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Chemopreventive Effect of *Coriandrum sativum* Fruits on Hepatic Toxicity in Male Rats

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Abstract: Coriander is recognized as one of the most important spices in the world. This study aimed to estimate the total phenolics content and the volatile chemical constituents of Sudanese *Coriandrum sativum* fruits as well as to assess *in vivo* and *in vitro* antioxidant activity of its aqueous extract and its hepatoprotective effects against paracetamol drug. Aqueous extract of Sudanese *Coriandrum sativum* fruits was subjected to quantitative determination of total phenolics content (TPC) and antioxidant activity by two different methods. Isolation and characterization of volatile compounds using GC and GC-MS was carried out. Biochemical and histological studies were carried out in rats for studying the effects of the plant infusion on panadol (paracetamol) induced free radicals and hepatotoxicity. Our results showed that aqueous extract of Sudanese *Coriandrum sativum* fruits posses high content of phenolic compounds and high antioxidant activity *in vitro*. GC-MS analysis enable the identification of seventy eight compounds with Sabinene(17.63%), camphor (15.5%), cis-beta-ocimene (10.11%), trans-beta-ocimene (5.64%), alpha pinene (4.69%) and norboreneolacetate (4.09%) as main constituents. The biochemical results showed that administration of paracetamol recorded a significant increase in plasma alanine amino transferase (ALT), aspartate amino transferase (AST), alkaline phosphates (ALP), gamma glutamyl Transferase (GGT), bilirubin, urea and creatinine with significant decrease in plasma total proteins, albumin and some antioxidant biomarkers [plasma total antioxidant capacity (TAC), catalase (CAT) and glutathione peroxidase (GPx)] compared to normal rates. ANOVA analysis indicated that rats which supplemented with aqueous extract and then administrated with paracetamol showed significant improvement in all biochemical parameters, which become near to control, the results were confirmed by histopathological examination of the liver tissue of control and treated animals.

Key words: *Coriandrum sativum* fruits · Volatiles compounds · GC-MS, Antioxidant · Paracetamol · Hepato-protection.

INTRODUCTION

Coriandrum sativum is an annual herb in the family Apiaceae. Coriander is native to southern Europe and North Africa to southwestern Asia. Coriander is recognized as one of the most important spices in the world. The oil of Coriander is used as a flavouring agent. The fruit has been used as a drug for indigestion, against worms and as a component of embrocations for rheumatism and pains in the joints. Fresh leaves are pungent and aromatic. The essential oil of coriander stimulates the secretion of gastric juices [1]. It has been reported to have a number of possible health attributes, particularly relating to the gastro-intestinal tract, but also

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as a possible diabetic remedy [2]. Many experimental studies have suggested anti-hyperglycemic effects of coriander seeds in streptozotocin-diabetic mice [3]. Gray and Flatt [4] reported that incorporation of coriander into the diet or in drinking reduced hyperglycemia of streptozotocin-diabetic mice and a seed decoction showed anti-hyperglycemic action in rats. In another study, Chithra and Leelamma [5] reported that feeding coriander seed (10%) protected against the 1, 2-dimethylhydrazine-induced colon and intestine tumors in rats. As a major constituent of a spice mix added to a diet (2%), ‘‘coriander", when fed to female Wistar rats for 8 weeks, ‘‘favorably enhanced” the activities of pancreatic lipase, chymotrypsin and amylase. Additionally, feeding the diet containing the spice mix significantly stimulated the bile flow and bile acid secretion [6]. A study found both the leaves and seed to contain antioxidants, but the leaves were found to have a stronger effect [7]. Drugs continue to be taken off the market due to late discovery of hepatotoxicity. Due to its unique metabolism and close relationship with the gastrointestinal tract, the liver is susceptible to injury from drugs and other substances. 75% of blood coming to the liver arrives directly from gastrointestinal organs and then spleen via portal veins which bring drugs in concentrated form. The liver plays a central role in transforming and clearing chemicals and is susceptible to the toxicity from these agents. Certain medicinal agents, when taken in overdoses and sometimes even when introduced within therapeutic ranges, may injure the organ. More than thousands drugs have been implicated in causing liver injury [8]. Chemicals often cause subclinical injury to liver which manifests only as abnormal liver enzyme tests. Paracetamol overdose is the most common cause of drug induced liver disease. Acetaminophen (paracetamol, also known by the brand name Tylenol and Panadol) is usually well tolerated in prescribed dose but overdose is the most common cause of drug induced liver disease and acute liver failure worldwide [9]. Excessive use of paracetamol can damage multiple organs, especially the liver and kidney. In this research we studied the effects of Sudanese *Coriandrum sativum* on panadol toxicity on liver, kidneys and some antioxidant biomarkers.

MATERIALS AND METHODS

Preparation of Plant Infusion: Dry fruits of *Coriandrum sativum*, Kazbara, were purchased from Omdurman market in Khartoum State, Sudan. The dry fruits under investigation were separately grounded and three grams of plant were infused with 100 ml freshly boiled water for 5 min. followed by filtration. The infusion filtrates of Coriandrum sativum were subjected to the following tests:

Quantitative Determination of Total Phenolics Content (TPC): by the Folin-Ciocalteu method [10].

Quantitative Determination of Antioxidant Activity: according to β-carotene bleaching method [11] and DPPH free radical scavenging assay [12].

