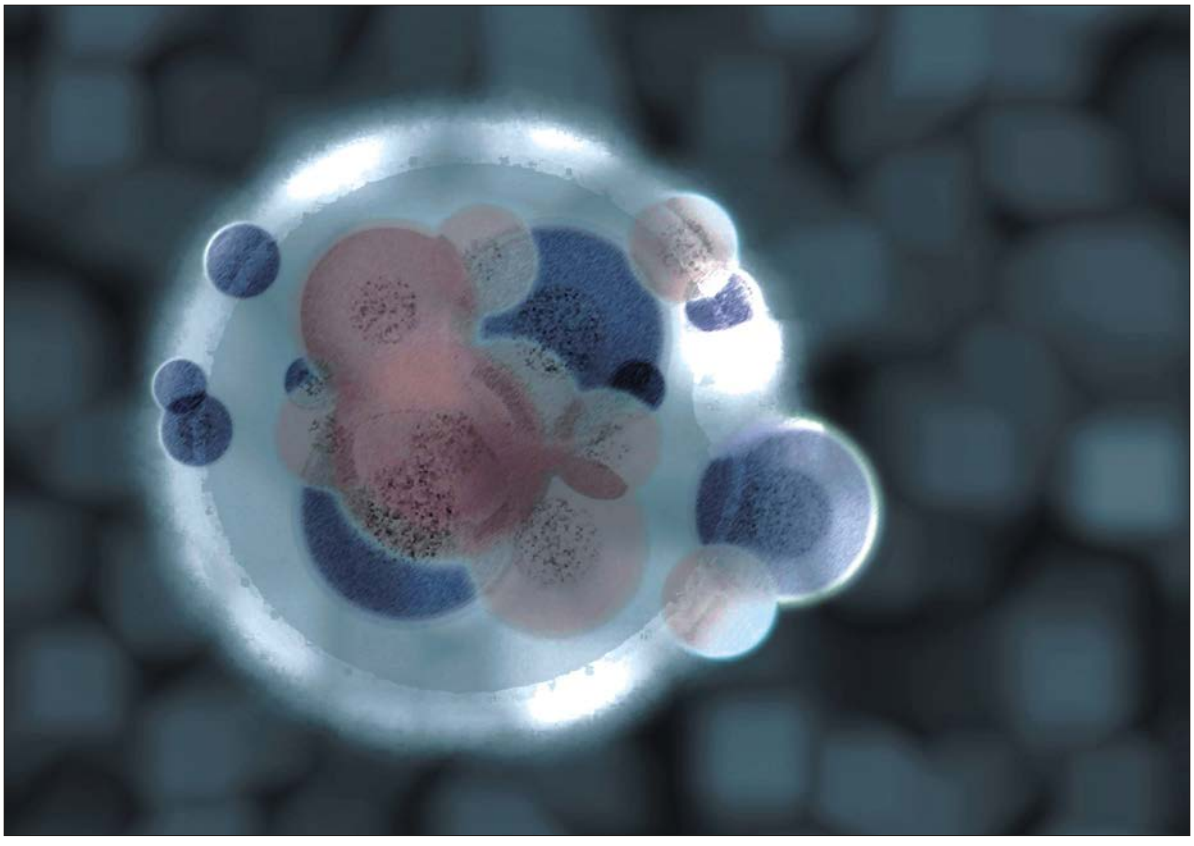

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Research Article
Prevention from Complications Associating Liver Toxicity by Carbon Tetrachloride Using a Blend of Plants in the Form of Dietary Supplement

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Abstract

Background and Objective: Carbon tetrachloride toxicity has been reported to cause several hepatocellular pathological changes. The present study was designed to investigate the efficiency of 2 formulas formed from several plant sources to protect against the health complications associating liver toxicity due to carbon tetrachloride injection. **Materials and Methods:** In this study 2 dietary supplements, the first constituted of green apple (25%), pomegranate (25%), red grape (25%), green tea (10%), tomato (10%) and artichoke (5%), the second of green tea (15%), licorice (25%), mint (25%), coffee (5%), lemon (15%) and grape fruit (15%) were used. The results were statiscally analyzed using SPSS-PC software, version 22. One-way analysis of variance (ANOVA) was used for comparison of different biochemical values. **Results:** Rats injected with carbon tetrachloride (1 mL kg^{-1} b.wt.) showed a significant decrease of serum protein and albumin, the activities of AST, ALT, γ-GT and ALP were significantly increased. Significant increase in most lipid parameters occurred while the high density lipoprotein was decreased. The antioxidant capacity of the 2 formulas were high. Inclusion of each of these 2 formulas with the diet of injected rats prevented most of the associated health complications as indicated by sound correction of most biochemical parameters describing the biochemical changes that occurred due to carbon tetrachloride injection, however the correction of the histopathological changes that occurred due to carbon tetrachloride injection was limited. **Conclusion:** It was recommended to use these 2 dietary supplements by patients suffering from liver diseases assuming that they will help a lot to ameliorate health complications associating the liver disease thus act as complementary medicine to the main route of therapy.

