

CHEMICAL CONSTITUENTS AND HEPATOPROTECTIVE EFFECT OF *CASSIA OCCIDENTALIS* L. CULTIVATED IN EGYPT

HANAA M. SAYED¹; MAHMOUD A. RAMADAN¹; MANAL M. SAYED²; SARY KH. ABD ELGHAFAR³ and HEBA H. SALEM¹

¹ Department of Pharmacognosy, Faculty of Pharmacy, Assiut University, Assiut 71526, Egypt.

² Department of Chemistry, Animal Health Research Institute, Assiut Lab., Egypt.

³ Department of pathology and Clinical Pathology, Faculty of Vet. Med. Assiut University, Assiut 71526, Egypt.

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ABSTRACT

This study was designed to investigate the hepatoprotective effects of the different successive extractives of the aerial parts of *Cassia occidentalis* L., on carbon tetrachloride (CCL₄) induced hepatotoxicity in adult Wistar rats. Sixty-six male albino rats were divided into eleven groups. First group received Tween only and served as control group. The second group injected with CCL₄ (1:1) in olive oil, at a dose of 2 ml/kg B.W. The third group injected with CCL₄ in the same dose and Silymarin (25 mg /kg) orally for 7 days and served as reference group. Groups from four to eleven served as test groups injected with CCL₄ in the same previous dose in addition to oral daily administration by one of either 200 or 400 mg /kg B.W. from each of the four extractives of *Cassia occidentalis* (total methanolic, chloroform, ethyl acetate and n- butanol extracts). Treatment started one day after the injection of CCL₄ and extended for two weeks. Parameters for liver function tests: Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT) and Bilirubinas as well as kidney function tests: Serum urea and Creatinine were determined spectrophotometrically. The different extractives of *C. occidentalis* L. showed improvement in both liver and kidney function indices, while ethyl acetate extractive either in 200 or 400 mg/kg B.W., showed the best results. Histopathology of liver tissue specimens supported these results. The biologically guided fractionation of *C. occidentalis* L. methanolic extract, revealed that the ethyl acetate fraction exhibited a significant dose dependant protective effect on both liver and kidney. The ethyl acetate fraction revealed activity approached that of silymarin (a known hepatoprotective agent). Chromatographic fractionation of the ethyl acetate fraction afford 6 purified compounds, identified on the basis of chemical and spectroscopic analysis as: naringenin (1), quercetin (2), 1,8-dihydroxy anthraquinone (3), 1,3,8-trihydroxy anthraquinone (4), chrysoeriol-7-O-rutinoside (5) and rutin (6). The isolated phenolics probably account for the hepatoprotective effect of the extract.

Key words: Chemical Constituents, Hepatoprotective, *Cassia Occidentalis*, Liver and kidney Function tests, rats.

INTRODUCTION

Liver diseases are considered a global problem especially in developing countries including Egypt. Hepatitis, viral infection, food additives, alcohol, toxic industrial chemical as well as air and water pollutants are the major risk factor of liver toxicity (Khan *et al.*, 2012). Treatment of liver diseases via synthetic medication is usually disappointing due to its sever undesirable effect upon prolonged administration. Therefore, herbal products gain more interest in this issue and most recent studies aim to characterize the health promoting properties of many plants, especially those rich in phenolics that known

to possess hepatoprotective and anti-inflammatory potencies.

Cassia is a genus of 40 species belonging to family Fabaceae, subfamily Caesalpinoideae (Tackhol, 1974). *C. occidentalis* L. (Coffee senra, Negro Coffee) as an Indian perennial diffuse under shrub, is used in several traditional medicine to cure various disease (Khare, 2007). This plant possess antibacterial, anthelmintic (Shao *et al.*, 2013), antifungal, antiviral (Cong *et al.*, 2014), antispasmodic, analgesic, antipyretic (Nakamura *et al.*, 2014), antiplatelet aggregation, antihistamine release, hepatoprotective and neuroprotective (Kim *et al.*, 2007 and Sung *et al.*, 2004). Phytochemical studies of *C. occidentalis* revealed the presence of diversity of constituents including tannins, saponins,

Corresponding author: Dr. SARY KH. ABD ELGHAFAR

E-mail address: sary64@windowslive.com

Present address: Department of pathology and Clinical Pathology, Faculty of Vet. Med. Assiut University, Assiut 71526, Egypt.

flavonoids, terpenes and anthraquinones (Bukhari *et al.*, 2014 and Yadava *et al.*, 2012).

Carbon tetrachloride (CCl₄) is one of the chemicals which cause liver damage through lipid peroxidation and oxidative stress (Moreira *et al.*, 2014). CCl₄ is a suitable chemical to induce oxidative liver toxicity in experimental animal model and its toxic effects was extensively studied by several investigators (Al-Dbass *et al.*, 2012 and Azeem *et al.*, 2010). In recent literatures it is documented that natural drugs with antioxidant potential can protect the liver from damage caused by CCl₄ (Andritoiu *et al.*, 2014; Shaker *et al.*, 2010 and Sanmugapriya and Vankataraman 2006).

The current study was planned to evaluate the hepato and kidney protective activities of the fractionated methanolic extract of the aerial part of *C. occidentalis* L. as well as, to isolate and identify the components of the potent extractive.

MATERIALS AND METHODS

1- Plant Material:

Aerial parts of *C. occidentalis* L. were collected in June 2013 from plants cultivated in the Experimental Station of Medicinal Plants, Faculty of Pharmacy, Assiut University. The plant was kindly authenticated by Prof. Dr. A.A. Fayed, Professor of Plant Taxonomy, Faculty of Science, Assiut University. Voucher specimen of the plant material was deposited at the Herbarium of Pharmacognosy Department, Faculty of Pharmacy, Assiut University.