Isolation and Characterization of Volatile Compounds: Two hundred grams of the dried fruits were ground to a fine powder using electric grinder; the essential oil was obtained by steam distillation in 3000 mL H_2O for 3 h by Clevenger apparatus. The oil was dried over anhydrous sodium sulphate and filtered. Extraction was carried out in duplicate and the results were averaged. Analysis of the volatiles of the plant infusions were done by using the chromatographic techniques, (GC) and (GC/MS).

Gas Chromatography (GC): The obtained volatiles samples were thermally desorbed, using a modified injector port, directly on the front of a (DB5) (60 m x 0.32 mm i.d) fused silica capillary column, in the oven of a Hewlet-packed HP 5890 gas chromatography and temperature increase from 45-240°C by the rate 4°C/min. Kovat’s indices were determined by co-injection of the sample with a solution containing homologous series of n-hydrocarbons (C_6-C_{26}) under the same conditions as described above. The separated components were identified by matching with NIST mass -spectral library data and by comparison of Kovat’s indices with those of authentic components and with published data [13]. The quantitative determination was carried out based on peak area integration. Retention indices (RI) of each compound were calculated from the standard alkane retention time and the peak retention time.

Gas Chromatography-mass Spectrometry (GC-MS): Analyses were performed on An HP model 6890 GC interfaced to an HP 5791A mass selective detector (GC/MS) was used for mass spectral identification of the GC components at (MS) ionization voltage of (70 eV. A 30 m x 0.25 mm i.d). (DF = 0.25 lm) DB wax bonded-phase fused-silica capillary column was used for (GC). The linear velocity of the helium carrier gas was 30 cm/s. The injector and the detector temperatures were 250°C. The oven temperature was programmed from 40 -240°C at 4°C/min and held for 50 min.

Biochemical Study:

Experimental Animals: Adult male Swiss rats with initial weights ranging from 120-150 g were used as experimental animals for biochemical and histological studies. All experimental animals were provided from the Breeding Unit of the National Research Centre (Cairo, Egypt). The animals housed individually in stainless steel wire mesh cages. They were maintained for one week, as an acclimatization period. Commercial standard pellets and tap water were supplied *ad libitum*.

Experimental Design: Twenty eight adult rats were used for studying the effects of the plant infusion on panadol (paracetamol) induced free radicals and hepato-toxicity [14]. The rats were equally divided into four groups (7 rats in each group):

Group 1: Normal control, rats were giving drinking tap water.

Group 2: Rats were supplemented with freshly prepared aqueous extract of *Coriandrum sativum* (3g/100ml water) for thirty days, to examine safety of plant extract concentration as a drink.

Group 3: Rats were intoxicated after 4 weeks by oral administration with paracetamol (2 g/kg. B.W) [14].

Group 4: Protected rats: where rats were maintained on drinking freshly prepared Sudanese *Coriandrum sativum* infusion (3 g/100 ml boiled water) for (28 days) instead of tap water and then rats were intoxicated by oral administration with paracetamol (2 g/kg. B.W).

The experiment duration was continued for 30 days. The rats were killed after two days from oral single dose of paracetamol administration.

Blood Sampling: Blood samples were withdrawn on heparinized tubes. Plasma was used for determination of liver and kidneys function and some antioxidant biomarkers. The (RBCs) was washed several times with cold saline solution. The packed RBCs were stored at 20°C for determination of Glutathione peroxidase.

Tissue Sampling and Processing: Liver was excised and rinsed with cold saline, blotted dry and weighed. A portion of the liver tissue was kept into 10% formalin for histological and morphometric examinations.

Biochemical Assays:

Antioxidant Capacity: Plasma total antioxidant capacity, plasma catalase and cellular glutathione peroxidase were determined by using assay kits [15-17].

Liver and Kidney Functions: Plasma total protein, albumin, alanine amino transferase (ALT), aspartate amino transferase (AST), alkaline phosphates (ALP), gamma-glutamyl transferase (GGT), total bilirubin and direct bilirubin were carried out by using assay kits [18-23]. Kidney functions (plasma Creatinine and urea) were also carried out [24, 25].

Histological Study: Livers were dissected out and fixed instantaneously in 10% formalin saline for 24 hours. The specimens were washed in tap water, dehydrated in ascending grades of ethanol, cleared in xylene, embedded in paraffin wax (melting point 58-60°C). Sections of 6µm thickness were prepared and stained with Haematoxylin and Eosin [26].

Histochemical Study:

1. *Total Proteins:* Mercury bromophenol blue method was applied for the histochemical demonstration of the total proteins [27].
2. *The Polysaccharide Inclusions*: Periodic acid Schiff method was applied for visualization of the polysaccharide materials [28].

Statistical Analysis: The data presented in the study was statistically evaluated as mean values for each group and its corresponding standard error of the mean (mean ± SE). Statistical evaluation of the difference between the groups means values were tested by ANOVA analysis. The significant level was set at $P < 0.05$ [29].

RESULTS

Total Phenolic Content and Antioxidant Activity: Table 1 showed that the amount of the total phenolic contents (TPC) in the aqueous extract was 1654 ± 3.4 mg GAE/L. The results of the *in vitro* antioxidant activity by β -carotene/linoleic acid assay, showed that *Coriandrum sativum* aqueous extract had strong antioxidant activity (84.6% at 400ug/ml), when compared with the standard reference Tert-butyl hydroquinone (TBHQ) (99.5% at the same concentration). Table 2 indicated the results obtained by DPPH free radical scavenging assay.

