and C, Melatonin and some medicinal plants act as preventive or protective agents as well (10-12).

Phytotherapeutic agent therapy has been widely recognized and practiced for ages, and in recent years, medical focus on plant research gained more attention all over the world (13-15). It is established that many people in the Middle East and Eastern countries use complementary and alternative medicines to cure various diseases (16). However, supplementary and alternative medicine is mainly based on empirical evi-

dence rather than scientifically controlled experimental studies. Herbs such as CS family *Theaceae*, HP family *Clusiaceae*, and UD family *Urticaceae* are used as alternative medicines in the treatment of many diseases as antidepressant, antimicrobial and anti-inflammatory agents, and for curing gastrointestinal disorders, hypertension (17, 18) and cancer (19, 20). In our study, we aimed to investigate the effects of herbal extracts on CCl₄ – induced experimental hepato- and nephrotoxicity in view of biochemical and histopathological observations. CCl₄ is well established to exert insidious effect via free radical overproduction thus causing oxidative burst. CCl₄ is first metabolized to CCl₃ in hepatocellular microsomes, thus triggering oxidative damage and lipid peroxidation, processes that has been shown to be alleviated by antioxidants.

The aim of the present study was to evaluate the protective effects of CS, HP and UD on oxidative stress induced by CCl₄ in rats by monitoring the status of blood and various tissues through biochemical analysis and histopathological examination and enlighten the possible mechanism engaged in this effect.

Materials and Methods

Plant extracts

The fresh aerial parts of HP and UD were collected from high latitude areas of South East Anatolia (37°14'08.3"N 38°41'49.5"E), CS was purchased from the herbal market of Sanliurfa - Turkey. The plants were identified and authenticated by a specialist in Biology Department. The voucher specimens were submitted for future reference. The herbs were cleaned, rinsed in tap water twice and dried in air circulated oven under 40°C and powdered into fine powder using electrical grinder. The powder was then extracted in 50 % ethanol for 24 hours and filtrated through a piece of clean moslini textile. The filtrate was dried with freeze dryer (Labanco Freezon 2.5 Plus Czech Republic). The extracts were weighed, pooled and stored in amber color containers and kept in a refrigerator at 4°C. The herbal extracts were prepared fresh whenever required.

Animals

Healthy Wistar albino rats each weighing 200–250 gr inbred in animal house of Biology department, Harran University were included in this study. The rats were housed in polypropylene cages with stainless steel covers, maintained under standard conditions (12/12 light/dark cycles, at 22±2°C and about 45–65% humidity). All animals were grouped randomly, 6 rats per cage, fed on rodent pellet chow and free tap water were provided *ad libitum*. The study was approved by the Institutional Animal Ethical Committee of Harran University Medical College. The investigation conformed to the principles according with international guidelines as prescribed by the Canadian Council on Animal Care (21). Experimental animals were randomly divided into five groups.

Experimental design and animal groups

Fifty eight rats were divided into five groups.
Group I: (n = 10) Sham group: Non-treated animals.
Group II: (n = 12) Control CCl₄ treated group; rats oral-

ly received 0.4 ml/kg CCl₄ diminished with pure olive oil (Sigma-Aldrich®Co. St. Louis, MO, USA).

Group III: (n = 12) *Hypericum perforatum* extract-treated group; rats orally received 0.4 ml/kg CCl₄ for ten consecutive weeks and treated with 200 mg/kg HP extract.

Group IV: (n = 12) *Urtica dioica* extract-treated group; rats orally received 0.4 ml/kg CCl₄ for ten consecutive weeks and treated with 200 mg/kg UD extract.

Group V: (n = 12) *Camellia sinensis* extract-treated group; rats orally received 0.4 ml/kg CCl₄ for ten consecutive weeks and treated with 200 mg/kg CS extract.

Histological examination

All rats were anaesthetized under ether at the end of the experiment and randomly chosen six rats to be tissue sampled were sacrificed by dislocation. Afterwards, liver and kidney specimens were obtained, the stomach was dissected along the greater curvature rinsed in ice-cooled saline, the tissue specimens were immersed in 10% formaldehyde for 24 hours. After routine tissue processing, the liver, kidney and stomach were sectioned in 5µm thickness, stained in hematoxylin and eosin (H&E). Masson Trichrome staining method was performed (22). Histological studies were carried out using a light microscope by a pathologist working with blind treatment protocol.

Biochemical assays

The blood samples of six rats chosen randomly were collected by cardiac puncturing. The rats that the blood samples were collected from were eliminated from the experiment due to stress-induced complications. The tubes with blood samples were left to clot for 30 minutes at room temperature. After this period, the serum was separated by centrifugation at 3000 rpm for 10 minutes for further analysis of various biochemical parameters namely alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), blood urea nitrogen (BUN), creatinine (Cr), and triglyceride levels using commercially available assay kits (Abbott, Chicago, IL, USA) with an Abbott Aeroset automated clinical chemistry analyzer (Chicago, IL, USA).

Remaining six rats in each group were sacrificed by dislocation; stomach, liver and kidney were removed immediately, the fundus of stomach was incised from the greater curvature side, and one portion of liver and kidney were immersed in 10% neutral formaldehyde for histopathological evaluation. 0.5 g from the other portion of the liver and kidney was weighed, rinsed in ice - cold normal saline followed by 0.15 M Tris - HCl with pH 7.4, blotted dry and weighed for biochemical evaluation. 10% of homogenate (w/v) was prepared in 0.15 M Tris-HCl buffer from each, weighed rinsed and homogenized in 5 ml phosphate buffer (pH: 7.4) and the homogenate was divided into two portions. The first portion was used to determine the activities of antioxidant enzymes (GSH, CAT, GST, SOD and GSH-Px) while the second portion was used for determination of the lipid peroxidation level (MDA content).