2- Plant Extraction:

Air dried powdered aerial parts of *C. occidentalis* L. (3 kg) was extracted by percolation with 80% methanol (4x6 L). The resulting methanolic extract was evaporated under vacuum, at a temperature not exceeding 40°C. The concentrate (300 gm) was suspended in distilled water (600 ml), transferred to a separating funnel and subjected to successive several solvent fractionation using *n*-hexane, chloroform (CHCl₃), ethyl acetate (EtOAc) and *n*-butanol. The different extractives were concentrated separately under reduced pressure to give 65, 35, 25 and 8 gm, respectively. The remaining aqueous extract was kept for further study (Harborne, 1973).

3- Instruments and Chemicals:

Nuclear magnetic resonance (NMR) spectra were recorded on Bruker Spectrophotometer at 400 MHz for ¹H and 100 MHz for ¹³C NMR. Digital VIS/Ultraviolet Spectrophotometer was recorded on Cecil, Cambridge, England. Olympus microscope CX31, Japan and Camera (Olympus, Camedia, C-5060, Japan) were also used.

All chemicals and solvents were of analytical grade. Silica gel G₆₀ (Prolabo, India) for column chromatography, precoated silver gel plates G₆₀ F₂₅₄ (Aluminum sheets, E-Merck, Germany), for thin layer chromatography (TLC). The biochemical kits were purchased from Bio-diagnostics, Cairo, Egypt.

4- Experimental Animals:

Sixty-six male Wistar albino rats (120-150 g) were obtained from the Animal House, Faculty of Medicine, Assiut University. All animals were acclimatized to the laboratory conditions, kept in controlled environment of air and temperature for 15 days before starting the experiments with access of water and diet *ad Libitum*.

5- Acute Toxicity Study of Plant Extract:

Three groups of rats (8 rats, each) were examined for the acute toxicity of the plant extract. One oral dose of *C. occidentalis* methanolic extract at concentrations of 200, 400 and 800 mg/kg B.W. were administered to rats. The mortality rate was calculated 24 hours after administration. The total methanolic extract of *C. occidentalis* aerial parts did not show any signs of toxicity and mortality up to 4 g/kg B.W. according to Lorke (1983).

6- Experimental Design:

C. occidentalis different extractives (5 g, each) were suspended in Tween in distilled water at the appropriate concentration prior to oral treatment. The administration regimen was started one day after the injection of CCl₄ and extended for two weeks. Sixty-six male Wistar albino rats were divided into 11 groups (6 animals, each).

Group 1: (Control group) received Tween only.

Group 2: (CCl₄ group) injected (I.P.) with one dose of CCl₄ in olive oil (1:1) 2 mg/kg B.W. according to Lutz *et al.*, (2003).

Group 3: (Silymarin reference group) injected (I.P.) with CCl₄ in the same dose and followed by Silymarin (25 mg/kg b.wt.) orally for 7 days according to Mohamed (2013).

Groups 4-11: Served as testing groups and injected with CCl₄ in the same previous dose and followed by oral daily administration by one of either 200 or 400 mg/kg B.w. from each of the four extractives. The treatment started one day after the injection of CCl₄ and extended for 2 weeks at the same time for the same duration.

7- Serum Sample Preparation:

Blood was collected from the retro-orbital plexus into clean free test tubes. Serum was prepared after

centrifugation of the blood samples at 3000 r.p.m for 10 minutes and stored at -20°C for further estimation of liver and kidney function tests.

8- Biochemical Estimations:

Serum AST, ALT and bilirubin as well as serum urea and creatinine were measured by using the appropriate test kits (Bio- diagnostics, Cairo, Egypt) spectrophotometrically. This was done according to the standard protocol of the suppliers.

9- Histopathological Examination:

Tissue specimens from liver were fixed in 10% neutral buffered formalin, dehydrated in a graded ethyl alcohol series, cleared with methyl benzoate and embedded in paraffin wax. Sections of $5\ \mu\text{m}$ were cut and stained with haematoxylin and eosin (Bancroft *et al.*, 1996). Stained sections were examined under light microscope and photographed using digital camera.

10- Statistical Analysis:

Data are expressed as mean \pm SE for all parameters. The data were analyzed using Graph Pad Prism Data Analysis program software package (Graph Pad Software, Inc., San Diego CA, USA) for multiple comparisons, one-way analysis of variance (ONE-WAY-ANNOVA) test was used.

11- Isolation of Ethyl Acetate Extractive Contents:

Fractionation of the ethyl acetate extractive (10 g) was done on silica gel column (150x5 cm, 400 g) and eluted successively with CH_2Cl_2 -methanol gradient to give several fractions screened by TLC and similar fractions were combined to afford four fractions (I-IV) (2, 1.5, 3 and 2.5 g, respectively). Fraction I (2 g) was rechromatographed on silica gel column (100x2 cm/80 g) with gradient elution using CH_2Cl_2 -MeOH in 2% increments to yield compounds (1) and (2). Fraction II (1.5 g) was rechromatographed on silica gel column (100x2 cm, 60 g) with gradient elution using CH_2Cl_2 -MeOH in 1% increments to yield compounds (3) and (4). Fraction III (3 g) were rechromatographed on silica gel column (100x2, 120 g), with gradient elution using CH_2Cl_2 -MeOH in 1% increment to yield compound (5) and (6). Fraction IV showed mixture of minor spots, was kept for further investigation.