Table 1: Total phenolic content (TPC) and *in vitro* antioxidant activity (A.A) of Sudanese *Coriandrum sativum* fruits determined by β-carotene method

		A.A at different concentrations by β-carotene method			
Item	TPC(mg GAE/L)	50 (µg/ml)	100 (µg /ml)	200 (µg /ml)	400 (µg /ml)
Extract0	1654 ± 3.4	52.0 ± 2.7	62.0 ± 2.4	78.4 ± 301	84.6 ± 2.8
♦?TBHQ	--	75.2 ± 3.1	85.0 ± 205	94.0 ± 3.4	99.5 ± 2.7

♦ Tert -butyl hydroquinone

Each value represents the mean ± S.E (Standard Error) and mean of three replicates.

Table 2: *In vitro* antioxidant activity (A.A) of Sudanese *Coriandrum sativum* fruits determined by DPPH free radical scavenging method.

	A.A at different concentrations by DPPH free radical scavenging method			
Item	50 (µg /ml)	100 (µg /ml)	200 (µg /ml)	400 (µg /ml)
Extract	53.03 ± 1.9	64.43 ± 2.4	75.53 ± 2.3	88.50 ± 2.9
♦TBHQ	76.53 ± 2.3	83.75 ± 2.5	95.36 ± 2.6	99.73 ± 2.6

♦ Tert -butyl hydroquinone

Each value represents the mean ± S.E (Standard Error) and mean of three replicates.

Table 3: Volatile compounds of Sudanese *Coriandrum sativum* fruits analyzed by GC-MS.

Peak no	K.I	Area %	Compound name
1	926	1.59	Cumene
2	933	4.69	alpha-pinene
3	943	0.13	beta-citronellene
4	947	0.25	beta-camphene
5	952	0.24	beta-camphene
6	972	17.63	Sabinene
7	980	0.19	beta-pinene
8	994	0.23	Myrecene
9	1002	2.59	delta-2-carene
10	1015	3.06	beta-phellendrene
11	1019	10.11	cis-beta-ocimene
12	1040	5.64	trans-beta-ocimene
13	1060	0.98	trans-3-pinanone
14	1078	0.10	Terinolene
15	1090	0.08	Nananal
16	1098	0.80	Linalool
17	1112	5.20	trans-sabinene hydrate
18	1121	0.63	cis-pinene hydrate
19	1128	4.09	Norboreneol acetate
20	1137	0.58	cis-beta-dihydroterpineol
21	1142	15.50	Camphor
22	1155	1.69	Nonanol
23	1157	3.19	Isoborneol
24	1164	0.66	borneol
25	1170	1.25	terpenin-4-ol
26	1196	0.17	carveol
27	1206	0.21	trans-piperitol
28	1215	0.07	iso-dihydrocarveol
29	1219	0.45	cis-sabinene hydrate
30	1244	0.12	Carvone
31	1252	1.80	Piperitone
32	1260	0.47	(E)-2-decanal
33	1278	0.55	thujyl acetate

Table 3: Continue

Peak no	K.I	Area %	Compound name
34	1282	1.28	Safrole
35	1286	0.02	bornyl acetate
36	1292	0.79	verbenyl acetate
37	1304	0.04	Dihydrocarveol acetate
38	1314	0.28	trans-dihydro-alpha-terpinyl acetate
39	1316	0.44	cis-2,3-pinanediol
40	1320	0.73	dihydrocitronellol acetate
41	1330	0.28	piperitol acetate
42	1333	0.06	delta-indanol
43	1335	0.35	iso-safrole
44	1341	0.28	verbanol acetate
45	1346	0.02	alpha-terpinyl acetate
46	1356	1.09	eugenol
47	1363	0.65	piperitenone oxide
48	1367	0.82	neryl acetate
49	1371	0.53	carvacrol acetate
50	1376	0.26	alpha-copaene
51	1408	0.91	(Z)-caryophyllene
52	1414	1.53	alpha-cis-bergamotene
53	1425	0.16	alpha-ionone
55	1438	0.27	aromadendrene
56	1445	0.28	alpha-patchoulene
57	1459	0.08	(E)-beta-farnesene
58	1465	0.06	9-epi-caryophyllene
59	1477	0.20	gamma-muurolene
60	1490	1.09	beta-selinene
61	1507	0.15	beta-bisabolene
62	1530	0.04	trans-calamene
63	1583	0.06	caryophyllene oxide
64	1652	0.34	alpha-cadinol
65	1684	0.19	epi-alpha-bisabolol
66	1712	0.11	14-dihydro-alpha-humulene
67	1718	0.08	(Z)-beta-santalol
68	1729	0.06	(Z)-nuciferol
69	1750	0.05	alpha-sinensal
70	1758	0.88	(E)-nuciferol
71	1759	0.02	Eupatoriochromene
72	1773	0.11	(E)-alpha-atlantone
73	1794	1.30	alpha-bisabolol acetate
74	1808	0.31	Cryptomeridiol
75	1908	0.29	2-phenylethylphenyl acetate
76	1924	0.78	Beyerene
77	2138	0.14	Osthol
78	2234	2.32	7-alpha-hydroxy-manool

K.I: Kovats index.