Lipid peroxidation levels

Lipid peroxidation levels measured as malon-

dialdehyde content was assayed by a fluorometric method as described by Hedge *et al.* (23) using 1,1,3,3-tetraethoxypropane as standard. The absorbance was followed with fluorometric reading at 520 nm of excitation and 555 nm of emission in Spectramax M5 microplate reader (Molecular Devices, USA). Results were given as nmol/mg tissue.

Glutathione levels

Glutathione level was measured using the method of Obrosova *et al.* (24), the reaction was initiated by the addition of 0.01 ml O-phthalaldehyde (OPA). The absorbance of reduced chromogen was followed by spectrofluorometric read. The differences in initial and final readings excitation 345 nm and emission 425 nm. Using Spectramax M5 microplate reader (Molecular Devices, USA). GSH level was determined from a standard curve and expressed as nmol/mg tissue.

Catalase activity

The activity of CAT was measured using its peroxidase activity according to the method of Johanson and Borg (25). All liver and kidney homogenates were diluted with buffer as described before in order to obtain an adequate dilution of the enzyme. The reaction was started by adding 1 ml of 30 mM hydrogen peroxide into the cuvette containing 2 ml of the enzyme dilution and the change in absorbance was followed at 550 nm by using a spectrophotometer (Double Beam Spectrophotometer U-2900 Tokyo, Japan).

Glutathione -S-transferase activity

GST activity was assayed spectrophotometrically at 25°C by following the conjugation reaction with glutathione in the first step of mercapturic acid synthesis. GST activity was measured at 340 nm absorbance by the method of Habig and Jacoby (26). A blank was run in absence of the enzyme. One unit of GST activity is 1 μm product formation per minute.

Superoxide dismutase activity

Tissue SOD activity was assayed by the method described by Kakkar *et al.* (27). Assay mixture contained 0.1 ml of sample, 1.2 ml of sodium pyrophosphate buffer (0.052 mM, pH 7.4), 0.1 ml phenazinemethosulphate (PMS) (186 μM), 0.3 ml of nitro blue tetrazolium (NBT) (300 μM). 0.2 ml of the supernatant following centrifugation (1500 x g, 10 min followed by 10,000 xg, 15 min) of 10% tissue homogenate was added to the reaction mixture. Enzyme reaction was initiated by adding 0.2 ml of NADH (750 μM). After incubation at 30 °C for 90 s, the reaction was stopped by the addition of 0.1 ml glacial acetic acid. Color intensity of the chromogene, the butanol layer was measured at 560 nm spectrophotometrically. Results are expressed as units/mg protein.

Glutathione peroxidase activity

Glutathione peroxidase activity was measured with NADPH oxidation, using a coupled reaction system consisting of glutathione, glutathione reductase, and cumenehydroperoxide (28). 100 μL of enzyme sample incubated for five minutes with 1.55 ml of 50 mM Tris buffer, pH 7.6 with 0.1 mM EDTA containing 0.25 mM

GSH, 0.12 mM NADPH and 1 unit glutathione reductase. The reaction was initiated by adding 50 μL of cumenehydroperoxide (1 mg/ml), and the rate disappearance of NADPH with time was determined by monitoring absorbance at 340 nm. Results are expressed as units/mg protein.

Statistical analysis

Data were analyzed using SPSS 15 for probability value of 0.05 and less was accepted as statistically significant. Values were expressed as means ± SEM and analysed statistically using Kruskal Wallis and Mann-Whitney U test (29).

Results

Effect of CS, HP, and UD on serum enzymes change in the activities of serum enzymes ALT, AST, LDH, BUN, creatinine and triglyceride in the serum of CCl₄-induced rats, as evidence from Figure 1-3. The serum marker enzymes were found to be significantly increased in CCl₄-induced liver and kidney damage when compared with the normal sham group ($p < 0.05$). Whereas treatment with CS, HP, and UD extract showed decreased activity of blood serum enzymes.

Malondialdehyde levels

Free radical formation resulting in lipid peroxida-

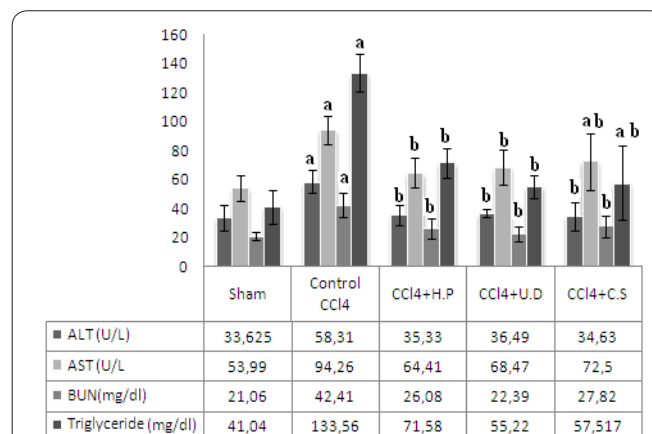


Figure 1. Changes in serum enzyme levels following ten weeks treatment in male rats; Values are expressed as means (± S.E.M) from six rats. ^a Statistically significant at $p < 0.05$ as compared to sham group. ^b Statistically significant at $p < 0.05$ as compared to CCl₄-treated group (n = 6).

