

Hepatoprotective Effect of Aqueous Extracts of Some Medicinal Plant Mixtures on CCl₄-Induced Liver Toxicity

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Abstract: The rhizomes of Ginger (*Zingiberofficinale*), Turmeric (*Curcuma longa*), Licorice (*Glycyrrhizaglabra*), the bark of Cinnamon tree, (*Cinnamomumzeylanicum*) and the calyces of red Roselle (*Hibiscus sabdariffa* L.) are herbs used in this hepatoprotective studies. This study evaluates the hepatoprotective activity of water extract mixtures using carbon tetrachloride (CCl₄)-induced liver injury in rats. In vitro antioxidant activity of plant water extracts was determined using DPPH. The water extract mixtures were administered for 10 days; on the 10th day all rats were challenged with CCl₄ except control group animals. Aspartate aminotransferase (AST), alanine aminotransferase (ALT), and albumin levels were determined to prove the hepatoprotective effect. The enzyme activities were significantly increased in CCl₄ treated rats. The four water extract mixtures exhibited significant ($P < 0.05$) protective effect against CCl₄-induced hepatotoxicity and nephrotoxicity by decreasing the levels of serum markers, specially AST and creatinine, respectively. On the other hand, the serum lipid profiles were slightly improved; HDL-cholesterol significantly ($P < 0.05$) increased in all the water extract mixtures used.

Keywords: Liver and kidney disorders, hepatoprotective activity, serum markers, CCl₄, histopathology.

I. Introduction

The liver, as a vital organ in the body, is primarily responsible for the metabolism of endogenous and exogenous agents. It plays an important role in drug elimination and detoxification. Liver damage may be caused by Xenobiotics, alcohol consumption, malnutrition, infection, anemia and medications [30]. Hepatotoxicity is defined as injury to the liver that is associated with impaired liver function caused by exposure to a drug, or another non-infectious agent [31]. Hepatotoxic agents can react with the basic cellular components and consequently induce almost all types of liver lesions. Toxins and drugs are among the basic etiopathogenetic agents of acute liver failure [19]. Nevertheless, chemical toxins (including acetaminophen, carbon tetrachloride, galactosamine and thioacetamide) are often used as the model substances causing experimental hepatocyte injury in both in vivo and in vitro conditions [14]. Carbon tetrachloride (CCl₄) is an occupational chemical reagent widely used as a solvent in insecticide industry and is correlated with high incidence of certain types of cancer [35]. The hepatotoxicity of halogenated hydrocarbons, particularly CCl₄, has been the subject of numerous investigations in experimental animals [41].

Free radicals in the form of reactive oxygen and nitrogen species are an integral part of normal physiology. Over-production of these reactive species can occur, due to oxidative stress brought about by the imbalance of the body antioxidant defense system and free radical formation. These reactive species can react with biomolecules, causing cellular injury and even death. They can lead to the development of chronic diseases such as cancers and those that involve the cardio and cerebrovascular systems [20]. Despite the fact that hepatic problems are responsible for a significant number of liver transplantations and deaths recorded worldwide, available pharmacotherapeutic options for liver diseases are very limited and there is a great demand for the development of new effective drugs. A number of studies have shown that the plant extracts having antioxidant activity protect against CCl₄ hepatotoxicity by inhibiting lipid peroxidation and enhancing antioxidant enzyme activity [45], [42], [21], [24], [46] and [38].

The goal of the present study was to examine the antioxidant and hepatoprotective activity of some medicinal plants water extract mixtures against oxidative stress induced by CCl₄ in rats.

II. Material And Methods

Materials:

a. Plant Materials

Plant materials were purchased from the herbal local market at in Taif governorate (rhizomes of Ginger (*Zingiberofficinale*), Turmeric (*Curcuma longa*), Licorice (*Glycyrrhizaglabra*), the bark of Cinnamon tree, (*Cinnamomumzeylanicum*) and the calyces of red Roselle (*Hibiscus sabdariffa* L.). All samples were

cleaned, dried, and powdered with an electrical grinder, then passed through sieve no.40 to remove the debris. The sieved powder was stored in airtight containers at room temperature.

b. Chemicals

1. All chemicals and reagents used in this study were of analytical grade.
2. DPPH (1, 1-DiPhenyl -2-Picryl Hydrazyl radical) was purchased from Aldrich, Company Germany.
3. The reagent kits (Bio-diagnostic systems GmbH, Germany) were purchased from local suppliers viz. Cholesterol, Triglycerides “TG”, High Density Lipoprotein “HDL”-cholesterol, Alanine Amino Transferase “ALT”, Aspartate Amino Transferase “AST”, Albumin, Urea, Creatinine, and Uric Acid.