Key words: Liver toxicity, carbon tetrachloride, dietary supplements, liver enzymes, liver histopathology

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Deaths due to liver cirrhosis have increased worldwide to varying extents over the past 30 years. This variation depends on several factors such as the population size, life style, medical care, food habits, alcohol consumption and perhaps the most contributing is the prevalence of viral infection and specifically virus C as the case in Egypt. The mortality rate due to liver cirrhosis in Egypt is perhaps the highest among other countries of the world reaching 72.2/ 100.000 people[1]. It is estimated that nearly 1 fifth (18.1%) of all deaths of the males in the age of 45-54 years old is due to liver cirrhosis. Chronic viral hepatitis usually lead to chronic liver injury which eventually develops to cirrhosis through specific and non-specific mechanisms. This may leads to liver failure[2,3]. Progressive accumulation and decreased remodeling of the extracellular matrix, along with other fibrogenic cells are also factors contributing to the development of cirrhosis. Carbon tetrachloride (CCl_4) also lead to the occurrence of liver toxicity causing several hepatocellular changes characteristic to liver diseases such as degeneration and centrilobular necrosis with the associated leakage of liver enzymes in plasma. Irrespective of the etiology of liver injury whether it is toxic-metabolic, viral, autoimmune, genetic disorders or nonalcoholic fatty liver disease, the end point if not properly handled is the development of cirrhosis[4]. Due to complications of the disease and despite the great progress in management, complete therapeutic approaches are lacking and there is an urgent need for non-traditional therapies to ameliorate these complications. Natural products or derivatives obtained from them are widely used now as novel therapy for the disease[5]. The advantage of these natural products is that if it is not able directly to cure the disease, most probably it will be able to relief most complications particularly if it is formed from different sources. In such a way, it will contain several bioactive compounds that are able to deal with a particular health complication. Several studies have been reported recommending the use of plant sources as remedy to the complications associating liver toxicity or diseases. Wang *et al.*[6], showed that apple polyphenols extracted from the unripe apple can improve acute hepatotoxicity induced by CCl_4. Choudhury *et al.*[7], proved that pomegranate protects against arsenic-induced p53-dependent ROS-mediated inflammation and apoptosis in liver cells. A combination of grape polyphenols and fish oils when given with the diet was found to reduce lipogenesis and glycolysis enzymes. It could also enhance beta-oxidation of fatty acid and signaling insulin thus ameliorating endoplasmic reticulum stress and protein oxidation[8]. An inverse association of coffee intake with fibrosis severity in nonalcoholic liver cirrhosis was reported by Marventano *et al.*[9] it is thus clear that a combination of different plants or herbs are needed all together to insure the presence of different bioactive compounds each may be specifically concerned with certain liver complications or signs.

The aim of the present study was to test the efficiency of 2 formulas formed from several plant sources to protect against complications of liver toxicity by carbon tetrachloride. The results of this study are hoped to be extended to humans suffering from viral infections thus performing similar actions protecting against the complication of liver diseases.

MATERIALS AND METHODS

The study was carried out in September 2016 at the Department of Nutrition and Food Science at the National Research Centre in Egypt.

Materials: The animals used in this experiment were Sprague Dawley male albino rats obtained from the animal house of the National Research Centre. The body weight of the animals ranged from 130-160 g.

Constituents, either plants or herbs used for composing the formula were:

- **Formula I:** Green apple, pomegranate, red grape, green tea, tomato and artichoke
- **Formula II:** Green tea, licorice, mint, coffee, lemon and grape fruit

All these constituents were purchased from the local market. Carbon tetrachloride (CCl_4) used for induction of hepatotoxicity in rats was obtained from Alpha chemical, Co., Mumbai (India). Corn oil in which CCl_4 was dissolved was obtained from the local market.

Most of the constituents of the diet formulated and introduced to the rats (starch, sugar, corn oil) were purchased from the local market, while casein was obtained from Screma Co., France. The salts and vitamins mixtures were used of analytical grade obtained from Fluka (Germany) and BDH (England) chemical companies.

Kits used for the estimation of the analyzed parameters were obtained from Biodiagnostics and Stanbio Laboratories company (Egypt).

Methods

Preparation of the formula: The selected ingredients (Green apple, pomegranate, red grape, tomato, artichoke,

mint, lemon and grape fruit) were cut into small pieces, dried in air ventilated oven at 60°C till complete dryness. The dried ingredients were then milled into fine powder in a mechanical grinder. The powdered ingredients were mixed together in suitable concentrations assumed to exert bioactive role and according to panel testing.

Composition of the formulas:

- **Formula I:** Green apple (25%), pomegranate (25%), red grape (25%), green tea (10%), tomato (10%) and artichoke (5%)
- **Formula II:** Green tea (15%), licorice (25%), mint (25%), coffee (5%), lemon (15%) and grape fruit (15%)

This powder was subjected to chemical analysis for determination of polyphenolic compounds prior to the feeding experiment.

The two formulas were extracted with methanol (99% S.d. Fine, chem. Ltd. POICHA) according to the method of Alvarez-Jubete *et al.*[10] and subjected to chemical analyses for determination of polyphenolic compounds. The results are given in Table 1. Total polyphenol content was determined by Folin-Ciocalteu assay according to the method of Ramful *et al.*[11]. Results were expressed in mg of tannic acid equivalent/mL of extract. Total flavonoid content was determined using a colorimetric method as described by Heimler *et al.*[12]. The results were expressed as mg of catechin/mL extract. Total antioxidant capacity of the 2 formulas was determined according to Locatelli *et al.*[13].