RESULTS

I- Biochemical Study:

Analysis of serum parameters of liver function tests (Table 1) demonstrated a decrease in serum concentration of ALT, AST and total bilirubin in groups which received CCl_4 and treated either with total methanolic extract or different extractives of *C. occidentalis L.* when compared with the group which received CCl_4 only.

The two groups which treated with EtoAc extractive (200 & 400 mg/ kg B.W.) showed the best improvement in liver function parameters in which there was marked significant decrease in ALT, AST and total bilirubin concentrations. Comparing this improvement in liver function parameters with those of silymarin treated group, no significant changed could be detected. Groups which treated with 200 mg /kg B.W. of CHCl_3 and 200 & 400 mg/kg B.W. of n-butanol extractive showed non-significant decrease in ALT values.

Analysis of serum parameters of kidney function tests (Table 2) showed non-significant decrease in serum urea values in groups which received CCl_4 and treated with all extractives when compared with those of the group which received CCl_4 alone.

Groups which treated with EtoAc extractive (200 & 400 mg/kg B. W.) showed a significant decrease in urea concentration when compared with that of CCl_4 alone. In addition, no significant changed in urea concentration could be detected in comparison with that of Tween and Silymarin groups.

Concerning serum creatinine level in groups which treated with 200 & 400 mg/ kg B.W. of EtOAc extractive, significant decrease was detected on comparison with that of CCl_4 received group. In addition, no significant changes could be recognized in serum creatinine level when compared with those of Tween and Silymarin groups. Other groups which treated with total methanolic, CHCl_3 and n-butanol extractives showed non-significant decrease in serum creatinine level in comparison with CCl_4 received group.

Histopathological Examination:

Microscopic examination of the liver sections of negative control group (Tween) revealed normal histological appearance of the liver tissue with normal hepatolobular architecture contained central vein and regular hepatic cell cords (Fig. 1A), while that from reference group (Silymarin plus CCl_4), showed mild vacuolar degeneration of the hepatocytes (Fig. 1B). In CCl_4 treated group the hepatocytes showed marked fatty degeneration with appearance of small and large cytoplasmic vacuoles (Fig. 1C). In group received CCl_4 along with 200 mg total methanolic extract, the liver cells showed moderate fatty degeneration (Fig. 1D), while in group treated with 400 mg total methanolic extract, the hepatocytes surrounding the central vein showed minute foci of necrosis (Fig. 2A). In the group treated with 200 mg and 400 mg CHCl_3 extractive, the hepatocytes showed very mild vacuolar degeneration (Fig. 2B&C). Liver examination of group treated with 200 mg EtOAc extractive showed normal hepatocytes with vesicular

nuclei and acidophilic cytoplasm (Fig. 2D), while in 400 mg EtOAc extractive treated group the liver showed congestion of the central vein with very mild vacuolar degeneration of the hepatocytes (Fig. 3A). In *n*-butanol extractive 200 mg treated group, the liver showed marked vacuolar degeneration of the hepatocytes and periaciner necrobiosis (Fig. 2B), while in group received 400 mg *n*-butanol extractive, the liver showed congestion of the central vein with marked vacuolar degeneration of the hepatocytes (Fig. 3C).

Isolated Compounds:

Fractionation of the ethyl acetate extractive of the methanolic extract of *C. occidentalis* L. that showed significant hepatoprotective effect resulted in isolation of 6 phenolic compounds (Fig. 4). All of them were identified basically on chromatographic, chemical and spectroscopic analysis as well as through comparison with published data.

Compound (1) was obtained as yellow amorphous powder (20 mg, MeOH), attained deep purple colour under UV and a yellow to orange colour with AlCl₃ spray. UV λ_{\max} nm: MeOH (287, 326), +NaOMe (245, 323), +NaOAc (284, 323), +NaOAc/H₃BO₃ (290, 390), +AlCl₃ (312, 382), +AlCl₃/HCl (305, 372). ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm: 5.42 (1H, dd, J= 14.4, 2.4, H-2), 3.25 (1H, dd, J= 12.8, 3.2, H-3a), 2.70 (1H, dd, J= 12.8, 3.2, H-3b), 5.86 (2H, s, H-6,8), 6.79 (2H, d, J= 8.4, H-3',5'), 7.31 (2H, d, J= 8.4, H-2',6'), 12.14 (1H, s, 5-OH). ¹³C NMR (100 MHz, DMSO-*d*₆) δ ppm: C-2 (78.88), C-3 (42.41), C-4 (196.81), C-5 (163.39), C-6 (96.28), C-7 (163.94), C-8 (95.46), C-9 (158.15), C-10 (102.19), C-1' (129.32), C-2',6' (128.80), C-3',5' (115.63), C-4' (167.22). The above mentioned data is in agreement with that reported for naringenin (Mabry *et al.*, 1970 and Agrawal (1989). This is the first report for its isolation from the studied plant.

Compound (2) was obtained as yellow amorphous powders (20 mg, MeOH), attained yellow colour under UV and intensified after AlCl₃ spray. UV λ_{\max} nm: MeOH (255, 370), +NaOMe (275, 425), +NaOAc (266, 390), +NaOAc/H₃BO₃ (259, 385), +AlCl₃ (272, 425), +AlCl₃/HCl (261, 423) nm. ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm: 6.18 (1H, d, J= 2, H₆), 6.4 (1H, d, J= 2, H₈), 6.88 (1H, d, J= 8.4, H₅), 7.53 (1H, d, J= 8.4, H₆), 7.67 (1H, d, J= 2, H₂). The above mentioned data is consistent with the literature data of quercetin (Mabry *et al.*, 1970 and Agrawal 1989). It was previously isolated from the plant.