Methods:

a. Extraction and preparation of some beverages:

The fine powdered samples were extracted by boiling water (1 part of powder to 100 parts of water) in a stoppered flask after shaking well. They were then allowed to stand for 10 minutes to cool, then filtered for analysis. The aqueous extract was used within 12h from filtration [4].

b. In vitro antioxidant activity using DPPH method

The hydrogen donating ability of individual and poly-herbal water extracts were examined in the presence of DPPH stable radical according to the method described by [29]. An aliquot of 1 mL 0.3 mM DPPH ethanol solution was added to 2.5 mL solution of different plant samples and allowed to react at room temperature. After 30 min, the absorbance values were measured at 517 nm. Ethanol (1 mL) plus plant extract solution (2.5 mL) was used as a blank; DPPH solution (1 mL, 0.3 mM) plus ethanol (2.5 mL) served as negative control. The concentration (mg/mL) of the extract required to scavenge the radicals was calculated by using the percentage scavenging activities. Percentage inhibition (I %)” was calculated using the formula of [8] which utilized the absorbance of samples and controls.

Animals:

A total of 36 male albinos Wister rats weighing 100-120g were used for the experiments. The rats were caged individually in wire-bottom stainless steel cages and kept under normal healthy laboratory conditions. Water was consumed ad libitum at room temperature, (22-24 °C) and fed a basal diet according to that recommended by [7], for one week, before and during the experiment. After the adaptation period, rats were randomly divided into six groups (6 rats in each) and kept in standard environmental conditions (22-24 °C and 12h light /dark cycle). Then rats were subjected to the hepatoprotective activity of water extracts of Turmeric, Ginger, Cinnamon, Roselle and Licorice mixtures against CCl₄ - induced hepatotoxicity, as described by [37] .

The rats were divided into six groups as follow:

- Group I. (g 1) served as untreated control.
- Group II. (g 2) rats were treated with hepatotoxic (CCl₄).
- Group III. (g 3) rats were treated orally with a mixture of Turmeric and Ginger water extracts (1:1)
- Group IV. (g 4) rats were treated orally with a mixture of Turmeric, Ginger and Licorice extracts (1:1:1)
- Group V. (g 5) rats were treated orally with a mixture of Cinnamon and Hibiscus water extract (1:1)
- Group VI. (g 6) rats were treated orally with a mixture of Cinnamon, Hibiscus and Licorice water extracts (1:1:1)

Body weight was recorded daily for ten days. On the 10th day, all animals received 1.5 ml/kg CCl₄ (1:1 of CCl₄ in corn oil) orally, except for rats in group 1. Biochemical parameters were estimated after 18 h of the last dose administration by fasting the experimental animals. Blood samples were withdrawn from the eye vein.

III. Biochemical Analysis

Blood samples were allowed to clot for 45 min at room temperature. Serum was separated by centrifugation at 2500rpm at 30°C for 15 min and utilized for the estimation of various biochemical parameters. Serum lipid profile i.e. Cholesterol [3], Triglycerides “TG” [16], High density lipoprotein “HDL” cholesterol [28], kidney function tests i.e. Urea [17], [49], and Uric Acid [16], liver function tests, i.e. serum Glutamic Pyruvic Transaminase (sGPT) or Alanine Amino Transferase “ALT” and Glutamic Oxaloacetic Transaminase (GOT), or Aspartate Amino Transferase “AST” [40] .

Histopathology Of The Liver:

Fresh liver samples were collected from sacrificed treated and control rats. Liver and kidney tissues were fixed in 10% neutral formalin, sectioned at 5-6 µm and stained with hematoxylin and counterstained with eosin. Stained sections were then examined under a light microscope for deformities and damage of hepatocytes due to CCl₄ induced toxification and plant extracts treatments.

IV. Results And Discussion

The radical-scavenging activity, measured by the molar ratio of antioxidant to DPPH radical required for 50% reduction in DPPH radical concentration at 30 min, is shown in the Table-1 and 2.