Compound listed in the order of elution from a DB_5 column, Retention indices relative to C_7-C_{20} *n*-alkanes on the DB-5MS column, identification based on retention index and comparison of mass spectra

Table 4: Effect of administration of paracetamol and the aqueous extract of Sudanese *Coriandrum sativum* fruits on liver enzymes in plasma

	Parameters			
Groups	AST (U/L)	ALT (U/L)	ALP (U/L)	GGT (U/L)
Control	61.57± 4.28[a]	16.43± 1.54[a]	169.09 ± 7.54[a]	23.20 ± 1.63[a]
Extract	61.17± 3.97[a]	15.50± 2.28[a]	166.87± 10.15[a]	23.49± 0.89[a]
Paracetamol	85.00 ± 5.48[b]	39.57± 2.64[b]	240.19 ± 8.49[b]	34.19 ± 1.15[b]
Paracetamol + extract	71.86 ± 3.63[c]	30.00 ± 2.38[c]	221.36± 13.09[c]	29.94 ± 1.56[c]

Data presented as mean ± SE

Values in the same column with the same superscripts are not significantly at ($P < 0.05$).

Table 5: Effect of administration of paracetamol and the aqueous extract of Sudanese *Coriandrum sativum* fruits on some liver bio-indicator in plasma.

	Parameters				
Groups	T. prot.(g/dl)	Alb(g/dl)	T. bili(mg/dl)	D. bil (mg/dl)	Ind. Bil (mg/dl)
Control	6.74±0.39 [a]	3.15±0.21[a]	0.34±0.03[a]	0.14±0.01[a]	0.19±0.02[a]
Extract	6.95± 0.33[a]	3.22± 0.20[a]	0.32± 0.03[a]	0.14± 0.01 [a]	0.18± 0.01[a]
Paracetamol	5.97 ±0.18[b]	2.88±0.19[b]	0.50±0.02[b]	0.24±0.02[b]	0.26±0.02[b]
Paracetamol + extract	6.63±0.25 [a]	2.98±0.35[a]	0.41±0.03[c]	0.19 ±0.02[c]	0.22±0.03[c]

Data presented as mean ± SE Values in the same column with the same superscripts are not significantly at ($P < 0.05$).

The aqueous extract showed strong DPPH free-radical scavenging activity (88.5% at 400ug/ml), compared with the standard reference (TBHQ) (99.73% at the same concentration).

Chemical Composition of the Essential Oil: The typical gas chromatogram (GC) profile of hydro distilled Sudanese *Coriandrum sativum* oils is shown in Fig. 1 and Table 3. Identification of volatile compounds of the hydro distilled *Coriandrum* sativum oils were carried out by comparison of their mass spectra and retention time with those of reference standard and published data. GC-MS analysis enable the identification of seventy eight compounds with Sabinene (17.63%), camphor (15.5%), cis-beta-ocimene (10.11%), trans-beta-ocimene (5.64%), alpha pinene (4.69%) and norboreneolacetate (4.09%) as the main constituents.

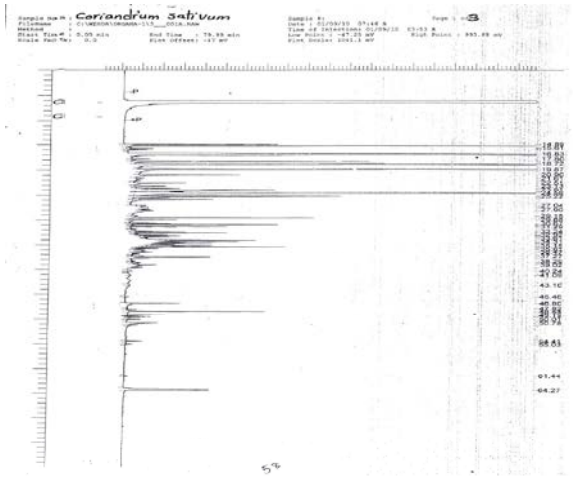

Fig. 1: Gas Chromatogram of volatiles in the hydrodistilled oil of Sudanese Coriandrum sativum fruits analyzed by GC-MS.

Biochemical Results: Table 4 showed the effect of administration of paracetamol and the aqueous extract of Sudanese *Coriandrum sativum* fruits on some liver enzymes. The results indicated that rats which administrated only paracetamol (group 3) recorded a significant increase in plasma AST, ALT, ALP and GGT (85±5.48, 39.57±2.64, 240.19±8.49 and 34.19±1.15) compared to normal control rats (group 1) (61.57±4.28, 16.43±1.54, 169.09±7.54 and 23.20±1.63), respectively. ANOVA analysis indicated that rats supplemented the aqueous extract then administrated with paracetamol (group 4) showed a significant decrease in plasma AST, ALT, ALP and GGT (71.86±3.63, 30±2.38, 221.36±13.09 and 29.94±1.56), respectively compared to the group of paracetamol intoxicated rats (group 3). Table 5 showed the effect of administration of paracetamol and the aqueous extract of Sudanese *Coriandrum sativum* fruits on some liver bio-indicators, total proteins, albumins and bilirubin group. A significant decrease in plasma total proteins and albumin was noticed in paracetamol rats (5.97± 0.18 and 2.88±0.19) compared with control group (6.74±0.39 and 3.15±0.21). A significant increase in plasma total bilirubin, direct bilirubin and indirect bilirubin were obtained in paracetamol rats (0.50±0.02, 0.24±0.02 and 0.26±0.02) compared with control group (0.34±0.03, 0.14±0.01 and 0.19±0.02), respectively. ANOVA analysis indicated that rats which supplemented the aqueous extract then the