Compound (3) was obtained as yellowish orange (10 mg, MeOH). UV λ_{\max} nm: MeOH (225, 277, 429). ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm: 7.83 (2H, t, J= 8, H-3,-6), 7.73 (2H, d, J= 8.4, H-4,5), 7.40 (2H, d, J=

8.4, H-2,7), 11.94 (s, OH). ¹³C NMR (100 MHz, DMSO-*d*₆) δ ppm: C-1,8 (161.83), C-2,7 (124.90), C-3,6 (137.93), C-4,5 (119.78), C-8a,9a (133.81), C-4a,5a (116.47), C-9 (191.62), C-10 (181.37). The above mentioned data is in agreement with that previously reported for 1,8-dihydroxy anthraquinone, which was previously isolated from the roots of the plant. (Chukwujekwu *et al.*, 2006).

Compound (4) was obtained as yellowish orange (10 mg, MeOH). ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm: 7.77 (1H, s, H₂), 8.13 (1H, s, H₄), 7.75 (1H, d, J= 7.6, H₃), 7.83 (1H, t, J= 7.6, H₆), 7.41 (1H, d, J= 8, H₇), 11.98 (OH). ¹³C NMR (100 MHz, DMSO-*d*₆) δ ppm: C₁-1 (161.5), C-2 (124.64), C-3 (165.92), C-4 (119.22), C-4a (134.24), C-5 (119.22), C-6 (138.13), C-7 (129.15), C-8 (161.83), C-8a (116.33), C-9 (191.62), C-9a (118.53), C-10 (181.37), C-10a (133.14). The above mentioned data is consistent with the literature data of 1,3,8-trihydroxy anthraquinone (Rogerio *et al.*, 2008). It is the first report for its isolation from the plant.

Compound (5) was obtained as yellow amorphous powder (15 mg, MeOH) attained deep purple colour under UV and a yellow colour with AlCl₃ spray. UV λ_{\max} nm: MeOH (267, 342), +NaOMe (270, 391), +NaOAc (269, 346), +NaOAc/H₃BO₃ (269, 350), +AlCl₃ (273, 347), +AlCl₃/HCl (275, 349, 290). ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm: 6.46 (1H, s, H-6), 6.76 (1H, s, H-3), 6.82 (1H, s, H-8), 7.13 (1H, d, J= 8.4, H-5'), 7.44 (1H, s, H-2'), 7.57 (1H, d, J= 8.4, H-6'), 5.08 (1H, d, J= 7.2, H-1') 4.53 (1H, d, J= 1.2, H-1'''), 3.87 (3H, s, OCH₃), 3.17-5.45 (m, sugar protons), 1.07 (3H, d, J= 6, H-6'''), 12.93 (1H, s, 5-OH). ¹³C NMR (100 MHz, DMSO-*d*₆) δ ppm: C-2 (163.41), C-3 (104.29), C-4 (182.42), C-5 (161.67), C-6 (100.05), C-7 (164.66), C-8 (95.27), C-9 (157.42), C-10 (105.91), C-1' (123.34), C-2' (112.71), C-3' (151.78), C-4' (147.23), C-5' (119.42), C-6' (123.34), C-1'' (100.37), C-2'' (73.56), C-3'' (76.06), C-4'' (70.03), C-5'' (76.72), C-6'' (66.50), C-1''' (100.99), C-2''' (71.20), C-4''' (72.51), C-5''' (68.80), C-6''' (18.27). On the basis of the above mentioned data, compound (5) was elucidated as chrysoeriol-7-O-rutinoside (Agrawal, 1989). It is the first report for its isolation from the plant.

Compound (6) was obtained as yellow amorphous powder (24 mg, MeOH) attained deep purple colour under UV and a yellow colour with AlCl₃ spray. UV λ_{\max} nm: MeOH (253, 357), +NaOMe (272, 400), +NaOAc (272, 380), +NaOAc/H₃BO₃ (260, 375), +AlCl₃ (275, 433), +AlCl₃/HCl (273, 404). ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm: 6.19 (1H, d, J= 2, H₆), 6.38 (1H, d, J= 2, H₈), 6.84 (1H, d, J= 8.4, H₅), 7.54 (1H, d, J= 8.4, H₆), 7.55 (1H, d, J= 2, H₂), 5.34 (1H, d, J= 7.2, H_{1''}), 3.05-5.33 (m, sugar protons), 4.39 (1H, d, J= 0.5, H_{1'''}), 0.99 (3H, d, J= 6.3, H_{6'''}), 12.60

(1H, s, 5-OH). ^{13}C NMR (100 MHz, DMSO- d_6) δ ppm: C-2 (156.88), C-3 (133.66), C-4 (177.78), C-5 (161.58), C-6 (99.16), C-7 (164.52), C-8 (94.11), C-9 (157.13), C-10 (104.37), C-1' (121.58), C-2' (115.65), C-3' (145.15), C-4' (148.82), C-5' (116.64), C-6' (122.09), C-1'' (101.54), C-2'' (74.49), C-3'' (76.24), C-4'' (70.40), C-5'' (75.79), C-6'' (67.41), C-1''' (101.13), C-2''' (70.77), C-3''' (70.95), C-4''' (72.25),

C-5''' (68.66), C-6''' (18.07). On the basis of the above mentioned evidence, compound (6) was elucidated as quercetin-3-O- α L-rhamnopyranosyl-(1-6)- β -D-glucopyranoside (rutin) (Mabry *et al.* and 1970, Agrawal, 1989). It is the first report for its isolation from the plant.