Table 1: In vitro antioxidant activity of individual medicinal plant water extract using DPPH

Medicinal plant water extract(1g/100ml boiled water)	DPPH % inhibition Mean±SD (n=3)
Ginger	56.47 ± 1.74
Turmeric	67.35 ±2.78
Licorice	73.65 ±1.28
Cinnamon	76.35 ±2.31
Hibiscus	91.20 ±0.33

Table 2: In vitro antioxidant activity of medicinal plant water extract mixtures using DPPH

Medicinal plant water extract mixtures (1g/100ml boiled water)	DPPH % inhibition Mean±SD (n=3)
Turmeric and Ginger extracts (1:1)	51.32 ± 1.88
Turmeric, Ginger and Licorice extracts (1:1:1)	55.12± 2.14
Cinnamon and Hibiscus (1:1)	90.33± 1.55
Cinnamon, Hibiscus and Licorice (1:1:1)	93.42± 1.93

The radical scavenging activity of the individual plant water extract was decreased in the following order: Hibiscus > Cinnamon > Liquorice > Turmeric > Ginger. While the radical-scavenging activity of plant water extract mixtures decreased in the following order: Cinnamon, Hibiscus and Liquorice (1:1:1) > Cinnamon and Hibiscus (1:1) > Turmeric, Ginger and Liquorice extracts (1:1:1) > Turmeric and Ginger extracts (1:1). In this respect, [48] and [51] indicated that many herbs and spices are an excellent source of phenolic compounds which have been reported to show good antioxidant activity. Therefore, they may serve as natural food preservatives. However, herbs and spices usually contain essential oils which show antioxidant activity but also carry flavor. Also [15] showed that the water extracts of selected parts of the Roselle plant and BHT as standards decreased in the following order: seeds > leaves > stems > calyces > BHT

Table 3: Effect of various medicinal plant water extract mixtures on serum lipid profile in CCl4 treated rats

Rat groups	Cholesterol mg/dl	Triglycerides TG mg/dl	HDL-cholesterol mg/dl	LDL-cholesterol mg/dl
g 1	81.60±18.3	92.14±9.51	37.90±18.8	80.50±5.85
g 2	*146.73±13.1	107.1±8.24	*28.98±3.74	98.06±33.9
g 3	*61.60±3.21	82.14±17.0	54.95±19.1	75.83±8.13
g 4	112.7±31.7	99.99±26.7	*61.69±28.0	81.24±10.8
g 5	107.1±27.8	92.85±31.4	*68.37±27.3	74.94±8.82
g 6	131.2±15.2	105±59.7	*67.63±25.7	82.48±14.5

Values are expressed as mean ± SD, (*) Significant at P<0.05 where g1 untreated rats (Control), g2 treated with CCl4, g3 treated with mix (Turmeric + Ginger water extract 1:1), g4 treated with mix (Turmeric +Ginger +Licorice 1:1:1), g5 treated with mix (Cinnamon+ Roselle 1:1) g6 treated with mix (Cinnamon+ Roselle+Licorice 1:1:1)

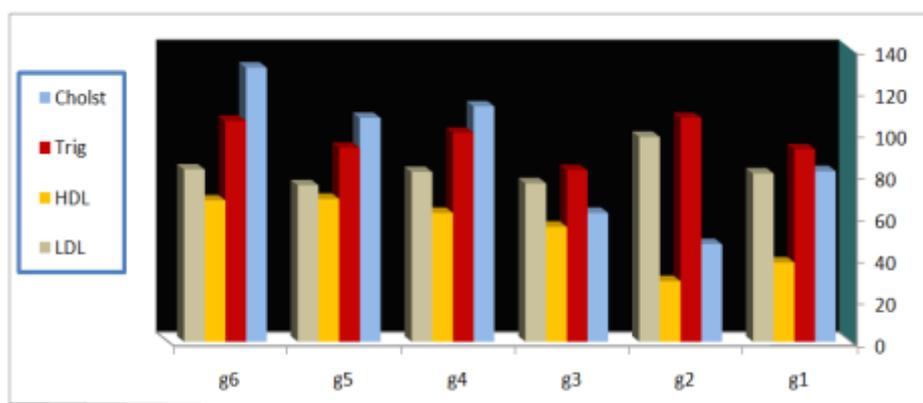


Fig 1: Effect of various medicinal plant water extract mixtures on serum lipid (mg/dl) profile in CCl4 treated rats.