Table 6: Effect of administration of paracetamol and the aqueous extract of Sudanese *Coriandrum sativum* fruits on kidneys function

	Parameters	
Groups	Plasma urea (mg/dl)	Plasma creatinine (mg/dl)
Control	37.78 ± 1.59[a]	0.71±0.04[a]
Extract	36.17 ± 2.17[a]	0.61±0.04[b]
Paracetamol	42.69±1.08[b]	0.76 ±0.03[c]
Paracetamol + extract	37.01±2.51[a]	0.67±0.04[b]

Data presented as mean ± SE Values in the same column with the same superscripts are not significantly at ($P < 0.05$).

Table 7: Effect of administration of paracetamol and the aqueous extract of Sudanese *Coriandrum sativum* fruits on some antioxidant biomarkers

	Antioxidant Biomarkers		
Groups	Plasma TAC (mM/L)	Plasma CAT (U/ml)	GPx in RBCs (U/ml)
Control	1.15 ± 0.13[a]	386.17 ± 28.02[a]	0.16 ± 0.03[a]
Extract	1.79 ± 0.19[b]	412.86 ± 50.00[a]	0.25±0.07[b]
Paracetamol	0.92 ± 0.06[c]	198.91 ± 19.54[c]	0.12 ± 0.02[c]
Paracetamol + Extract	1.12 ± 0.15[a]	289.43 ± 41.89[d]	0.16 ± 0.03[a]

Data presented as mean ± SE Values in the same column with the same superscripts are not significantly at ($P < 0.05$).

paracetamol showed a significant increase in total proteins and albumin with a significant decrease in bilirubin, which become near to normal control. Results of liver function tests, as shown in Tables 4 and 5, revealed that supplementation of the aqueous extract to rats (group 2) had no significant effect on all the tested parameters included in liver function tests (AST, ALT, ALP, GGT, total proteins, albumin, total bilirubins, direct bilirubin, indirect bilirubin) compared to control rats. Table 6 showed the results of kidneys function tests, rats which were supplemented with aqueous extract (group 2) recorded a significant decrease in plasma creatinine with non significant change in plasma urea level (36.17±2.17, 0.61±0.04) compared to control (37.78±1.59 and 0.67±0.04). A significant increase in plasma urea and creatinine occurred in group of paracetamol intoxicated rats (42.69±1.08 and 0.76±0.03) compared to control group, with a significant decrease occurred in rats protected with *Coriandrum sativum* (37.01±2.51 and 0.67±0.04) (group 4). The effect of aqueous extract of Sudanese *Coriandrum sativum* fruits and the administration of paracetamol on some antioxidant biomarkers (plasma total antioxidant capacity (TAC), plasma catalase activity (CAT) and cellular glutathione peroxidase activity (Gpx)) are shown in Table 7. ANOVA analysis indicated that rats supplemented with aqueous extract (group 2) recorded a significant increase in TAC and GPx with non significant change in CAT activity (1.79 ± 0.19 mM/L, 412.86±50.00U/ml and 0.25±0.07U/ml) compared to control (1.15±0.13mM/L, 0.16±0.03U/ml and 386.17±28.02 U/ml), respectively. Administrated only paracetamol recorded a significant decrease in TAC (0.92 ± 0.06), CAT (198.91 ± 19.54) and Gpx (0.12 ± 0.02), compared to normal control rats. Rats supplemented with aqueous extract and then administrated with paracetamol showed a significant increase in TAC (1.12±0.15), CAT (289.43± 41.89) and GPx (0.16±0.03) levels compared with paracetamol toxicated rats. On the other hand, compared to normal control, the values of aqueous extract supplemented group return back near to control values in all previous parameters.

Histological Results: The hepatoprotective effect of *Coriandrum sativum* was confirmed by histopathological examination of the liver tissue of control and treated animals. Administration of paracetamol showed several apoptotic cells, focal necrosis associated with lymphocytic infiltration (Fig. 2-C). On the other hand, liver of rats treated with paracetamol showed portal tracts with dilated and congested veins. Periportal necrosis of the hepatocytes that surround the portal areas and inflammatory infiltration were also seen (Fig.2-D). Examination of liver of rats treated with *Coriandrum sativum* and then treated with paracetamol indicated normal hepatic lobules structure with exception of a little lymphocytic infiltration in the portal and periportal areas in some rats (Fig. 2-E, F).

Histochemical Results:

Total Proteins: Examination of sections of the liver of the control rats displayed the proteinic inclusions in the hepatocytes as grayish blue irregular particles of various sizes against weakly to moderately stained ground cytoplasm. The nuclear chromatin and the nucleoli are densely stained indicating their rich content of proteinic constituents (Fig. 3-A).