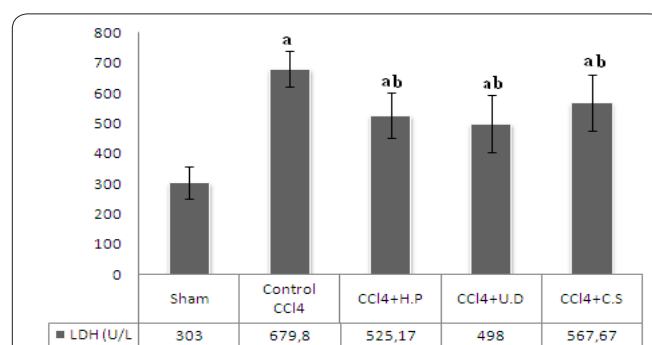


Figure 2. Changes in serum LDH levels following ten weeks treatment in male rats; Values are expressed as means (± S.E.M) of six rats. ^a Statistically significant at $p < 0.05$ as compared to sham group. ^b Statistically significant at $p < 0.05$ as compared to CCl₄-treated group (n=6).

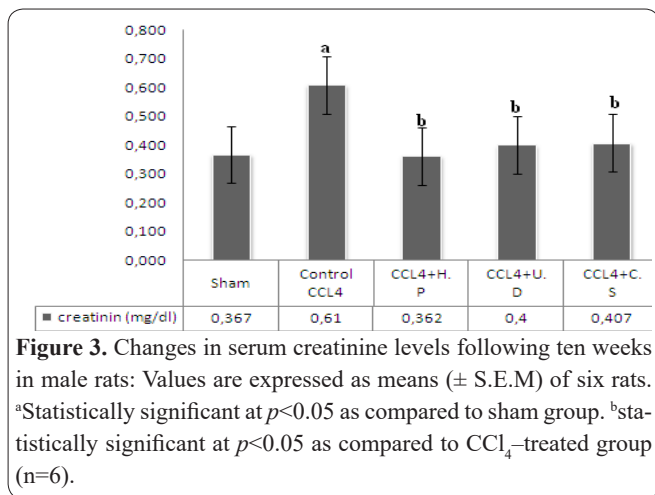


Figure 3. Changes in serum creatinine levels following ten weeks in male rats: Values are expressed as means (\pm S.E.M) of six rats. ^aStatistically significant at $p < 0.05$ as compared to sham group. ^bstatistically significant at $p < 0.05$ as compared to CCl₄-treated group (n=6).

tion, measured as MDA content in rat liver and kidney homogenates is shown in Table 1. MDA level in the liver and kidney homogenates were increased in CCl₄ – rat groups in comparison, as shown in Table 1. MDA level 26.31 \pm 4.78 in kidney increased to 41.52 \pm 3.37 and 26.68 \pm 7.57 in liver increased to 43.17 \pm 7.87 nmol/g wet weight. Treatment with CS, HP and UD inhibited MDA levels to 20.51 \pm 0.95, 27.98 \pm 1.58, and 32.39 \pm 3.1 in kidney homogenates; and 16.65 \pm 1.75, 17.22 \pm 0.71 and 18.92 \pm 71 nmol/g wet weight protein in liver homogenates, respectively.

Effect of CS, HP, and UD on CCl₄-induced changes in the activities of antioxidant enzymes of liver and kidney

While the activities of antioxidants, (GSH, CAT, GST, SOD and GSH-Px) significantly ($p < 0.05$) decreased in CCl₄ control group compared to control sham group. Treatment with CS, HP, and UD extract show statistically significant, increases in liver and kidney tissue, data are presented in (Table 1). The liver and kidney antioxidant enzyme activities were significantly decreased ($p < 0.05$), meanwhile the sham control group of rats maintained optimal value activity of the anti oxidants studied.

Table 1. Changes in hepato-nephrotoxicity of CCl₄ treated rats, and treatment with herbal extract of CS, HP and UD.

Groups	Organ	MDA (nmol/g wet weight)	GSH (nmol/ml)	CAT (IU/ml)	GST (EU/ml)	SOD (EU/ml)	GSH-Px (nmol/min/ml)
Sham control	Kidney	26.31 \pm 4.78	550.51 \pm 22.67	45.69 \pm 4.97	1.75 \pm 0.38	0.19 \pm 0.01	217.71 \pm 12.99
	Liver	26.68 \pm 7.57	1070.18 \pm 58.01	40.27 \pm 4.49	4.65 \pm 0.38	0.16 \pm 0.01	322.49 \pm 16.11
	Kidney	41.52 \pm 3.37 ^a	371.93 \pm 29.03 ^a	30.12 \pm 5.78 ^a	1.12 \pm 0.10 ^a	0.14 \pm 0.02 ^a	183.60 \pm 16.76 ^b
CCl ₄ control	Liver	43.17 \pm 7.87 ^a	710.60 \pm 79.59 ^a	24.80 \pm 4.62 ^a	2.58 \pm 0.34 ^a	2.58 \pm 0.34 ^a	275.0 \pm 25.0 ^b
	Kidney	20.51 \pm 0.45 ^{ab}	538.54 \pm 71.48 ^b	48.74 \pm 3.29 ^b	1.36 \pm 0.10 ^b	0.16 \pm 0.01 ^{ab}	211.57 \pm 9.05 ^b
CCl ₄ + <i>C. sinensis</i>	Liver	16.65 \pm 1.75 ^{ab}	747.20 \pm 87.51 ^a	31.91 \pm 2.87 ^{ab}	3.04 \pm 0.72 ^{ab}	0.15 \pm 0.01	292.25 \pm 39.43
	Kidney	27.98 \pm 1.58 ^b	522.50 \pm 67.97 ^b	50.78 \pm 3.83 ^b	1.46 \pm 0.09 ^b	0.15 \pm 0.01 ^a	223.94 \pm 18.01 ^b
CCl ₄ + <i>H. perforatum</i>	Liver	17.22 \pm 0.71 ^b	766.85 \pm 102.97 ^a	27.66 \pm 6.04 ^a	3.72 \pm 0.91 ^{ab}	0.17 \pm 0.01 ^b	302.08 \pm 29.18
	Kidney	32.39 \pm 3.19 ^b	536.14 \pm 64.50 ^b	41.60 \pm 5.94 ^b	1.67 \pm 0.11 ^b	0.16 \pm 0.02 ^a	227.71 \pm 20.66 ^b
CCl ₄ + <i>U. dioica</i>	Liver	18.92 \pm 0.71 ^b	883.45 \pm 117.20 ^a	30.98 \pm 7.16 ^a	3.81 \pm 0.49 ^{ab}	0.14 \pm 0.01 ^a	308.80 \pm 19.01 ^b