Results in table (3) and Fig.(1) revealed that serum cholesterol, triglycerides and LDL cholesterol increased while HDL cholesterol decreased in CCl4 intoxicated rats group compared with control group (P<0.05). Combination of ginger with turmeric in CCl4 treated rats (g2) showed significant decrease in serum cholesterol level (P<0.05) comparing with CCl4 intoxicated group (g2). It is clear that the plant water extract combinations of all treated rat groups used in the study increased the HDL-cholesterol level. Water extract combinations of groups 4, 5 and 6 recorded significant increase HDL-cholesterol level comparing with CCl4 intoxicated group (g2).

The hyperlipidemic effect of CCl4 noticed in the serum of CCl4 intoxicated group, might suggest enhanced lipogenesis. Since cholesterol and triglycerides were increased significantly. The observed hyperlipidemia might reflect the impairment of liver cells to metabolize lipids and reduced transformation of cholesterol to bile acid and excretion. In the present study, ginger and turmeric water extract combination reduced serum cholesterol level. These results are in agreement with [43] and [9] who reported that ginger acts as a hypolipidemic agent in cholesterol fed rabbits. Also, [18] observed that cholesterol synthesis was inhibited by some ginger extract derived metabolite, or by secondary mediator.

[39] stated that oral administration of a turmeric extract inhibited LDL oxidation and had hypocholesterolemic effects in rabbits with experimental atherosclerosis.

In this respect,[5] demonstrated that pretreatment with different herbal extracts provided various levels of protection against the hepatocellular damage resulting from administration of CCl4

Table 4: Effect of various medicinal plant water extract mixtures on serum urea, creatinine and uric acid in CCl4 treated rats.

Rat groups	Urea mg/dl	Creatinine mg/dl	Uric acid mg/dl
g1	33.63±13.69	0.95*±0.33	1.20±0.37
g2	52.49±26.94	*1.92±0.16	1.70±0.45
g3	42.27±28.01	*0.95±0.31	1.78±0.25
g4	29.60±11.60	*1.19±0.44	2.07±0.51
g5	45.70±6.29	*0.90±0.48	1.97±0.34
g6	45.26±24.36	*0.80±0.05	2.05±0.38

Values are expressed as mean ± SD; () Significant at P<0.05. Where g1 untreated rats (Control), g 2 treated with CCl4, g3 treated with a mixture (Turmeric + Ginger 1:1), g4 treated with a mixture (Turmeric + Ginger + Licorice 1:1:1), g5 treated with a mixture (Cinnamon+ Roselle 1:1) g 6 treated with a mixture (Cinnamon+ Roselle+Licorice 1:1:1).

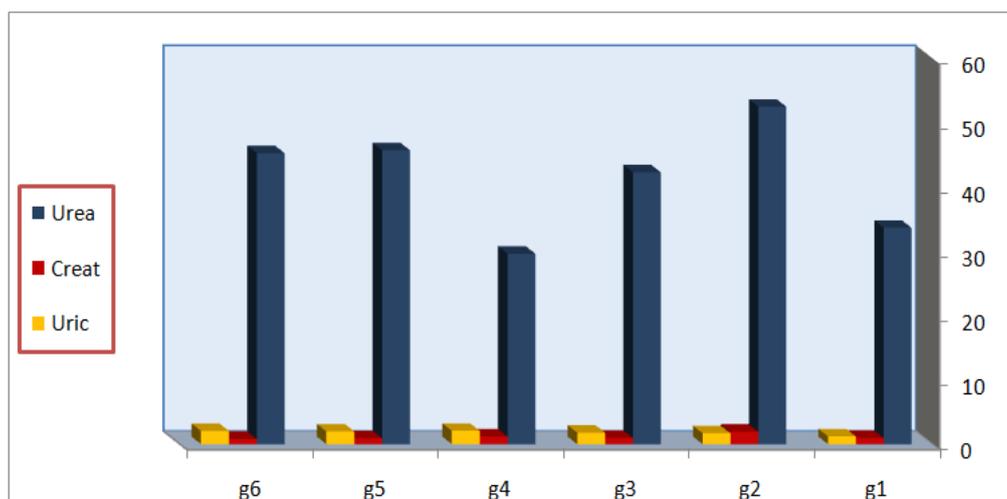


Fig (2):Effect of various medicinal plant water extract mixtures on serum urea (mg/dl),Creatinine(mg/dl) and Uric Acid(mg/dl) in CCl4- treated rats