Preparation of the diet: The standard control diet was prepared according to Reeves *et al.*[14] and then each formula was added to the diet at two levels (10 and 20%) on the expense of starch (Table 2).

Animal experiment: The experiment was carried out on 42 rats. A group of 7 rats was separated to serve as -ve normal control (Group I).

The remaining 35 rats were classified as follows:

- **Group II:** Fed on a standard diet and served as +ve control
- **Group III:** Fed on a standard diet containing 10 g/100 g diet of formula I
- **Group IV:** Fed on a standard diet containing 20 g/100 g diet of formula I
- **Group V:** Fed on a standard diet containing 10 g/100 diet of formula II
- **Group VI:** Fed on a standard diet containing 20 g/100 g diet of formula II

Groups from 2-6 were injected with CCl_4 (1 mL kg^{-1} b.wt.) twice a week for 4 weeks[15].

Animals were kept in stainless steel cages in temperature controlled room at 25°C. Light was adjusted day and night. Food and water were allowed *ad libitum* to the animals during the whole experiment which lasted for 4 weeks.

During the experiment, the food consumption and body weight of the animals were measured.

The experimental procedure was carried out according to the Institutional Animals Ethics Committee of the National Research Centre, Egypt.

Table 1: Concentrations of total polyphenoles, flavonoids and antioxidant activity% for the prepared formulas

	Total polyphenols as tannic acid (mg mL^{-1})			
Formula	Methanol extract	HCl extract	Flavonoids (Catechin) (mg mL^{-1})	Antioxidant activity (DPPH assay) (%)
1	2.36	0.923	0.16	23.7
2	0.85	0.558	0.13	72.9

Table 2: Composition of the diets given to different groups of rats (g/100g)

	Groups					
Ingredients (g/100 g)	1	2	3	4	5	6
Casein	15	15	15	15	15	15
Corn oil	10	10	10	10	10	10
Sucrose	10	10	10	10	10	10
Maize starch	56	56	46	36	46	36
Cellulose	4	4	4	4	4	4
Salt mixture	4	4	4	4	4	4
Vitamin mixture	1	1	1	1	1	1
Formula I	-	-	10	20	-	-
Formula II	-	-	-	-	10	20
Total	100	100	100	100	100	100

At the end of the experiment (4 weeks) the rats were fasted overnight. All animals were sacrificed by cervical decapitation. Blood samples were collected from each rat from the retro-orbital vein and were received into clean dry centrifuge tubes. Serum was separated by centrifugation at 3000 rpm (Sigma laborzentrifuge Gmbh, WestGermany, model 2-15 3360 osterode/Hertz)for 15 min and kept in deep-freezer at 20°C until used for biochemical analysis.

Liver and kidney of the sacrificed rats were taken, washed with saline, weighed and then liver was immersed in 10% formalin solution and kept for histopathological examination.

Nutritional and biochemical assessment: The nutritional assessment included food consumption, gain in body weight, change in organs weight relative to body weight and the feed efficiency ratio calculated as the result of dividing the gain in body weight by the food consumption.

The biochemical evaluation was done as follows.

Liver function was assessed by determination of serum albumin according to Doumas *et al.*[16], total protein according to Henry[17] and bilirubin according to Walter and Gerade[18]. Serum aspartate aminotranferase (AST) and alanine aminotransferase (ALT) were determined according to the procedure given by Reitman and Frankel[19]. Gamma Glutamyltransferase (γ-GT) was estimated according to Saw *et al.*[20] and alkaline phosphatase (ALP) according to Belfield and Goldberg[21]. Lipid peroxidation was measured as malondialdehyde(MDA)following the procedure described by Satoh[22] and total antioxidant capacity (TAC) according to Koracevic *et al.*[23]. Most lipid parameters were determined including, triglycerides according to Fossati and Prencipe[24]and total cholesterol according to Allain *et al.*[25]. Low density lipoprotein cholesterol (LDL-C) and high density lipoprotein cholesterol (HDL-C) were also determined following the procedure described by Levy[26], Burstein and Scholnick[27], respectively.

Statistical analysis: Data are presented as Mean±SE. Statistical analysis of the data was performed using SPSS-PC software, version 22 (Chicago, IL). Unpaired student's t-test was used to compare biological differences. Meanwhile one-way analysis of variance (ANOVA) was used for comparison of different biochemical values in various experimental groups. It was followed by Duncan's multiple range tests to clarify the significance. p-values less than 0.05 were considered significant.

RESULTS

Nutritional evaluation: Nutritional evaluation of the effect of carbon tetrachloride injection and the possible protective action of the 2 formulas was done by following the food intake, the growth rate of the animals represented by the change in body and organs weights.

Food intake, gain in body weight and feed efficiency ratio: The values of food intake of rats, gain in body weight and the calculated feed efficiency ratio for the different groups are shown in Table 3. Injection of rats with carbon tetrachloride caused a non significant reduction in food consumption associated with a significant $p<0.05$ reduction in body weight as shown in Table 3. The feed efficiency ratio was also decreased.