a: Compared with control sham group $p < 0.05$, b: Compared with CCl₄- induced control group $p < 0.05$.

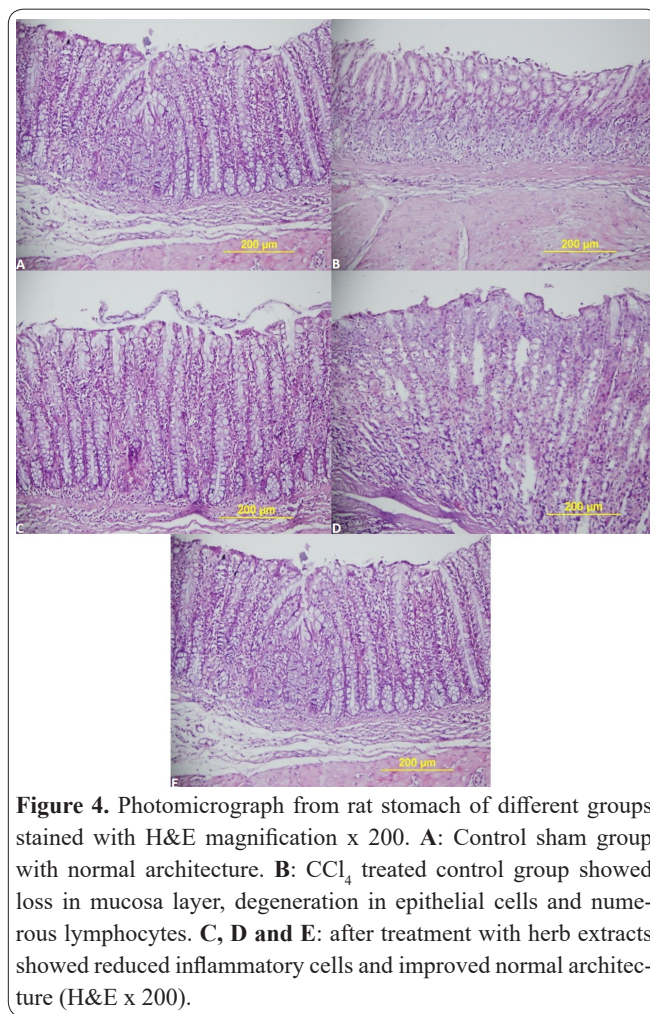


Figure 4. Photomicrograph from rat stomach of different groups stained with H&E magnification x 200. **A:** Control sham group with normal architecture. **B:** CCl₄ treated control group showed loss in mucosa layer, degeneration in epithelial cells and numerous lymphocytes. **C, D and E:** after treatment with herb extracts showed reduced inflammatory cells and improved normal architecture (H&E x 200).

Histopathological findings

Oral administration of 0.4ml/kg CCl₄ attenuated with pure olive oil to rats caused prominent histopathological damage in the stomach, liver and kidney compared with sham control group rats. Histological sections obtained from target organs were shown in (Fig 4-6). Section from control sham group showed normal architecture, while CCl₄ treated group showed marked deleterious histological changes.