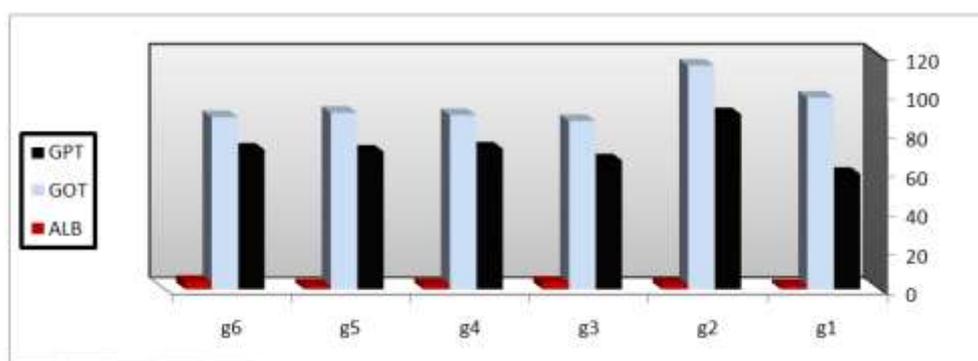
The presented data (Table 4 and Fig 2) showed that administration of CCl4 to rats caused a significant increase in urea ,creatinine, and uric acid compared with control group .Moreover, concomitant administration of plantwater extract and CCl4 to rats caused a significant decrease in uric acid, urea and creatinine level compared to CCl4 group except in case of uric acid which showed an increase in all treated rat groups. Administration of herbal water extracts caused significant decrease in serum creatinine level versus the control group.

In this respect, the presence of abnormally high levels of urea, uric acid and creatinine in serum are possible indicators of hepatic and/or kidney injuries induced through CCl4 treatment[32]. The serum creatinine level does not rise until at least half of the kidney nephrons are damaged or destroyed [10]. [25] reported that chronic renal injuries and urea elevations developed in rats after CCl4 intoxication. The present study revealed that administration of water extracts significantly restored the levels of creatinine in serum. Similar investigations were also documented that different plant extracts significantly recovered the renal injuries induced through CCl4 intoxication [32]. The protective effects plant water extract mixtures against the CCl4-induced renal injury could be attributed to its high levels of polyphenols and other antioxidants like flavonoids.

Table 5: Effect of various plant water extract mixtures on serum enzymes GPT, GOT, and albumin in CCl4 treated rats.

Rat groups	GPT(ALT) (IU/L)	GOT(AST) (IU/L)	Albumin(ALB) (g/dL)
g1	59.25±25.30	98.50±33.55	1.76±1.01
g2	90.00±17.50	*115.2±3.774	*2.27±1.35
g3	66.25±20.80	*86.50±10.66	3.34±0.81
g4	72.25 ±33.00	*89.50±6.80	2.21±0.29
g5	70.75±9.70	*90.75±17.34	1.82±0.65
g6	71.75±13.70	*88.50±10.37	*3.72±0.15

Values are expressed as mean ± SD, () Significant at P<0.05. Where g1 untreated rats (Control), g2 treated with CCl4, g3 treated with mix (Turmeric + Ginger water extract 1:1), g4 treated with mix (Turmeric + Ginger + Liquorice 1:1:1), g5 treated with mix (Cinnamon+ Roselle 1:1), g6 treated with mix (Cinnamon+ Roselle+ Liquorice 1:1:1)



Fig(3):Effect of various plant water extract mixtures on serum enzymes GPT, GOT, and albumin in CCl4 treated rats.

Results in Table 5 and Fig 3 showed the effect of different treatments of polyherbal water extracts on serum liver enzymes activity. Values were expressed as mean ± SD, and (*) significant at P<0.05. Change in GOT was significant at P<0.05 in g2 vs. g3, g4, g5, g6. Albumin was significant at P<0.05 for g2 vs. g6. No significant difference in serum GPT activity was noticed in animals treated with polyherbal extracts in comparison with CCl4 control group. On the other hand, animals treated with the four polyherbal preparations revealed a significant decrease (P<0.05) in GOT activity compared with CCl4 control group. Animals treated with CCl4 showed changes in serum albumin concentration compared with the control, while animal's intake plant water extract mixtures containing Roselle+Cinnamon +Liquorice (Group 6) showed a significant increase (P<0.05) in albumin compared with CCl4 treated group.