Table 1: Serum ALT (U/L), AST (U/L) and total bilirubin (mg/dL) values in different experimental groups.

Parameters	Groups			Total	Total	CHCl ₃	CHCl ₃	EtOAc	EtOAc	<i>n</i> -butanol	<i>n</i> -butanol
	Tween	Silymarin	CCl ₃	200	400	200	400	200	400	200	400
ALT	18.78	22.23	36.24	28.35	28.75	31.18	28.80	26.75	24.83	33.69	35.41
Mean \pm SE	$\pm 1.06^a$	$\pm 1.29^b$	$\pm 2.02^c$	$\pm 1.30^b$	$\pm 1.66^b$	$\pm 1.85^c$	$\pm 1.55^b$	$\pm 0.89^b$	$\pm 2.55^b$	$\pm 1.43^c$	$\pm 2.41^c$
AST	44.61	51.53	78.33	59.42	58.68	57.28	54.21	56.95	55.94	59.69	59.05
Mean \pm SE	$\pm 1.46^a$	$\pm 1.89^b$	$\pm 2.30^c$	$\pm 1.62^b$	$\pm 1.15^b$	$\pm 1.16^b$	$\pm 0.70^b$	$\pm 0.52^b$	$\pm 1.81^b$	$\pm 1.17^b$	$\pm 0.96^b$
Total bilirubin	0.52	0.57	0.88	0.76	0.78	0.82	0.85	0.68	0.60	0.78	0.76
Mean \pm SE	$\pm 0.10^a$	$\pm 0.15^a$	$\pm 0.15^b$	$\pm 0.33^c$	$\pm 0.13^c$	$\pm 0.19^c$	$\pm 0.09^c$	$\pm 0.12^a$	$\pm 0.23^a$	$\pm 0.14^c$	$\pm 0.12^c$

Notes: Values are means bearing different superscripts within same raw were significantly different ($P \leq 0.001$).

Table 2: Serum urea (mg/dL) and creatinine (mg/dL) values in different experimental groups.

Parameters	Groups			Total	Total	CHCl ₃	CHCl ₃	EtOAc	EtOAc	<i>n</i> -butanol	<i>n</i> -butanol
	Tween	Silymarin	CCl ₃	200	400	200	400	200	400	200	400
Urea	20.78	29.3	55.9	50.35	40.75	38.8	34.08	29.05	27.03	46.60	43.01
Mean \pm SE	$\pm 0.96^a$	$\pm 0.82^a$	$\pm 0.76^b$	$\pm 1.30^b$	$\pm 0.66^b$	$\pm 0.85^b$	$\pm 1.05^b$	$\pm 0.89^a$	$\pm 2.55^a$	$\pm 1.43^b$	$\pm 1.41^b$
Creatinine	0.40	0.38	0.58	0.48	0.42	0.46	0.42	0.39	0.36	0.45	0.49
Mean \pm SE	$\pm 0.06^a$	$\pm 0.01^a$	$\pm 0.04^c$	$\pm 0.06^c$	$\pm 0.01^c$	$\pm 0.08^c$	$\pm 0.08^c$	$\pm 0.05^a$	$\pm 0.03^a$	$\pm 0.07^c$	$\pm 0.06^c$

Notes: Values bearing different superscripts within same raw were significantly different ($P \leq 0.001$).