The stomach histopathology section obtained from

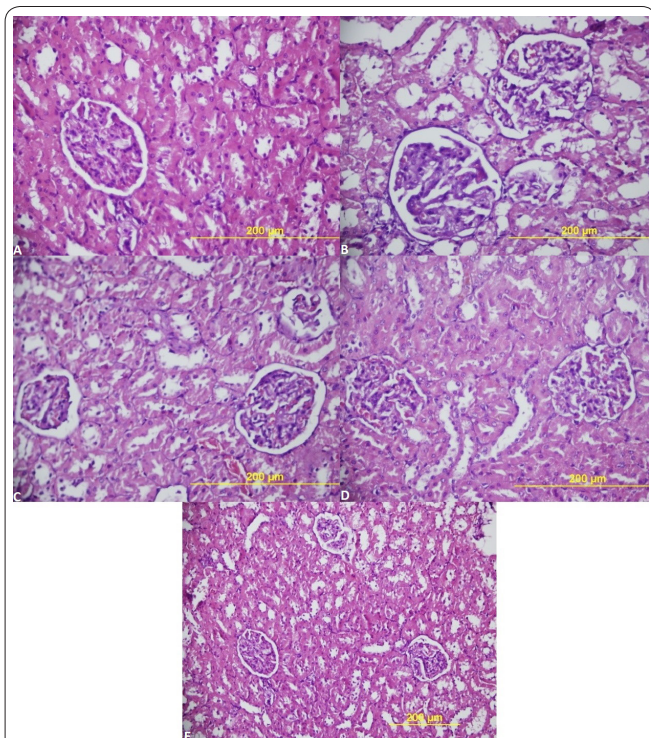


Figure 5. Photomicrographs of rat kidneys from different groups stained with H&E. **A:** Kidney tissue of sham control with normal glomeruli with an intact Bowman's capsule and proximal convoluted capsule. **B:** Kidney tissue of animal orally treated with 0.4 ml/kg CCl_4 showed tubular-brush border loss, interstitial edema, glomerular hyper cellularity and inflammatory cell infiltration. **C, D and E:** Kidney tissue from groups treated with herb extracts with attenuated necrosis reduced inflammatory cells and improved tubular and glomerular architecture (H & E x 200).

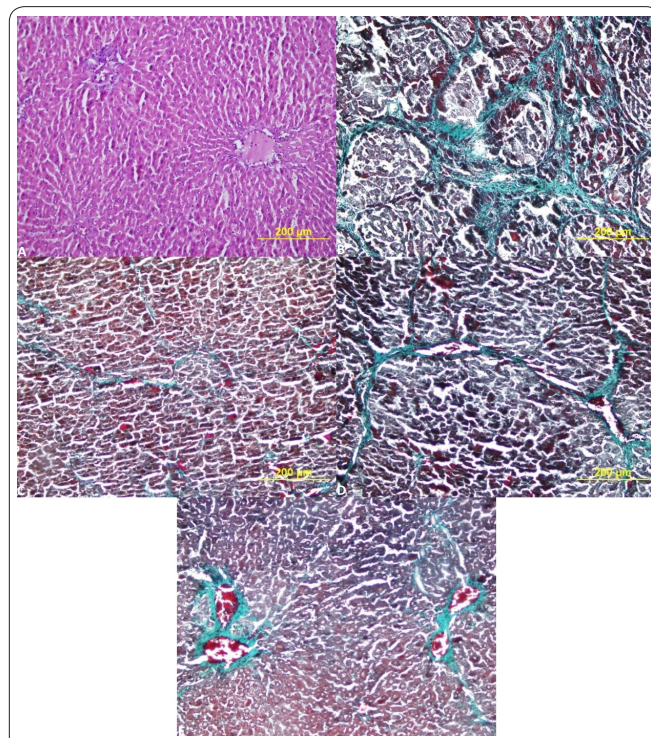


Figure 6. Effect of CS, HP and UD extract on CCl_4 induced liver damage in rats. **A:** showing normal architecture of hepatic cells; **B:** CCl_4 treated showing inflammatory infiltration, fatty changes and necrosis; **C:** Liver of CCl_4 - induced and treated with CS showing normal architecture with less fatty changes; **D:** Liver of CCl_4 - induced and treated with HP showing a sample of reduced inflammatory infiltration and little necrosis; **E:** Liver of CCl_4 - induced treated with UD showing a sample of reduced inflammatory and moderate necrosis (A, H & E x 200) (B, C, D, E, Masson trichrome x 200).

rats stained by H&E method, show damage to the mucosa, degeneration in epithelial cells and numerous lymphocytes (Fig 4 B). The histopathological sections of rat kidney stained by H&E method shows tubular-brush border loss, interstitial edema, glomerular hyper cellularity and inflammatory cell infiltration (Fig 5 B). Liver histopathology sections of the rats stained by Masson method presents the lobules of liver were separated and surrounded by fibers (pseudolobules) and there were severe necrosis and fatty degeneration of hepatocytes (Fig 6 B). No damage was distinguished in sections obtained from groups treated with CS, HP and UD extracts.

Discussion

Although the relationship between liver disease and kidney function is known, it has not been clearly explained (30, 31). The probabilities are that the mediators accumulating in the liver disease may affect the kidney directly, or that the kidney lesions enlarge as a secondary condition to the altered liver function (32, 33). *In vitro* and *in vivo* studies indicate that CCl_4 increases lipid peroxidation, and reduced GSH/GSSG ratio in kidney cortex as well as renal microsomes and mitochondria. Therefore, a number of chemicals, drugs and environmental toxicants can cause severe damage in many organs of the body via highly reactive substances such as free radicals (34). CCl_4 is one of chemical toxic elements used with industrial purposes, and in induced animal models to cause severe cellular damages in va-

rious organs such as stomach, kidney and liver through metabolic activation of highly reactive substances such as different enzymes and free radicals (35). CCl_4 is metabolized by cytochrome P450 2E1 to carbon trichloride (CCl_3), which contains a highly reactive derivative (Cl_3COO), which are assumed to initiate free radical-mediated lipid peroxidation.

The accumulation of lipid peroxidation products causes hepatic and renal damage (36), these radicals resulting in severe cellular damage play a significant role in pathogenesis of diseases by initiating a chain of lipid peroxidation reactions caused by insulation of hydrogen from fatty acids. This evidence suggests that various enzymatic and non-enzymatic systems have been developed by mammalian cells to strive with reactive oxygen species (ROS) and other free radicals (37). However, when oxidative stress exceeds the defense capacities against ROS, it becomes insufficient (38).