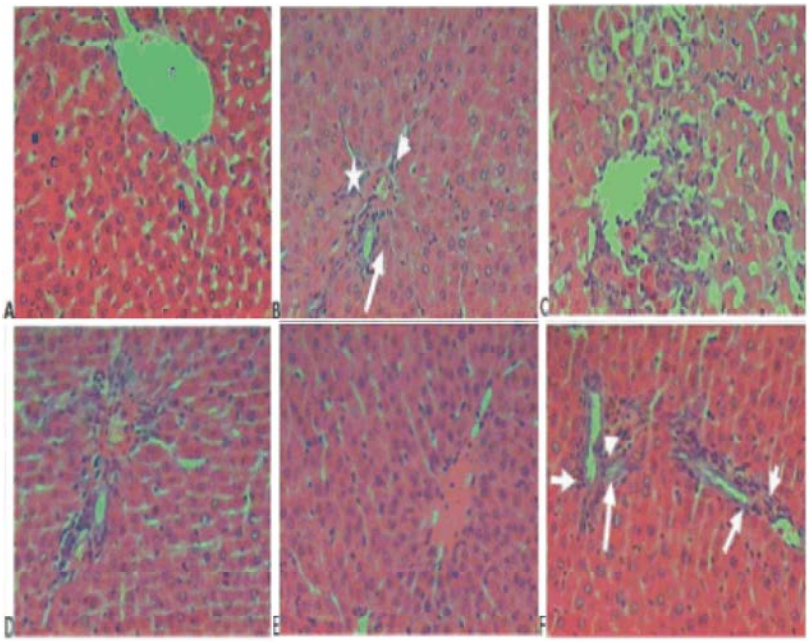

Fig. 2: Micrograph of sections of liver of A) control shows the architecture of a hepatic lobule. The central vein (CV) lies at the centre of the lobule surrounded by the hepatocytes (HC) with strongly eosinophilic granulated cytoplasm (CY) and distinct nuclei (N). Between the strands of hepatocytes the hepatic sinusoids are shown, B) control shows the portal area, portal artery (arrows), portal vein (arrowhead) and bile duct (asterisk) are noticed, C) rat treated with Panadol shows several apoptotic cells (arrows) focal necrosis (arrowheads) associated with lymphocytic infiltration (asterisk), D) rat treated with Panadol shows a portal tract with dilated and congested vein (arrow). Notice the periportal necrosis of the hepatocytes that surround the portal area (long arrow) and the inflammatory infiltration (arrowhead), E) rat treated with *Coriandrum sativum* shows normal structure of the hepatocytes and blood sinusoids, F) rat treated with *Coriandrum sativum* and Panadol shows normal structure of the hepatocytes with a little lymphocytic infiltration in the portal and periportal areas (H & E stain-X 300).

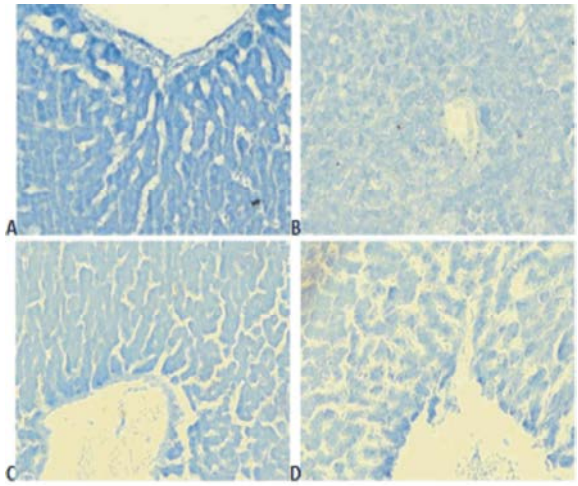

Fig. 3: Photomicrographs of sections of liver of A) control rat shows the normal proteinic contents distribution, B) rat treated with Panadol shows that inclusions are diminuted and acquire pale stainability in the hepatocytes, C) rat treated with *Coriandrum sativum* shows normal distribution of proteinic inclusions in the hepatocytes, D) rat treated with *Coriandrum sativum* and Panadol shows the stainability of the proteinic inclusions that relatively diffused in both of the cytoplasm and nucleus (Bromophenol blue reaction- X 400).

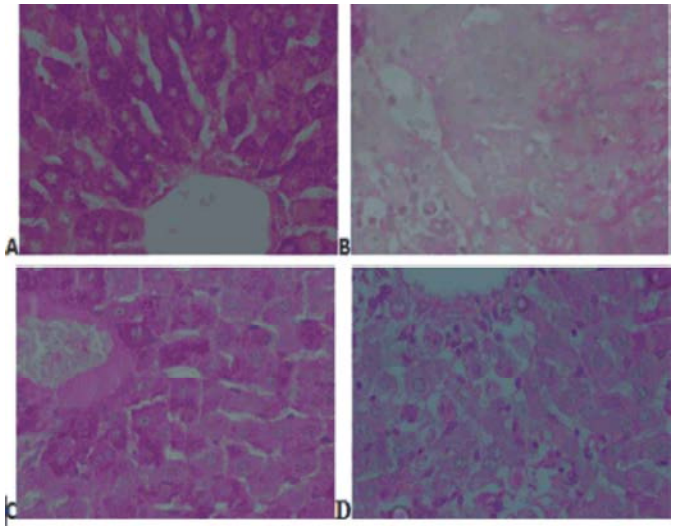

Fig. 4: Photomicrographs of sections of liver of A) control rat showing the normal abundance of glycogen in the cell of the hepatic lobule, B) rat received Panadol shows marked depletion of the polysaccharide inclusions, C) rat treated with *Coriandrum sativum* showing normal distribution of the polysaccharide inclusions in the hepatocytes, D) rat treated with *Coriandrum sativum* and Panadol shows the polysaccharides inclusions displayed diffuse stainability. A few number of the hepatocytes display dense stainability than the others (PAS/H- X 300).