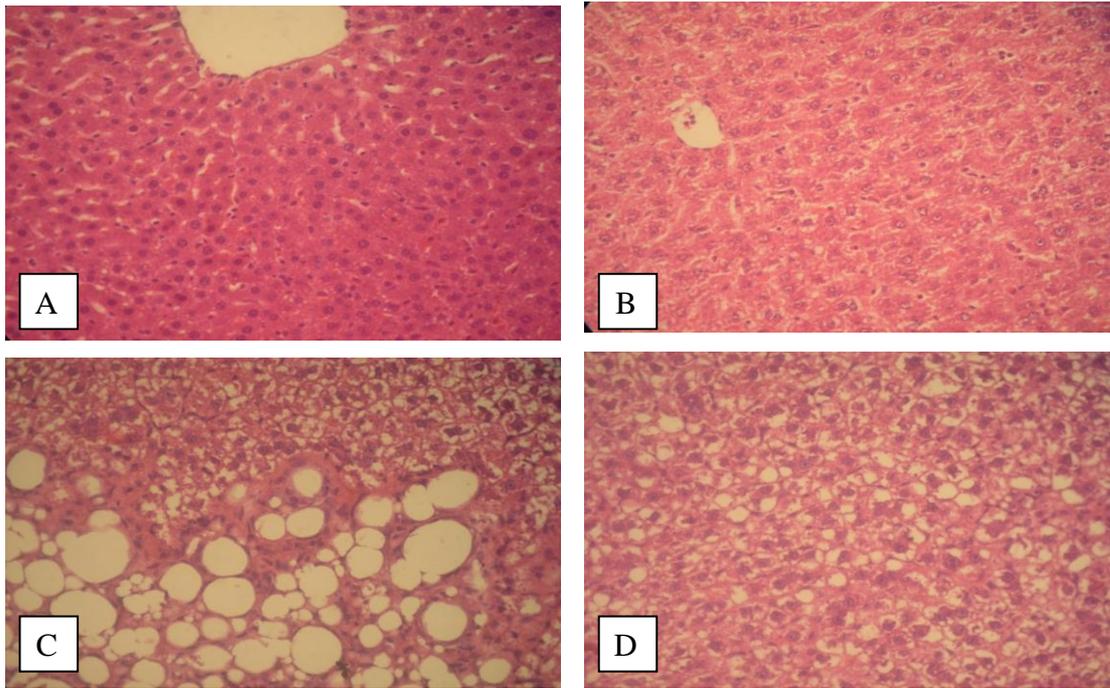


Fig.1: Photomicrograph liver section, (A) from negative control (Tween) group showing normal histological appearance, notice the central vein and hepatic cords. (B) from reference group (Silymarin) showing mild vacuolar degeneration of the hepatocytes. (C) from CCl_4 group showing marked fatty degeneration in the hepatocytes. (D) from total methanolic extract 200mg treated group showing moderate fatty degeneration of the hepatocytes.

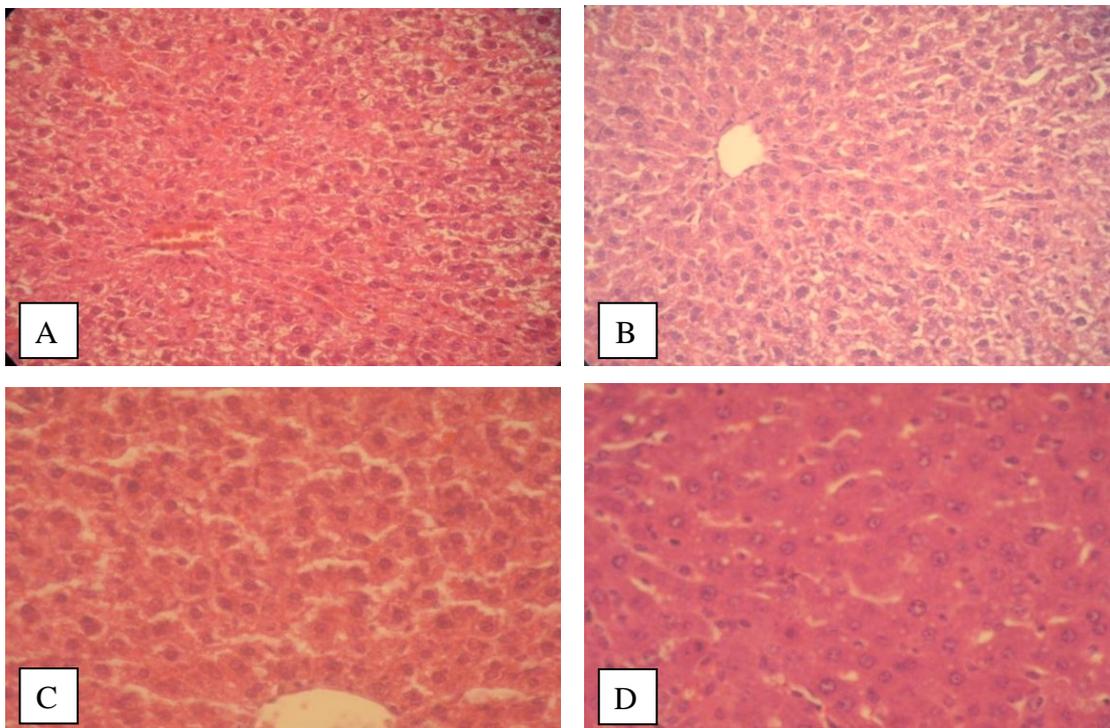


Fig.2: Photomicrograph liver section, (A) from total methanolic extract 400mg treated group showing minute foci of hepatocytes necrosis surrounding the central vein. (B) from CHCl_3 extractive 200mg treated group showing very mild vacuolar degeneration of the hepatocytes. (C) from CHCl_3 extractive 400mg showing very mild vacuolar degeneration of the hepatocytes. (D) from EtoAc extractive 200mg showing normal hepatocytes with vesicular nuclei and acidophilic cytoplasm. H&E. X 40.