Treatment with CCl_4 decreased the hepatic xenobiotic-metabolizing enzyme activity in rats however, as shown in Table 1 treatment with CCl_4 caused significant elevation in serum AST, ALT and LDH levels, these changes are attributed to the cytotoxic effects on both liver and kidney. It is clearly known that transaminases such as ALT and AST are intracellular enzymes found in blood only in small amounts. The liver damage by CCl_4 could be estimated by high concentrations of these enzymes as the damage may result in the exudation of these enzymes into the plasma. Eventually CCl_4 may guide to liberate these enzymes into serum, therefore

high levels of ALT and AST indicate liver damage (39). On the other hand, the injury in kidney is demonstrated by improved major renal function of the proximal tubule which is the active resorption of sodium ions from the tubular lumen (40). Changes in kidney structure and function are often encountered in severe liver disease (41). The possibility of these changes by precipitating CCl_4 in liver may affect the kidney directly (36) or that the renal lesions developed as a secondary event to the modified liver function (41). Triglyceride and LDH levels are elevated in tissue necrosis especially in liver and nephritic syndrome (42, 43). Urea, an end product of protein metabolism, is extracted by the kidney. Blood urea nitrogen is directly related to protein intake and nitrogen metabolism and inversely related to the rate of excretion of urea. Glomerular filtrate is the same as in plasma, but its tubular reabsorption is inversely related to the rate of urine formation, so nitrite production has been reported to increase in renal disease (44). Therefore, BUN and creatinine are directly related to protein intake and nitrogen metabolism and inversely related to the renal disease, the concentration of these parameters are significantly higher in CCl_4 -treated rats. In this study, the herb extracts of CS, HP and UD showed significant improvement in creatinine and BUN levels ($p < 0.05$) and decreased the elevated levels of serum creatinine and BUN and protect the liver and kidney against CCl_4 -induced toxicity in isolated rat liver and kidney tissue. It became clear that various enzymatic and non-enzymatic array have been developed by mammalian tissue cells to deal with ROS and other free radicals (37) and the defense system effected by reduction of the antioxidant levels of GSH, CAT, SOD and GSH-Px (45). On other hand it has been noticed that detoxification decreased by production of GST (46). Recent work has suggested that cytosolic protein GST may be highly specific for the cells of proximal tubules to assess renal injury (47). It is found in high concentration, released into the urine in case of tubular injury (48). CCl_4 induced-nephrotoxicity promote oxidative stress which can elevate the formation of a variety of vasoactive mediators that can affect the renal function directly by initiating renal vasoconstriction or decreasing the efficacy of glomerular capillary ultrafiltration which causes reduced glomerular filtration rate (4). The alleviation of lipid peroxidation in CCl_4 -induced rats are protected by CS, HP and UD. The results of our study show that treatment with CS, HP and UD extracts in rats reduces the subsequent CCl_4 -induced injury in liver, kidney and stomach, as demonstrated by normalizing the histopathology and improvement in antioxidant enzyme status of the liver and renal functions, by increasing levels of GSH, CAT, GST SOD and GSH-Px and reduction of MDA level.

The mechanism of pathophysiology following CCl_4 -induced liver and kidney injury are most likely multifactorial and subsidiary involving hypoxia, inflammation or free radical production (4). Recent studies indicate that the prime ingredient of CS, polyphenol, comprise antioxidant capacity by inhibiting oxidative stress in both liver and kidney (49, 50). HP extract, used as antidepressant medication, also demonstrates antioxidant activity (51). Previous work suggested that treatment with HP repair the balance between the rate of lipid peroxidation and the activation of GSH-Px as

countervailing antioxidant protective mechanism (52). In this study, it has been observed that treatment with HP perform significant decrease in serum enzymes and increase antioxidant enzymes capacity. Previous studies suggest that an extension of the biologic investigation for prostate cancer and substantiate the usefulness of correlations of binding of selected biologic probes to features derived from the assessment of integrated optical density and syntactic structure analysis (53). In our study we suggested that treatment with UD extract reduced the serum enzymes and MDA level and elevated the activity of antioxidant enzymes, due to its major content of lectin which is a mitogenic activator in acceptance of previous work Le Moal *et al* (54).

In conclusion, CS, HP and UD diminish dysfunction caused by CCl_4 administration in rat liver and kidney tissues. We assume CS, HP and UD effectuation operates by increasing antioxidant enzyme levels and antioxidant capacity. CS, HP and UD administration decreased the severity of histopathological changes. Moreover, reformation is monitored in CS, HP and UD administered groups by biochemical measurements especially in liver and kidney functions and antioxidant enzyme status. In addition, we think that further investigation should be conducted for evaluation of these substances for potential preventive treatment of liver and kidney dysfunction.

Disclosure

No conflict of interest declared by the authors.

Author's Contribution

Hypothesis is produced by Sahabettin Selek, İsmail Koyuncu, Sedat Meydan and Hifa Gulru Caglar. Experiments and data collection are performed by İsmail Koyuncu, Fatmanur Koktasoglu, Alime Sarikaya and Metin Demirel. Data analysis and manuscript preparation are conducted by Sahabettin Selek, İsmail Koyuncu, Sedat Meydan and Hifa Gulru Caglar.