Addition of formula 1 (10%) to the diet of animals in group 3 did not cause any change in food intake, gain in body

Table 3: Body weight gain (BWG), food intake (FI) and feed efficiency ratio (FER) of the different experimental groups

Group	Body weight gain (g)	Total food intake (g)	Food efficiency
G1(-ve)			
Mean±SE	75.70±21.51	378.63±17.62	0.199±0.021
G2(+ve)			
Mean±SE	45.12±4.13	344.34±7.43	0.131±0.013
p-value	<0.05	N.S	<0.05
G3 (10%)			
Mean±SE	41.92±6.01	330 .10±10.97	0.128±0.021
p-value	N.S	N.S	N.S
G4 (20%)			
Mean±SE	44.12±4.09	265.0±11.85	0.166±0.013
p-value	N.S	<0.005	N.S
G5 (10%)			
Mean±SE	35.70±3.35	267.91±16.03	0.133±0.009
p-value	N.S	<0.005	N.S
G6 (20%)			
Mean±SE	1.64±7.81	255.03±10.52	0.0002±0.031
p-value	<0.005	<0.005	<0.005

N.S: non-significant

Table 4: Relative liver weight and kidney weight in the different experimental groups

Groups	Liver	Kidney
G1(-ve)		
Mean±SE	3.12±0.121	0.85±0.06
G2(+ve)		
Mean±SE	4.76±0.123	0.82±0.05
p-value	<0.0005	N.S
G3 (10%)		
Mean±SE	5.12±0.163	0.87±0.03
p-value	N.S	N.S
G4 (20%)		
Mean±SE	5.29±0.234	0.81±0.02
p-value	N.S	N.S
G5 (10%)		
Mean±SE	4.97±0.246	0.79±0.06
p-value	N.S	N.S
G6 (20%)		
Mean±SE	5.84±0.22	1.06±0.06
p-value	<0.005	<0.05

N.S: Non-significant

Table 5: Concentration of serum albumin, bilirubin and total protein of rats in the different experimental groups

	Parameters		
Treatments	Albumin (g dL^{-1})	Bilirubin (g dL^{-1})	Total protein (g dL^{-1})
G1 (-ve control)	3.51 ± 0.18^{a}	0.15 ± 0.009^{b}	6.90 ± 0.16^{b}
G2 (+ve control)	2.65 ± 0.16^{a}	0.30 ± 0.01^{a}	5.70 ± 0.14^{c}
G3 (F1 10%)	3.47 ± 0.04^{a}	0.16 ± 0.01^{b}	7.04 ± 0.15^{ab}
G4 (F1 20%)	3.45 ± 0.11^{a}	0.17 ± 0.02^{b}	7.16 ± 0.25^{ab}
G5 (F2 10%)	3.09 ± 0.14^{a}	0.18 ± 0.01^{b}	7.37 ± 0.15^{a}
G6 (F2 20 %)	3.20 ± 0.08^{a}	0.16 ± 0.01^{b}	7.05 ± 0.14^{ab}

All values represented as Mean±SE, Means with different letters are significantly different ($p<0.05$)

weight or the food efficiency ratio. The same observation was noted when the dose was increased to 20%, the food intake was even less. Similar effect was noticed in rats given formula 2 with the diet. A non-significant change occurred when the dose was 10% but the food intake was markedly decreased (267.91±16.03 g).

Regarding the organ weights (Table 4), a significant increase ($p<0.0005$) in the weight of the liver was noticed in rats injected with carbon tetrachloride. However, no similar increase occurred in the kidney weight. No significant change occurred in these parameters when the formula was given with the diet. Only, a significant $p<0.05$ increase occurred in liver and kidney weights of rats given formula 2 with the high concentration.

Biochemical evaluation

Antioxidant power of the 2 formulas: Total polyphenol of the 2 formulas was estimated once for the methanol extract and the other for the HCl extract (Table 1). Results were expressed as mg tannic acid/mL and flavonoid content as mg catachin/mL. The DPPH% was also determined. The values obtained for total polyphenol as tannic acid in methanol extract amounted to 2.36 for formula 1 and 0.85 mg mL^{-1} for formula 2 as shown in Table 1. In case of the HCl extract the values obtained were 0.923 and 0.558 mg mL^{-1}, respectively. The values of the flavonoids as catachin amounted to 0.16 for formula 1 and 0.13 mg for formula 2. The values obtained for antioxidant activity (1, 1- diphenyl-2-picrylhydrazine, DPPH %) for formula 1 amounted to 23.7and that for formula (2) 72.9.

Serum total proteins, albumin and bilirubin: Injection of carbon tetra chloride caused a significant $p<0.05$ decrease of serum protein and albumin but recorded significant increase in bilirubin as shown in Table 5. The serum total protein dropped from a value of 6.90±0.16-5.70±0.14 g dL^{-1} and that for albumin from 3.51±0.18-2.65±0.16 g dL^{-1}. Serum bilirubin elevated from 0.15±0.009-0.30±0.01 mg dL^{-1}. It can be noticed that addition of any of the 2 formulas either with the low or the high concentration could protect against the drop in either serum total protein or albumin.