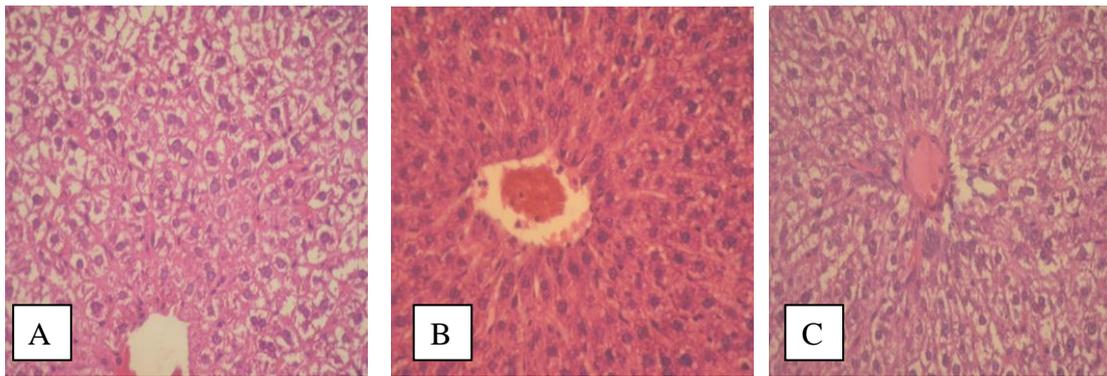
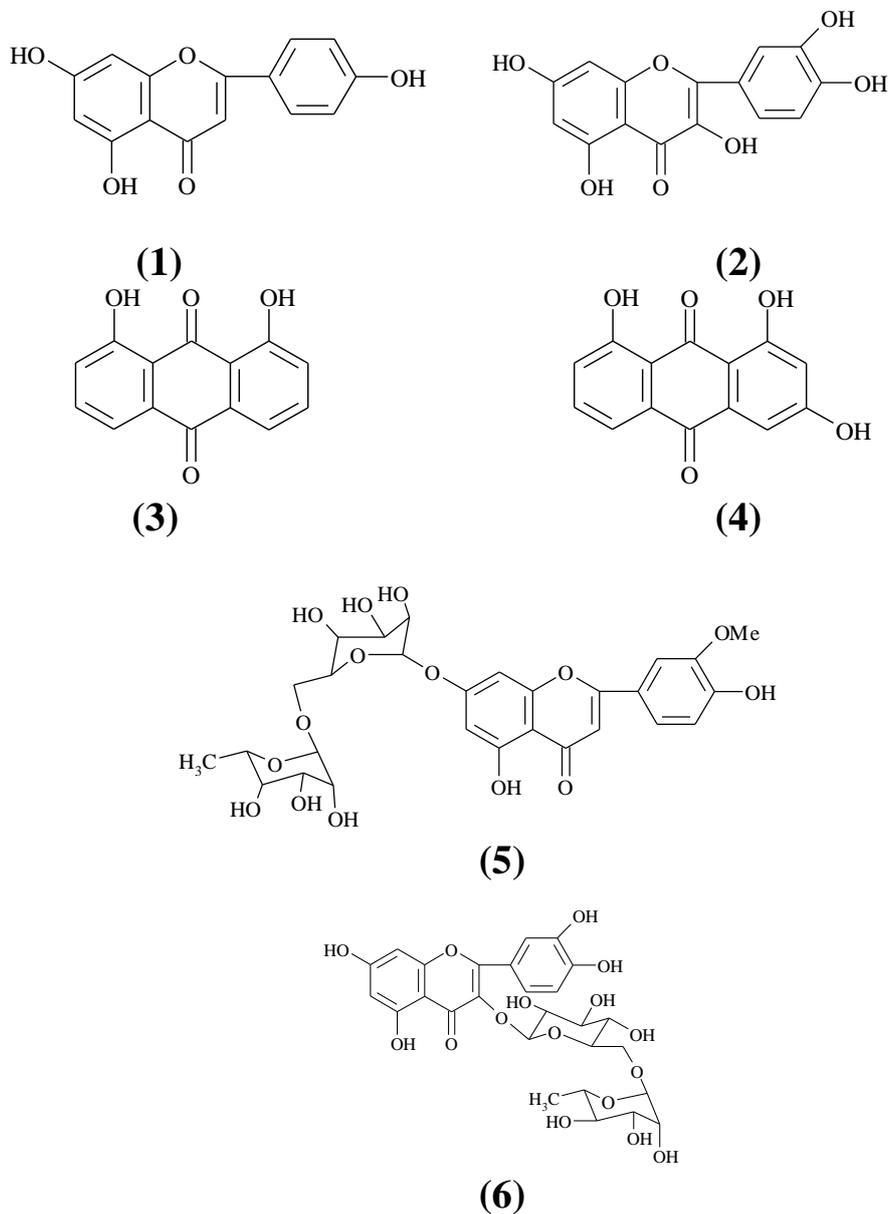


Fig.3: Photomicrograph of liver section (A) from EtoAc extractive 400mg treated group showing congestion of the central vein with very mild vacuolar degeneration of the hepatocytes. (B) from n-butanol extractive 200 mg treated group showing marked vacuolar degeneration of the hepatocytes and periaciner necrobiosis. (C) from n-butanol extractive 400mg treated group showing congestion of the central vein and marked vacuolar degeneration of the hepatocytes. H&E. X 40.

Fig. 4:



DISCUSSION

Our study revealed that treatment with ethyl acetate extractive of *Cassia occidentalis* L. either in a dose 200 or 400 mg/kg B.W. gave the best results in the liver function parameters in the form of marked significant decrease in ALT, AST and total bilirubin concentrations in comparison with CCl₄ treated group, and had no significant changes in values of ALT, AST and bilirubin in comparison with Silymarin treated group. Similarly, previous studies revealed significant results. Bhakta *et al.* (1999) studied the hepatoprotective effect of the *n*-heptan extractive of *Cassia fistula* leaves (400 mg/kg B.W.) in rats by inducing hepatotoxicity with carbon tetrachloride: liquid paraffin (1:1). He found that the extract (400 mg/kg B.W.) has been showed to possess protective effect by lowering the serum levels of (SGOT and SGPT), bilirubin and alkaline phosphatase (ALP). Jafri *et al.* (1999) also studied the effect of aqueous ethanolic extract of *Cassia occidentalis* leaves and reported an improvement in liver function parameters (AST, ALT and alkaline phosphatase) after liver damage induced by paracetamol in rats.