As shown in Table (5) and Fig.3, the activities of GOT and GPT in the serum increased significantly in CCl4 intoxicated group. The study revealed that acute CCl4 intoxication resulted in a decrease in serum albumin contents. Similar findings were recorded in liver diseases [47]. [44] reported that albumin was the most abundant plasma protein produced by hepatocytes; its depreciation usually reflects decreased hepatic synthesis. This fall is often attributed to hepatic impairment of albumin synthesis. The decrease may also be due to leakage in kidney function leading to the release of albumin with the urine, as well as significant increase in the activities of serum liver transaminase enzymes (GOT and GPT) of CCl4 treated group were noticed. These effects may reflect hepatocellular damage. [6] used polyherbal formulation YAK samples on paracetamol induced liver toxicity, and reported significant increase in serum levels of sGOT, sGPT, GGT and ACP. A similar effect, using *Carissa carandas* L. root extract against CCl4 and paracetamol induced toxicity, was reported by [24], where serum marker enzymes were decreased as well as bilirubin.

[36] studied the induced hepatotoxicity of *Amorphophalluspaeoniifolius* methanol and aqueous extracts on rats and reported significant reduction in sGPT, sGOT, sALP and bilirubin. Similar findings were reported by [34] using some extracts on paracetamol induced toxicity. Animal studies have demonstrated Turmeric's hepatoprotective effects from a variety of hepatotoxic insults, including carbon tetrachloride [13] and [33] in rats with CCl₄-induced acute and sub-acute liver injury, Curcumin administration significantly decreased liver injury in test animals compared to control. The reduction of GOT and GPT activities by the plant water extracts is an indication of repair of hepatic tissue damage induced by CCl₄. In accordance with those obtained by [22] who found that serum transaminases returned to normal with the healing of hepatic parenchyma and regeneration of hepatocytes. The ethanol extract induced suppression of the increased ALT and AST activities. Thus, administration of ethanol, or aqueous extracts of cinnamon revealed hepatoprotective activity against the toxic effect of CCl₄, which was also supported by histological studies. [27] showed that *Hibiscus sabdariffa* dried flowers extract had a protective effect against liver damage (fibrosis) induced by CCl₄. Concentrations of dried flower extracts of *Hibiscus sabdariffa* 1-5% was given for 9 weeks, whereas induction of CCl₄ was carried out for 7 weeks. They added that the Roselle extract could reduce liver damage including steatosis and fibrosis, while also lowering plasma level of aspartate aminotransferase (AST or GOT) and Alanine aminotransferase (ALT or GPT), and restore glutathione content decreased and inhibit lipid peroxidation product during CCl₄ administration. *Hibiscus sabdariffa* was also relatively non-toxic with LD₅₀ > 5000 mg/kg BW [2] .

V. Histopathological Changes For Liver And Kidney

a. Histopathological changes of liver

A photomicrograph of control liver g1 tissue (Plate L1) showed normal arrangement of the hepatic cords around the central vein with red blood cells within the hepatic sinusoids and normal Kupffer cell number and location. Liver tissue of group 2 (Plate L2): animal (+ Control) showed somewhat symmetrically dilated and congested central vein with ballooning degenerated pericentral hepatocytes containing intracytoplasmic vacuoles and a perivenular mononuclear inflammatory cell infiltration. The peripheral hepatocytes showed a pinkish granular cytoplasm and vesicular nuclei.

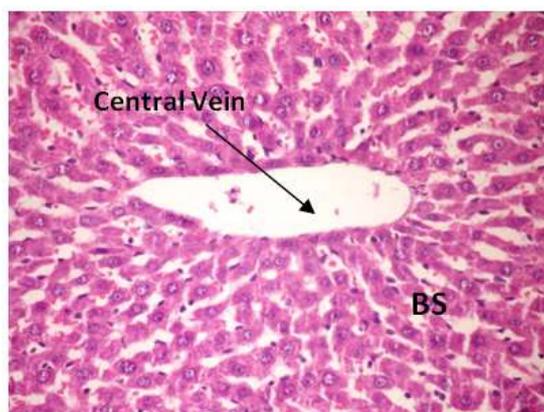


Plate L1. Liver of control group g1, untreated rat showing the normal histological structure of hepatic lobule (H and E X400).