Treatment with an oral dose of panadol caused reduction in the protein inclusions as compared with the control rats (Fig. 3-B). On the other hand, oral administration of *Coriandrum sativum* showed normal distribution of the protein inclusions in the hepatocytes (Fig.3-C). In some rats, oral administration of panadol plus *Coriandrum sativum* displayed diffuse stainability. A few number of the hepatocytes display dense stainability than the others (Fig.3-D).The above results revealed that panadol caused reduction in the protein materials in the liver of the treated rats and this reduction was decreased with the treatment of *Coriandrum sativum*.

Liver Polysaccharides: Examination of liver thin sections of control rat stained according to Periodic Acid Schiff`s technique (PAS) showed the abundance of polysaccharide materials (glycogen) in the hepatocytes. The nuclei of the hepatocytes give negative Periodic Acid Schiff`s reaction indicating the absence of polysaccharides (Fig. 4-A). Treatment with panadol induced faint homogeneous stainability of the polysaccharide inclusions in the hepatocytes of rats (Fig. 4-B). Oral administration *Coriandrum sativum* indicated normal distribution of the polysaccharide inclusions in the hepatocytes (Fig. 4-C). Oral administration with panadol plus *Coriandrum sativum* displayed diffuse stainability. A few number of the hepatocytes display dense stainability than the others (Fig. 4-D). The above results revealed that panadol caused reduction in the polysaccharide materials in the liver of rats. The degree of this reduction is decreased with the treatment of *Coriandrum sativum*.

DISCUSSION

The present research is a study of the phytochemical activity of aqueous extract of Sudanese *Coriandrum sativum* fruits. The plant was subjected to determination of total phenolic contents and *in vitro* antioxidant activities as well as chemical composition of the essential oils. Chemopreventive effect of aqueous extract of Sudanese *Coriandrum sativum* fruits on hepatic toxicity in male rats was also determined as well as kidney functions and some antioxidant biomarkers. The aqueous extract of Sudanese *Coriandrum sativum* fruits posses high amount of phenolic compounds (1654±3.4 mg GAE/L). It is well known that phenolic compounds play an important role as strong antioxidants. Antioxidants are a group of substances, which when present at low concentration, in relation to oxidizable substances, significantly inhibit or delay oxidative processes. As shown in Table 1, there was a high inhibiting effect for the oxidation of linoleic acid and the subsequent bleaching of β-carotene by coriander extract compared to TBHQ at the

same concentration. This may be attributed to terpene hydrocarbons and oxygenated compounds. It is well known that oxygenated-terpenes exhibited a higher antioxidant power in comparison to the other identified classes [30]. Abd El Mageed *et al.* [31] reported that coriander essential oil have a strong antioxidant effect for reduction of DPPH radical and bleaching of β-carotene. Also, Ruberto and Baratta [32] proved that monoterpenes have antioxidant activity similar to that found with the standard α-tocopherol. GC-MS study showed that essential oil of coriander contains mono-terpenes in considerable concentration (Table 3). In addition, anti-oxidative activities observed in volatile oil could be due to the synergistic effect of many compounds that may be present in the system to produce a broad spectrum of anti-oxidative activities that create an effective defense system against free radical attack [33]. The main constituents of essential oils of Sudanese *Coriandrum sativum* differed from those in the literature. Many factors affect the constituents of essential oils from different habitats. Knowledge of the factors that determine the chemical variability for each plant species is very important. These include: Physiological variations, environmental conditions, geographic variations, genetic factors and elevation and amount of plant material and species. The essential oil composition of *Coriandrum sativum* fruits obtained by hydro-distillation was studied at three stages of maturity by GC-FID and GS-MS. Essential oil yield showed marked increase during maturation process and forty one compounds were identified. Geranyl acetate (46.27%), linalool (10.96%), nerol (1.53%) and neral (1.42%) were the main compounds at the first stage of fotvoity (immature fruits). In the middle stage, linalool (76.33%), cis-dihydrocarvone (3.25%) and geranyl acetate (2.85%) were reported as the main constituent. Essential oils at the final stage of maturity (mature fruits) mainly consisted of alinalool (87.54%) and cis-dihydrocarvane (2.36%). Additionally, accumulation of monoterpene alcohols and ketons was observed during maturation process of Coriander fruit [34]. Ishikawa *et al* [35] identified 33 compounds from the water-soluble portion of the methanol extract of coriander fruit. Two photosensitizing furanocoumarins have been isolated and characterized from coriander. In a study of 15 samples of coriander for volatile contents, the oil distilled from the Polish variety of coriander (*C. sativum* var. micrcarpum) met the requirements of the British Pharmacopoeia [36]. The predominant constituent of essential oil of coriander is linalool, which forms approximately two thirds of the oil [37]. Typical compositional analysis of coriander oils as follows: alcohols:- linalool (60-80%), geraniol (1.2-4.6%), terpinen-4-ol (trace-3%), α-terpineol (<0.5%), hydrocarbons:- terpinene (0.2-8.5%), camphene (trace-1.4%), myrcene (0.2-2%), Ketones(7-9%): camphor (0.9-4.9%), esters: geranyl acetate(0.1-4.7%), linalyl acetate (02.7%), coumarins/furanocoumarins: umbelliferone, bergapten. Coriander oil was reported to contain approximately 30% terpene hydrocarbons and 70% oxygenated compounds [38]. The BACIS [39] reports the presence of 122 constituents in coriander, although the final number may be>200. The 18 main components constitute approximately 97% of the total oil. When reconstituted in the concentrations found in the natural sample, the reconstituted oil did not give the odor impression of coriander oil [40]. Hence, a major sensory effect of the oil apparently comes from the remaining trace constituents that occur, on average, in concentrations of about 0.01% or less. Although, mono and polyunsaturated fatty acids are minor constituents of the oil, they contribute to the characteristic aroma of the oil [41]. Coriander oil may have future use as a free radical scavenger, preventing oxidative deterioration in foods. Coriander oil was shown to have greater activity against the radical generating activity of 1, 1-diphenyl-2-picrylhydrazyl in several oils. The order of effectiveness among various oils in inhibiting free radicals was coriander> black cumin>cottonseed> peanut> sunflower> walnut> hemp seed> linseed> olive> niger see [42]. Hepatotoxicity of paracetamol has been attributed to the formation of toxic metabolites, when a part of paracetamol activated by hepatic cytochrome P-450 a highly reactive metabolite; N-acetyl-p-benzen-qunone imine is generated. Generally, this metabolites disactivates antioxidant enzymes such as catalase, glutathione peroxidase and superoxide dismutase [43]. N-acetyl-p-benzoquinone imine (NAPQI) is normally detoxified by conjugation with reduced glutathione to form mercapturic acid which is excreted in urine. Toxic over dose of paracetamol depletes hepatic reduced glutathione content so that free NAPQI binds covalently to cellular macromolecules causing acute hepatocellular necrosis. The NAPQI then cause acylation or oxidation of cytosolic membrane protein and generation of reactive oxygen which leads to further oxidation of protein thiols and lipid peroxidation, DNA fragmentation and ultimately cell necrosis [44]. Damage to the liver is not due to the drug itself but to a toxic metabolite NAPQI, which is produced by cytochrome P450 enzymes in the