The histopathological examination of the liver in different treated groups demonstrated that CCl₄ induced severe pathological changes either in the form of fatty changes or necrobiosis of the hepatocytes. These changes were subsided in Silymarin and other extractive treated groups. The treatment with ethyl acetate extractive of *Cassia occidentalis* aerial parts either in a dose 200 or 400 mg/kg B.W. gave the best improvement, in which the hepatic tissue appeared more or less normal. In Silymarin, methanolic extract and chloroform extractive treated groups, there were mild pathological changes in the form of congestion of vasculature and vacuolar degeneration of hepatocytes. While in the group which treated with *n*-butanol extractive, no histological improvement could be detected. From our results, it was clear that the histopathological findings of liver examination was positively correlated and supported the biochemical analysis of liver function parameters in different treated groups.

Our results of kidney function tests showed non significant decrease in serum urea values in all treated groups in comparison with CCl₄ treated group except the groups which treated with 200 and 400 mg/kg B.W. EtOAc extractive showed significant decrease in urea concentration in comparison with CCl₄ received group, and had no significant changes in comparison with Tween and Silymarin groups. There was significant decrease in serum creatinine levels in group treated with 200 and 400 mg/kg B.W. of EtOAc extractive groups in comparison with CCl₄ received group, and showed no significant changes in comparison with Tween and Silymarin groups. The

other groups (Total methanolic, CHCl₃ and *n*-butanol extractive) showed non significant decrease in serum creatinine levels in comparison with CCl₄ received group. Mirtes *et al.* (2011) demonstrated that oral administration with hydroalcoholic extract of *Cassia occidentalis* for 30 days (0.10, 0.50 and 2.5 g/kg) in male and female rats did not change the biochemical profile (creatinine and urea) which is good indicators of kidney functions and they reported that *Cassia occidentalis* extract did not induce any damage to the kidney and it is not toxic and safety for use by human. Also similar results was obtained by Hilaly *et al.* (2004). He found normal kidney function after oral sub-acute treatment by *Cassia occidentalis* extract. Fid'ele Ntchapda *et al.* (2015) revealed that subchronic treatment with aqueous extract of *Cassia occidentalis* leaves 400 mg/kg B.W. for 7 days in rats improved the kidney function index of urea and creatinine. The hepatoprotective and nephroprotective effects *Cassia occidentalis* extracts could be attributed to its antioxidant activity (Usha *et al.*, 2007 and Silva *et al.*, 2008). The histopathological findings in our results confirmed these effects. The treatment with ethyl acetate extractive of *Cassia occidentalis* either in a dose 200 or 400 mg/kg B.W. gave the best protection.

CONCLUSION

Cassia occidentalis ethyl acetate extractive have hepatoprotective and nephroprotective effects. This could be attributed to the antioxidant activity of its phenolic contents (compounds 1-6). Although the ethyl acetate extractive of *Cassia occidentalis* could be considered as a promising source of natural hepatoprotective agents, further application in medical practice should be confirmed by conveying pharmacological and placebo controlled clinical studies.

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المركبات الكيميائية والتأثير الوقائي الكبدى لمستخلص نبات الكاسيا أكسيدنتالس (زنجية القهوة) المزروعة فى مصر

هناء محمد سيد ، محمود أحمد رمضان ، منال محمد سيد ، سارى عبد الغفار ، هبه حامد سالم

E-mail: sary64@windowslive.com Assiut University web-site: www.aun.edu.eg

صممت هذه الدراسة لمعرفة التأثير الوقائي لمستخلصات نبات زنجية القهوة على التسمم الكبدى المحدث بمادة رابع كلوريد الكربون فى الفئران البيضاء. تم استخدام ٦٦ فأر قسمت إلى ١١ مجموعته (٦ فأر لكل مجموعة). المجموعة الأولى تم حقنها بالمذيب (Tween) ٢ مجم/كجم من وزن الجسم فى التجويف البريتونى كمجموعة ضابطة. المجموعة الثانية يتم حقنها CCL_4 المذاب فى زيت الزيتون ٢ مجم/كجم من وزن الجسم . المجموعة الثالثة يتم حقنها CCL_4 بنفس الجرعة بالإضافة إلى العلاج بمادة Syllimarine ٢٥ مجم/كجم من وزن الجسم عن طريق الفم لمدة أسبوع. المجموعات من الرابعة حتى الحادية عشر تعامل مثل المجموعة الثانية CCL_4 وفى اليوم التالى تعطى مستخلصات النباتات (Total methanolic, Chloroform, ethyl acetate) (and n- butanol serum) بتركيز ٢٠٠ ، ٤٠٠ مجم/كجم من وزن الجسم بالفم لكل مستخلص من نبات زنجية القهوة لمدة أسبوعين. فى نهاية التجربة تم ذبح الفئران وفصل السيرم لقياس وظائف الكبد AST , ALT and bilirubin . وكذا قياس وظائف الكلى urea and creatinine . كما تم أخذ عينات من الكبد للفحص الباثولوجى. أظهرت النتائج ان هناك تحسن فى وظائف الكبد والكلى بعد المعالجة بالمستخلصات المختلفة للنبات. هذا وقد اعطت المعالجه بمستخلص ethyl acetate أفضل النتائج. وقد عضدت نتائج الفحص الباثولوجى ماتم الحصول عليه من نتائج بيوكيميائيه. وقد اظهر التحليل الكروماتوجرافى لمستخلص ال ethyl acetate العديد من المركبات تم التنقيه والتعرف على ستة منها وهى

naringenin (1), quercetin (2), 1,8-dihydroxy anthraquinone (3), 1,3,8-trihydroxy anthraquinone (4), chrysoeriol-7-O-rutinoside (5) and rutin (6).

هذا وقد اعزى التأثير الكبدى الوقائي لهذه المركبات الفينولية المستخلصه.