References

1. Teschke R. Liver Injury by Carbon Tetrachloride Intoxication in 16 Patients Treated with Forced Ventilation to Accelerate Toxin Removal via the Lungs: A Clinical Report. *Toxics*. 2018;6(2):25.
2. Edwards M, Keller B, Kauffman F, Thurman R. The involvement of Kupffer cells in carbon tetrachloride toxicity. *Toxicology and applied pharmacology*. 1993;119(2):275-9.
3. Johnston DE, Kroening C. Mechanism of early carbon tetrachloride toxicity in cultured rat hepatocytes. *Pharmacology & toxicology*. 1998;83(6):231-9.
4. Weber LWD, Boll M, Stampfl A. Hepatotoxicity and Mechanism of Action of Haloalkanes: Carbon Tetrachloride as a Toxicological Model. *Critical Reviews in Toxicology*. 2003;33(2):105-36.
5. Persson JO, Terelius Y, Ingelman-Sundberg M. Cytochrome P-450-dependent formation of reactive oxygen radicals: isozyme-specific inhibition of P-450-mediated reduction of oxygen and carbon tetrachloride. *Xenobiotica; the fate of foreign compounds in biological systems*. 1990;20(9):887-900.
6. Adewole S, Salako A, Doherty O, Naicker T. Effect of melatonin on carbon tetrachloride-induced kidney injury in wistar rats. *African Journal of Biomedical Research*. 2010;10(2).
7. Esterbauer H, Gebicki J, Puhl H, Jurgens G. The role of lipid peroxidation and antioxidants in oxidative modification of LDL. *Free*

- Radic Biol Med. 1992;13(4):341-90.
8. Tirkey N, Pilkhwai S, Kuhad A, Chopra K. Hesperidin, a citrus bioflavonoid, decreases the oxidative stress produced by carbon tetrachloride in rat liver and kidney. *BMC Pharmacol.* 2005;5(1):2.
 9. Dahanukar S, Kulkarni R, Rege N. Pharmacology of medicinal plants and natural products. *Indian journal of pharmacology.* 2000;32(4):S81-S118.
 10. Gomes A, Vedasiromoni JR, Das M, Sharma RM, Ganguly DK. Anti-hyperglycemic effect of black tea (*Camellia sinensis*) in rat. *J Ethnopharmacol.* 1995;45(3):223-6.
 11. Packer JE, Slater TF, Willson RL. Direct observation of a free radical interaction between vitamin E and vitamin C. *Nature.* 1979;278(5706):737-8.
 12. Tanaka H, Hirose M, Kawabe M, Sano M, Takesada Y, Hagiwara A, et al. Post-initiation inhibitory effects of green tea catechins on 7,12-dimethylbenz[a]anthracene-induced mammary gland carcinogenesis in female Sprague-Dawley rats. *Cancer Lett.* 1997;116(1):47-52.
 13. Akbay P, Basaran AA, Undeger U, Basaran N. In vitro immunomodulatory activity of flavonoid glycosides from *Urtica dioica* L. *Phytother Res.* 2003;17(1):34-7.
 14. Ernst E, Rand JI, Barnes J, Stevinson C. Adverse effects profile of the herbal antidepressant St. John's wort (*Hypericum perforatum* L.). *Eur J Clin Pharmacol.* 1998;54(8):589-94.
 15. Tahri A, Yamani S, Legssyer A, Aziz M, Mekhfi H, Bnouham M, et al. Acute diuretic, natriuretic and hypotensive effects of a continuous perfusion of aqueous extract of *Urtica dioica* in the rat. *J Ethnopharmacol.* 2000;73(1-2):95-100.
 16. Khan AU, Gilani AH. Antidiarrheal, antisecretory, and bronchodilatory activities of *Hypericum perforatum*. *Pharm Biol.* 2009;47(10):962-7.
 17. Capasso R, Borrelli F, Capasso F, Mascolo N, Izzo AA. Inhibitory effect of the antidepressant St. John's Wort (*hypericum perforatum*) on rat bladder contractility in vitro. *Urology.* 2004;64(1):168-72.
 18. Gulcin I, Kufrevioglu OI, Oktay M, Buyukokuroglu ME. Antioxidant, antimicrobial, antiulcer and analgesic activities of nettle (*Urtica dioica* L.). *J Ethnopharmacol.* 2004;90(2-3):205-15.
 19. Lin JK, Liang YC, Lin-Shiau SY. Cancer chemoprevention by tea polyphenols through mitotic signal transduction blockade. *Biochem Pharmacol.* 1999;58(6):911-5.
 20. Siddiqui IA, Saleem M, Adhami VM, Asim M, Mukhtar H. Tea beverage in chemoprevention and chemotherapy of prostate cancer. *Acta Pharmacol Sin.* 2007;28(9):1392-408.
 21. Olfert ED, Cross BM, McWilliam AA. Guide to the care and use of experimental animals: Canadian Council on Animal Care Ottawa; 1993.
 22. Goldner J. A modification of the Masson trichrome technique for routine laboratory purposes. *The American journal of pathology.* 1938;14(2):237.
 23. Hegde K, Henein M, Varma S. Establishment of the mouse as a model animal for the study of diabetic cataracts. *Ophthalmic research.* 2003;35(1):12-8.
 24. Obrosova IG, Fathallah L, Greene DA. Early changes in lipid peroxidation and antioxidative defense in diabetic rat retina: effect of dl- α -lipoic acid. *European Journal of Pharmacology.* 2000;398(1):139-46.
 25. Johansson LH, Borg LA. A spectrophotometric method for determination of catalase activity in small tissue samples. *Anal Biochem.* 1988;174(1):331-6.
 26. Habig WH, Pabst MJ, Jakoby WB. Glutathione S-transferases. The first enzymatic step in mercapturic acid formation. *J Biol Chem.* 1974;249(22):7130-9.
 27. Kakkar P, Das B, Viswanathan PN. A modified spectrophotometric assay of superoxide dismutase. *Indian J Biochem Biophys.* 1984;21(2):130-2.
 28. Tappel AL. Glutathione peroxidase and hydroperoxides. *Methods in enzymology.* 1978;52:506-13.
 29. Zar JH. *Biostatistical analysis*, 663 pp. Prentice Hall, Englewood Cliffs; 1999.
 30. Lundh HA. Sequence Comparison between Kidney and Liver Lesions in the Rat following Carbon Tetrachloride Poisoning. *Journal of Occupational and Environmental Medicine.* 1964;6(3):123-8.
 31. Gentilini P, La Villa G. Liver-kidney pathophysiological interrelationships in liver diseases. *Digestive and Liver Disease.* 2008;40(12):909-19.
 32. Salomon MI, Zak FG. The kidney in hepatic disease and in gout: clinical and pathologic aspects. *J Am Geriatr Soc.* 1966;14(5):475-89.
 33. Striker GE, Smuckler EA, Kohnen PW, Nagle RB. Structural and functional changes in rat kidney during CCl₄ intoxication. *Am J Pathol.* 1968;53(5):769-89.
 34. Walker G, Kunz D, Pignat W, Wiesenberg I, Van den Bosch H, Pfeilschifter J. Tetranactin inhibits interleukin 1 beta and cAMP induction of group II phospholipase A2 in rat renal mesangial cells. *Eur J Pharmacol.* 1996;306(1-3):265-70.
 35. Zimmerman HJ, Kodera Y, West M. Rate of Increase in Plasma Levels of Cytoplasmic and Mitochondrial Enzymes in Experimental Carbon Tetrachloride Hepatotoxicity. *J Lab Clin Med.* 1965;66(2):315-23.
 36. Aleynik SI, Leo MA, Ma XL, Aleynik MK, Lieber CS. Poly-enylphosphatidylcholine prevents carbon tetrachloride-induced lipid peroxidation while it attenuates liver fibrosis. *J Hepatol.* 1997;27(3):554-61.
 37. Recknagel RO, Glende EA, Jr., Dolak JA, Waller RL. Mechanisms of carbon tetrachloride toxicity. *Pharmacol Ther.* 1989;43(1):139-54.
 38. Rincon AR, Covarrubias A, Pedraza-Chaverri J, Poo JL, Armandariz-Borunda J, Panduro A. Differential effect of CCl₄ on renal function in cirrhotic and non-cirrhotic rats. *Exp Toxicol Pathol.* 1999;51(3):199-205.
 39. Williamson EM, Okpako DT, Evans FJ. *Selection, preparation and pharmacological evaluation of plant material*: John Wiley & Sons; 1996.
 40. Imai M, Kokko JP. Sodium chloride, urea, and water transport in the thin ascending limb of Henle. Generation of osmotic gradients by passive diffusion of solutes. *J Clin Invest.* 1974;53(2):393-402.
 41. Laks MM, Pincus IJ, Goldberg D. Renal excretion of bilirubin in the common duct ligated dog. *Gastroenterology.* 1963;44(4):469-74.
 42. Balch PA. *Prescription for nutritional healing*: Penguin; 2006.
 43. Butt AA, Michaels S, Greer D, Clark R, Kissinger P, Martin DH. Serum LDH level as a clue to the diagnosis of histoplasmosis. *The AIDS reader.* 2002;12(7):317-21.
 44. Cattell V, Cook T, Moncada S. Glomeruli synthesize nitrite in experimental nephrotoxic nephritis. *Kidney Int.* 1990;38(6):1056-60.
 45. Halliwell B, Gutteridge JM. *Free radicals in biology and medicine*: Oxford University Press, USA; 2015.
 46. Yamamoto Y, Yamashita S. Plasma ubiquinone to ubiquinol ratio in patients with hepatitis, cirrhosis, and hepatoma, and in patients treated with percutaneous transluminal coronary reperfusion. *Biofactors.* 1999;9(2-4):241-6.
 47. Branten AJ, Mulder TP, Peters WH, Assmann KJ, Wetzels JF. Urinary excretion of glutathione S transferases alpha and pi in patients with proteinuria: reflection of the site of tubular injury. *Nephron.* 2000;85(2):120-6.
 48. Tsukahara H, Toyo-Oka M, Kanaya Y, Ogura K, Kawatani M, Hata A, et al. Quantitation of glutathione S transferase-pi in the urine of preterm neonates. *Pediatr Int.* 2005;47(5):528-31.