Enzymes activities: Four enzymes were analyzed namely AST, ALT, γ-GT and ALP. The activities of these enzymes in serum of control rats and the treated ones are shown in Table 6. The activities of these enzymes were significantly $p<0.05$ increased due to carbon tetra chloride injection as shown in Table 6. The values obtained for AST, ALT, γ-GT and ALP for G relative to values of G1 for controls. Addition of formula 1 or 2 to the diet

Table 6: Changes in serum hepatic enzyme activites of rats in the different experimental groups

	Parameters			
Treatments	AST (u L^{-1})	ALT (u L^{-1})	γ-GT (u mL^{-1})	ALP (u mL^{-1})
G1 (-ve control)	32.60±2.04^{e}	21.11±1.63^{d}	2.60±0.31^{b}	75.00±2.12^{c}
G (+ve control)	119.11±4.28^{a}	112.12±4.50^{a}	4.61±0.35^{ab}	155.12±9.01^{a}
G3 (F1 10%)	100.03±1.54^{b}	91.70±1.82b^{c}	2.63±0.28^{b}	117.51±6.24^{b}
G4 F1 20%)	83.92±2.04^{d}	87.00±1.97^{c}	3.54±0.50^{ab}	90.53±5.42^{c}
G5 (F2 10%)	80.61±1.212^{d}	84.60±1.98^{c}	4.21±0.67^{ab}	89.64±0.92^{c}
G6 (F2 20%)	93.34±0.80^{c}	96.10±2.02^{b}	6.98±2.65^{a}	91.13±5.71^{c}

All values represented as Mean±SE, Means with different letters are significantly different ($p<0.05$)

Table 7: Concentration of serum lipids of rats in the different experimental groups

	Parameters			
Treatments	Total cholesterol (TC) (mg dL^{-1})	Triglycerides (mg dL^{-1})	HDL-C (mg dL^{-1})	LDL-C (mg dL^{-1})
G-1(-ve control)	78.11±1.72^{c}	44.12±4.73 ^{c}	51.230±4.61^{a}	21.14±0.93^{e}
G-2(+ve control)	148.63±10.31^{a}	80.51±4.42^{a}	24.310±1.72^{c}	87.63±4.51^{a}
G3 (F1 10%)	87.71±2.80^{bc}	63.82±4.63^{b}	27.720±2.24^{c}	48.70±0.87^{b}
G4 (F1 20%)	89.00±3.73^{bc}	74.91±2.02^{a}	41.910±3.12^{ab}	35.20±2.2^{cd}
G5 (F2 10%)	91.90±2.72^{bc}	70.81±3.81^{ab}	37.810±3.13^{b}	40.20±1.12^{c}
G6 (F2 20%)	93.62±2.81^{b}	72.40±2.90^{ab}	46.730±3.52^{ab}	31.80±1.51^{d}

All values represented as Mean±SE, Means with different letters are significantly different ($p<0.05$)

Table 8: Concentration of total antioxidant capacity (TAC) and malondialdehyde (MDA) of rats in the different experimental groups

	Parameters	
Treatments	Total antioxidant (TAC) (mM L^{-1})	Malondialdehyde (MDA) (nmol mL^{-1})
G1 (-ve control)	4.01±0.10^{a}	5.91±0.33^{b}
G2 (+ve control)	2.27±0.01^{d}	8.92±0.30^{a}
G3 (F1 10%)	3.29±0.14^{c}	5.34±0.27^{b}
G4 (F1 20%)	3.70±0.15^{ab}	5.23±0.32^{b}
G5 (F2 10 %)	3.55±0.12^{bc}	5.72±0.26^{b}
G6 (F2 20 %)	3.34±0.14^{bc}	5.13±0.34^{b}

All values represented as Mean±SE, Means with different letters are significantly different ($p<0.05$)

of carbon tetrachloride rats caused improvement of the activities of these enzymes, their values returned to near normal values.

Serum Triglycerides, TC, LDL and HDL cholesterol: A significant $p<0.05$ increase in most lipid parameters occurred a result of injection with carbon tetrachloride (Table 7). The high density lipoprotein was decreased significantly. The values obtained were 44.12±4.73, 78.11±1.72, 21.14±0.93 mg dL^{-1} for triglycerides, TC and LDL-C, respectively for control animals. The similar values for injected rats were 80.51±4.42, 148.63±10.31 and 87.63±4.51 mg dL^{-1}. The HDL decreased from 51.23±4.61-24.31±1.72 mg dL^{-1}.

Addition of any of the 2 formulas to the diet caused some sort of correction to these parameters. The values obtained were near to normal values.

Serum total antioxidants and malondialdehyde: The serum total antioxidants of rats injected with carbon tetrachloride were significantly $p<0.05$ decreased while the serum malondialdehyde was significantly increased as shown in Table 8. The values obtained were 4.01±0.10 mM L^{-1} for total antioxidants of control rats and 5.91±0.33 nmol mL^{-1} for malondialdehyde. Similar values for injected rats were 2.27±0.01 and 8.92±0.30 nmol L^{-1}. Addition of any of the 2 formulas to the diet caused a change in the values of these fore mentioned parameters, the value obtained were more near to normal values.