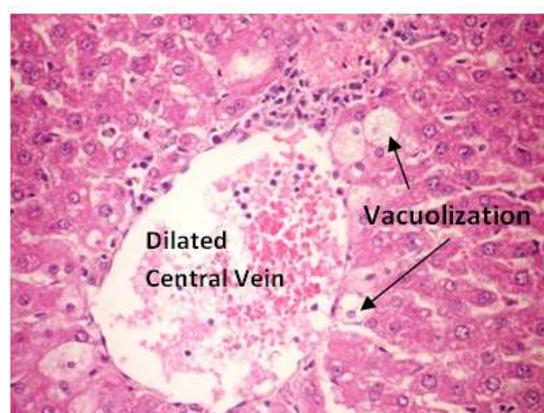


Plate L2. Liver of rat from CCl₄ group g2(+control) showing cytoplasmic vacuolization of hepatocytes and oval cells hyperplasia (H and E X400)

Liver tissue of rats given a water extract of Turmeric: Ginger (1:1) mixture in group 3 (Plate L3) and Turmeric: Ginger: Licorice (1:1:1) mixture of group 4 (Plate L4) animal showing dilated congested central vein with Kupffer phagocytic cell hyperplasia and disruption of the hepatocytic cords. On the other hand the animal hepatic tissues of groups 5 and 6 (Plates L5 and L6) showing a normal callipered central vein. The central and peripheral hepatocytes showed pinkish cytoplasmic granules and vesicular nuclei. The Kupffer phagocytic cells were proliferated.



Plate L3. Liver of rat from group g3 showing Kupffer cells activation (H and E X400).

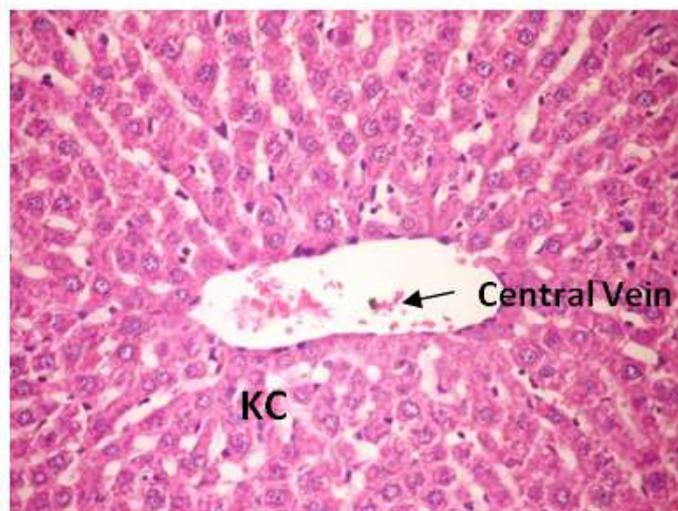


Plate L4. Liver of rat from group g4 showing Kupffer cells activation and dilatation of hepatic Sinusoids (H and E X400).

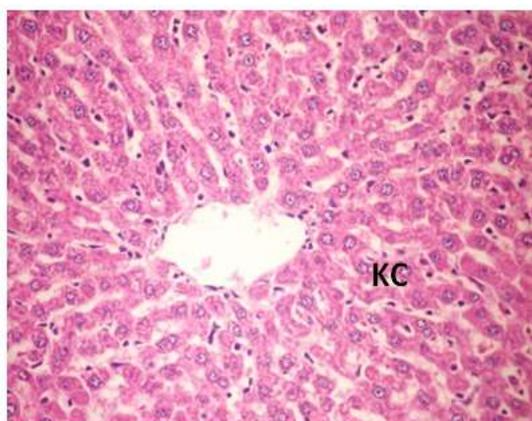


Plate L5. Liver of rat from group g5 showing Kupffer cells activation (H and EX400).

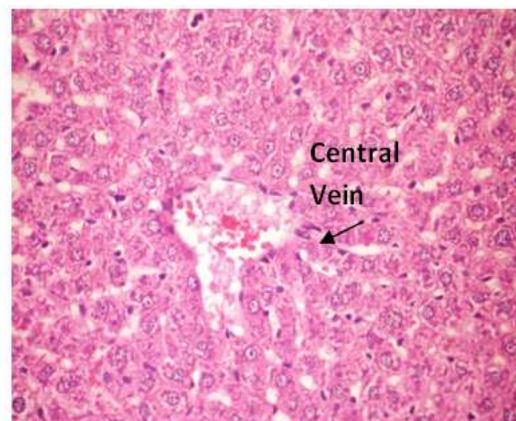


Plate L6. Liver of rat from group g6 showing Kupffer cells activation (H and E - X400).

It could be concluded that hepatoprotected groups g3,g4,g5and g6 had less pathological changes than the intoxicated group 2 and also, the activity of the reticulo-endothelial system inside the liver (Kupffer cells) was much more prominent in the hepatoprotected groups than in the unprotected group g2.