liver [45]. In normal circumstances this metabolite is detoxified by conjugating with glutathione in phase 2 reaction. In overdose large amount of NAPQI is generated which overwhelm the detoxification process and lead to damage to liver cells. Paracetamol hepatotoxicity was reflected in an increase ($P<0.05$) in serum ALT, AST, ALP, GGT activity and significant decrease ($P<0.05$) in total proteins and albumin level. CO-administration of the aqueous extract of Sudanese *Coriandrum sativum* fruits (30 mg/ml water) decreased the elevated serum enzyme activities and increased total protein and albumin. Bilirubin is one of the most useful clinical markers to diagnose the severity of necrosis, is a measure of binding conjugation and excretory capacity of hepatocytes. The level of serum bilirubin was significantly increased in paracetamol treated group and returns back near to normal level in aqueous extract treated rats group. Antioxidants constituents of the plant material act as radical scavengers and help in converting the radicals to less reactive species. The liver breaks down or modifies toxic substances and most medicinal products in a process called drug metabolism. This sometimes results in toxication, when the metabolite is more toxic than its precursor. Excess consumption of certain toxic chemicals such as antibiotics, chemotherapeutic, peroxidised oils, acetaminophen, aflatoxin, carbon tetrachloride, chlorinated hydrocarbon, alcohol lead to infection and autoimmune disorder. Biochemical marker (i.e. Alanine transferase (ALT), alkaline phosphatase (ALP) and bilirubin) are used to indicate liver damage. Liver injury is defined as rise in either ALT levels more than three times of upper limit of normal, ALP levels more than twice or total bilirubin levels more than twice. The hepatoprotective effect of *Coriandrum sativum* was confirmed by histopathological examination of the liver tissue of control and treated animals. In the liver section of the rats intoxicated with paracetamol (Fig.3), there was disarrangement and degeneration of normal hepatic cells with intense centrilobular necrosis. The liver section of rats treated with aqueous extract of coriander and intoxicated with paracetamol showed moderate hepatoprotective activity (Fig. 4.). The above results revealed that panadol caused reduction in the polysaccharide and protein materials in the liver of the treated rats. The degree of this reduction is decreased with the treatment of *Coriandrum sativum.* Histopathological examination of liver of rats treated with *Coriandrum sativum* showed normal structure of the hepatocytes and blood sinusoids. Drugs or toxins that have a pharmacological hepatotoxicity are those that have predictable dose-response curves (higher concentrations cause more liver damage) and well characterized mechanisms of toxicity such as directly damaging liver tissue or blocking a metabolic process. As in the case of acetaminophen overdose, this type of injury occurs shortly after some threshold for toxicity is reached [46]. The biochemical and histological results pointed to the safety effect of aqueous extract of Sudanese *Coriandrum sativum* fruits and non toxic effect as was evident from non significant change in all liver function tests. In addition *Coriandrum sativum* extract increased some antioxidant biomarkers (TAC and GPx), where extract posses high amount of phenolic compounds, this pointed to the potential antioxidant activity of extract, which strongly contributes to its significant hepatoprotective activity.

CONCLUSION

From chemical, biochemical and histological studies, our results showed that aqueous extract of Sudanese *Coriandrum sativum* fruits posses antioxidant activity and hepato-protective effects towards Paracetamol induced hepato-toxicity in rats. Coriander may have future use as a free radical scavenger, preventing oxidative stress and prevent many diseases.

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