Histopathological examination: Microscopically, liver of rats from control negative group revealed the normal histological structure of hepatic lobule (Fig. 1). In contrary, liver of control positive rats showed congestion of central vein, steatosis of hepatocytes, apoptosis and strands of collagen fibers deposition between the hepatic lobules (Fig. 2). Moreover, liver of rats from group 3 revealed steatosis of hepatocytes, apoptosis and fine strands of collagen fibers deposition (Fig. 3) but to less extent. Examined sections from group 4 still showed steatosis of hepatocytes (Fig. 4) and apoptosis less marked than the other groups. Moreover, liver of rats from group 5 also revealed congestion of central vein, steatosis of hepatocytes and apoptosis (Fig. 5). That of rats from group 6 still shows steatosis and apoptosis (Fig. 6).

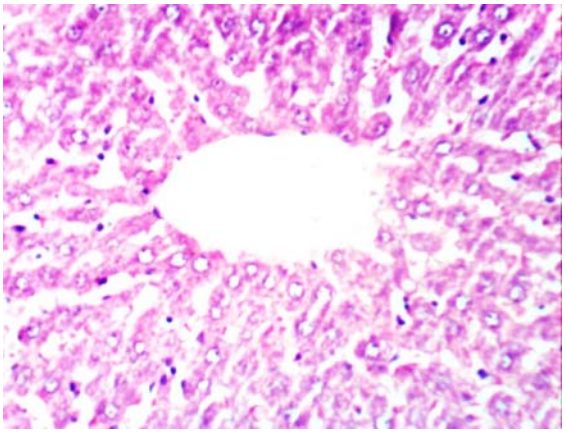

Fig. 1: Liver of rat from control negative group showing the normal histological structure of hepatic lobule (H and E X 400)

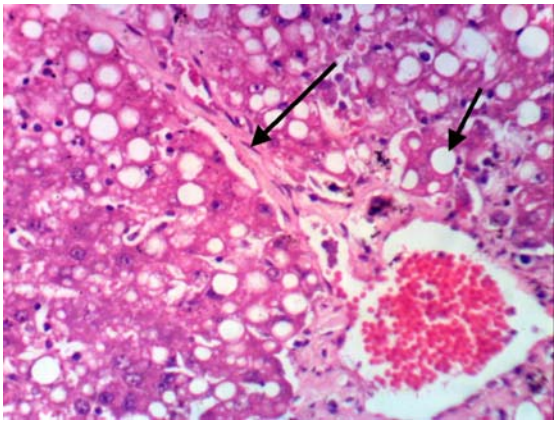

Fig. 2: Liver of rat from control positive group showing congestion of central vein, steatosis of hepatocytes and strands of collagen fibers deposition between the hepatic lobules (H and E X 400)

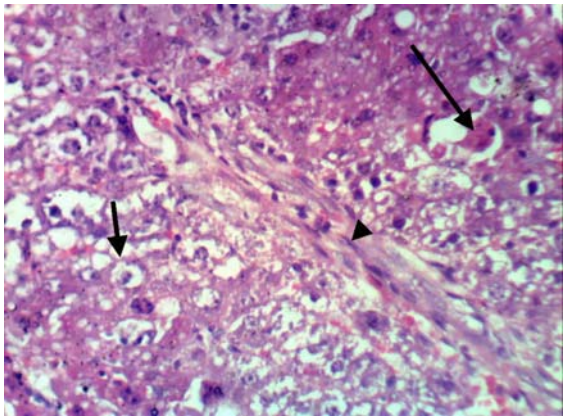

Fig. 3: Liver of rat from group 3 showing steatosis of hepatocytes, apoptosis of hepatocytes and fine strands of collagen fibers deposition (H and E X 400)

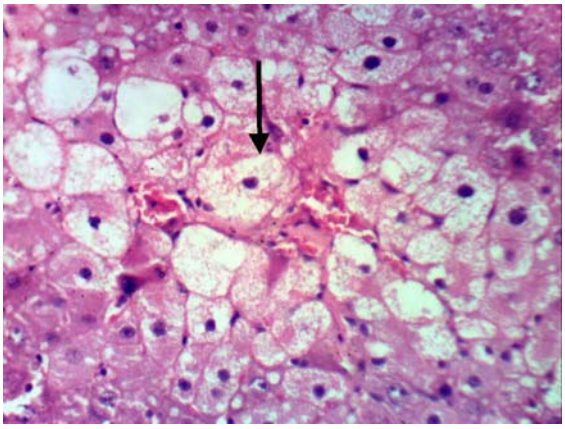

Fig. 4: Liver of rat from group 4 showing marked steatosis of hepatocytes (H and E X 400)