Histological results reported in the current study confirmed the biochemical results and indicated that CCl₄ induced severe histological changes in the hepatic tissues. Similar histological changes in the liver have been documented previously [11,12]. The acute hepatotoxic effects induced by CCl₄ administration were confirmed histopathologically, revealing extensive hepatocellular degeneration and necrosis, fatty changes, inflammatory cell infiltration, congestion, and sinusoidal dilatation. It could be observed that treatment with medicinal plant water extract mixtures (MPWEM) used under this study effectively protected rats against CCl₄-induced hepatic toxicity. Treatment with (MPWEM) prevented the necrosis and the other histopathological changes induced by CCl₄ toxicity.

a. Histopathological changes of kidney

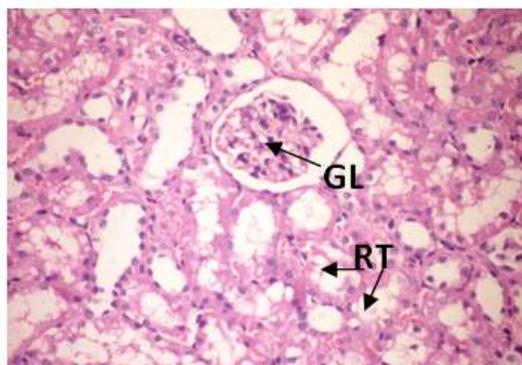


Plate K1. Kidney of -control g1, untreated rat (control+) showing the normal histological structure parenchyma (H and E X400).

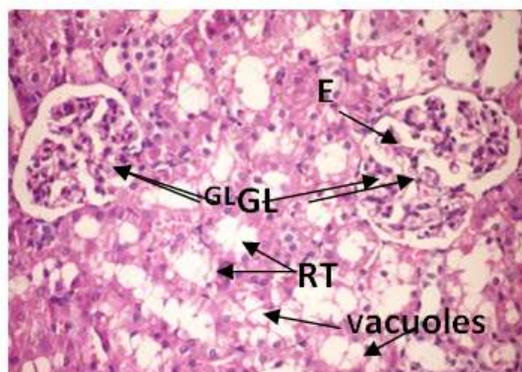


Plate K 2. Kidney of rat from group g2 showing vacuolization of epithelial lining of renal renal tubules RT and endothelial lining glomerular tufts(GL)(H and E X400).



Plate K 3. Kidney of rat from group g3 showing congestion of glomerular tuft and intertubular blood vessels (H and E X400)

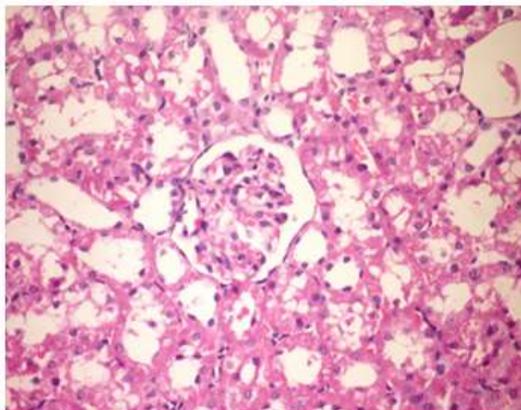


Plate K 4. Kidney of rat from group g4 showing minute vacuolization of epithelial lining tubules and endothelial lining renal glomerular tuft (H and E X400).

As shown in the above photomicrographs, CCl₄ renal intoxication was associated with severe glomerular and tubulo-interstitial necrosis which was characterized by hydropic degeneration of the glomerular and tubular cells with complete obliteration of the tubular lumen (from hydropic degeneration and tubular casts) (Plate K1) when compared to normal rat kidney (Plate K6). However, oral pretreatments with medicinal plant water extract mixtures (MPWEM) ameliorated renal histological lesions (Plates K2 to K6) with the least change in renal architecture. Literature has shown medicinal plants with nephron-protective properties to mediate their protection via antioxidant and/or free radical scavenging activities due to the high concentration of alkaloids, flavonoids and phenolic compounds they contain [1]. Equally, saponins have been reported to protect liver and kidneys against carbon tetrachloride intoxication [23]. In conclusion, the present study demonstrated that aqueous extract of some medicinal plant water extract had a preventive effect in CCl₄-induced liver damage. However, it is necessary to determine other parameters such as oxidative stress markers and molecular biology assays to confirm our findings.

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