49. Mei Y, Wei D, Liu J. Reversal of multidrug resistance in KB cells with tea polyphenol antioxidant capacity. *Cancer Biol Ther.* 2005;4(4):468-73.
50. Shapiro H, Singer P, Halpern Z, Bruck R. Polyphenols in the treatment of inflammatory bowel disease and acute pancreatitis. *Gut.* 2007;56(3):426-35.
51. Mennini T, Gobbi M. The antidepressant mechanism of *Hypericum perforatum*. *Life sciences.* 2004;75(9):1021-7.
52. Barnes J, Anderson LA, Phillipson JD. St John's wort (*Hypericum perforatum* L.): a review of its chemistry, pharmacology and clinical properties. *J Pharm Pharmacol.* 2001;53(5):583-600.
53. Obertreis B, Ruttkowski T, Teucher T, Behnke B, Schmitz H. Ex-vivo in-vitro inhibition of lipopolysaccharide stimulated tumor necrosis factor-alpha and interleukin-1 beta secretion in human whole blood by extractum urticae dioicae foliorum. *Arzneimittelforschung.* 1996;46(4):389-94.
54. Riehemann K, Behnke B, Schulze-Osthoff K. Plant extracts from stinging nettle (*Urtica dioica*), an antirheumatic remedy, inhibit the proinflammatory transcription factor NF- κ B. *FEBS Letters.* 1999;442(1):89-94.