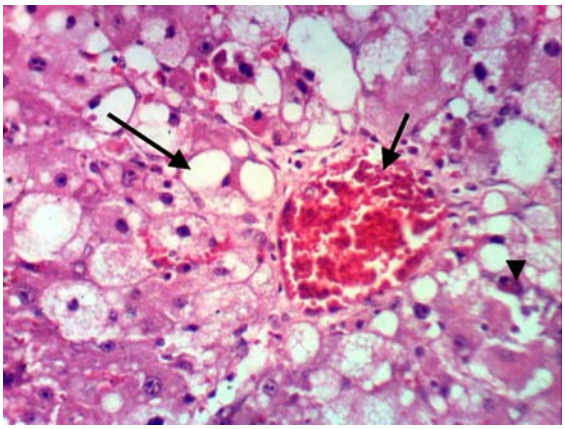

Fig. 5: Liver of rat from group 5 showing congestion of central vein, steatosis of hepatocytes and apoptosis of hepatocytes (H and E X 400)

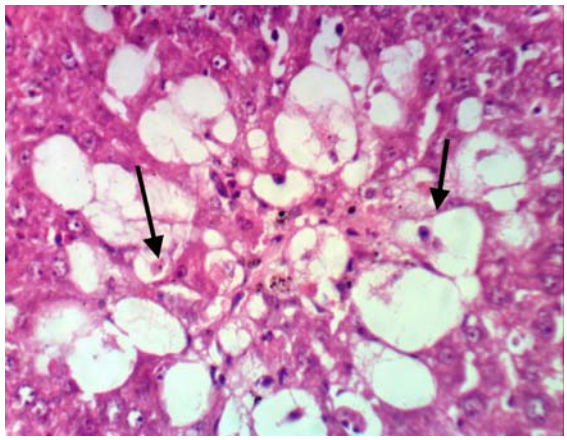

Fig. 6: Liver of rat from group 6 showing steatosis and apoptosis of hepatocytes (H and E X 400)

DISCUSSION

In the present study liver toxicity was induced in rats. Most of the metabolic changes reported for carbon tetrachloride toxicity was induced in the model animals used in the experiment. Carbon tetrachloride toxicity caused several hepatocellular changes similar to what happens in liver diseases such as liver degeneration and centrilobular necrosis with the associated leakage of liver enzymes in plasma and elevation of the activities of each of AST, ALT, ALP and bilirubin including the fatty layer degeneration of liver. Several previous studies used carbon tetrachloride to induce a model of liver toxicity in animals[28-30]. It has been stated that cytochrome P-450 dependent mixed oxidase in the endoplasmic reticulum activate carbon tetrachloride and generate trichloromethyl free radical (CCl_3 and Cl_3COO). These free radicals cause damage to the endoplasmic reticulum and other membrane increasing their permeability to calcium ion and results in disturbances of calcium homeostasis and necrotic cell death[31-33]. There was a marked elevation of each of AST, ALT, ALP and γ-GT. Such elevation is an indication to cell membrane damage and leakage of these enzymes in circulation. Serum or plasma γ-GT is closely related to hepatic steatosis and considered as a surrogate marker of non-alcoholic fatty liver disease (NAFLD)[34]. The increased activity of the AST enzyme indicates loss of functional integrity of the liver while increased activity of APT indicates intra and extra hepatic biliary obstruction of the liver[35]. On the other hand, serum γ-GT which is a hepatobiliary enzyme synthesized in epithelial cells of the intrahepatic duct was significantly $p<0.05$ increased. This enzyme is considered as a marker of NAFLD. It has been reported that increased deposition of liver fat induces hepatocellular damage and stimulate the synthesis of γ-GT[36]. The increased activity of this enzyme enhances mitochondrial damage and free radicals that cause significant oxidative stress and pro-inflammation[37]. In addition, serum γ-GT is involved in cellular glutathione (GSH) metabolism thus, elevated levels of γ-GT is expected to generate reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2), superoxide (O_{2^-}) and hydroxyl (OH^-) anion radicals through its interaction with free iron and induces low grade hepatic inflammation by hepatic steatosis[38]. Elevated level of serum bilirubin is due to its leakage from hepatocytes to plasma, usually because of hepatic obstruction to bile outflow and cholestasis. Injection with carbon tetrachloride caused a significant $p<0.05$ increase in the level of indirect bilirubin. The latter compound which is a metabolic end product of heme breakdown is a powerful antioxidant that protect against oxidation stress by inhibition of NADPH oxidase that increase superoxide production[39,40]. The serum albumin and plasma protein of carbon tetrachloride injected rats were decreased indicating the reduced capability of the liver to synthesize proteins[41]. As expected the total antioxidant capacity of blood (TAC) was decreased and the serum malondialdehyde (MAD) was increased. The decreased antioxidant capacity is believed to be as mentioned before due to activation of carbon tetrachloride and generation of trichloromethyl free radical. This is further confirmed by the increased serum malondialdehyde reflecting the increased oxidation product in the body[42]. Since the liver is the principle site for formation and clearance of lipoprotein, the oxidation stress generated in the body as a result of carbon tetrachloride injection affect the liver function causing an abnormal lipid pattern represented by increases in triglycerides, total and low density lipoprotein cholesterol. The toxic effect of carbon tetrachloride on the liver is not only restricted to the function of the liver but affected body weight. The growth rate of the animals was markedly affected in spite of the non-significant decrease in food intake. The food efficiency ratio was markedly decreased[15,43].

In brief, it can be stated that injection of rats with carbon tetrachloride caused acute liver injury with most of the surrounding pathological changes and symptoms (see plate showing the histopathological changes Fig. 2). A further derangement in liver function is the drop in serum albumin indicating the incapability of the liver to synthesize proteins which is one of the main functions of the liver when it is healthy.

Bioactive components present in food can provide protection against so many complications associating liver toxicity thus provide positive impacts on health[44] and influence physiological or cellular activities in animals and humans. In this study a number of plant sources were collected that were reported to contain bioactive compounds able to exert health effects such as being anti-inflammatory, anti-oxidant anti-atherosclerotic, immunmodulators, anti-proliferative or protect from liver necrosis and cirrhosis[45]. Estimation of the total polyphenol, flavonoid content or the percentage antioxidant activity as DPPH% proved that these two formulas contain considerable amounts of polyhenols with appreciable antioxidant characters[46]. Among the ingredients used for composing these formulas, apple which was reported to contain phenolic compounds and pentacyclic triterpenes, such as ursolic acid that possess antioxidant, antitumor, anti-inflammatory and antibacterial properties[47]. Anthocyanins, ellagic acid derivatives and hydrolysable tannins present in pomegranate juice are responsible for the antioxidant activity of this fruit[48]. Red or white grapes are also rich in polyphenols with marked antioxidant capacity[49,50]. Tomato is an excellent source of minerals, vitamins C and E,

B-carotene, lycopene, flavonoids, organic acids, phenolics and chlorophyll[51] all of these compounds have health value. Heads of artichoke showed a high nutritional value and antioxidant activity[52]. Several health benefits were reported for coffee. The regular intake of coffee decreased incidence of liver fibrosis[53] and it also mitigates glucose intolerance, hypertension, cardiovascular remodeling and fatty liver[54].

Licorice extract may play an important role in medicine by scavenging free radicals, stimulating activities of antioxidant enzymes and arresting production of inflammatory cytokine, subsequently protecting the liver against CCl_4-induced damage. The components (triterpene, saponins, glycyrrhizin acid) in single or in combination with other components present in the licorice extract might be responsible for the reduction of hepatotoxicity in treatment groups[55].

The chemical components of peppermint are rich in phenolic compounds, such as mono- and dicaffeoylquinic acids and flavonoids which responsible for the antioxidant activity[56].The health benefits of lemon are due to its many nourishing elements like vitamin C, vitamin B, phosphorous, proteins and carbohydrates. Lemon is a fruit that contains flavonoids, which have antioxidant and cancer fighting properties[57]. Lemon consists of 68% dilemonene, a powerful antioxidant[58]. Also grapefruit juice is an excellent source of many phytochemicals and nutrients that contribute to a healthy diet[59].

The results obtained from this study show that including groups of these plants in the diet of animals protect against most of the associated health complications that occurred due to injection with carbon tetrachloride. Regarding the oxidation state in the body, the results showed that the serum total antioxidants were increased relative to the intoxicated rats. In the same time the serum malondialdehyde were decreased. This is an indication that including the formulas with the diet of the intoxicated animals added to the antioxidant power of the body. This is expected since most of the plant sources used for formulation of these dietary supplements was reported to contain antioxidant compounds either as antioxidant vitamins, phenolic compounds or any other compounds with antioxidant characters. Based on this and due to scavenging of free radicals by these antioxidant compounds so many of the health hazards occurred due to free radical damaging effect were prevented[60]. The integrity of the cell membranes was preserved indicated by decreased activity of the enzymes leaked in circulation due to lysing of the cells such as AST, ALT, γ-GT and ALP. In general the liver function was improved indicated by increased serum total protein and albumin. The serum bilirubin was decreased showing less heamolysis of blood hemoglobin due to decreased oxidation load in the body as a result of inclusion of the dietary supplements with the diet. This means that the plant sources used for formulation of the 2 dietary supplements succeeded to protect the liver against most complications associating liver toxicity by carbon tetrachloride. This shows that they can be safely given to patients suffering from liver diseases to help protect against complications associating the disease. In the same time, they are safe and exert no health hazards on the body. In spite of this, the histopathological examination of the liver showed that these plants have limited capacity to protect from the pathological lesions occurred due to carbon tetrachloride injection, however still there is some sort of protection because the histopathological lesions that occurred due to carbon tetrachloride injection were less pronounced when any of the formulas were given.

CONCLUSION

Injection of rats with carbon tetrachloride exerted several hepatocellular changes similar to what happens in liver diseases such as liver degeneration and centrilobular necrosis with the associated leakage of liver enzymes in plasma and elevation of the activities of each of AST, ALT, ALP and bilirubin including the fatty layer degeneration of liver. These biochemical changes were induced by generation of trichloromethyl free radical (CCl_3 and Cl_3COO). Such health complications could be prevented by including one of the two dietary supplements used in this study.

It is thus recommended to use these two formulas as dietary supplements for patients suffering from liver diseases. This will offer additional protection of the body from the associating complications.

SIGNIFICANCE STATEMENTS

Carbon tetrachloride injection caused acute liver injury with most of the surrounding pathological changes and symptoms. The bioactive components present in the plant sources used for formulation of the 2 dietary supplements succeeded to protect the liver from carbon tetrachloride toxicity, thus provide positive impacts on health.

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