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Chemical characterization, antioxidant and antihepatotoxic activities of *Calliandra* haematocephala (Hassk.), growing in Egypt

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ABSTRACT

This study aims to investigate the major constituents of hexane fraction, of Calliandra haematocephala (Hassk.) leaves. In addition to, the hepatoprotective and antioxidant activities of the total alcohol extract against carbon tetrachloride (CCl_4) -induced liver damage in vivo. C. haematocephala leaves were extracted with methanol, and hexane, fraction of methanol was separated and two major bands and were identified by GC/MS, MS, ¹HNMR. Fatty acids were isolated, methylated and identified by GC/MS. In vivo the hepatoprotective and antioxidant activities of methanol extract against CCl₄-induced liver injury was evaluated in rats based on the analysis of biochemical parameters and histopathological studies. The results illustrated that the analysis of hexane fraction led to isolation of lupeol and mixture of sterols. Four major fatty acids (palmitic, oleic, linoleic and linolenic,) were determined. Oral administration of the extract at doses 100 and 200 mg/kg bw. caused significant decrease in the levels of serum ALT, AST, GGT and bilirubin as well as albumin was significantly elevated. Moreover, the extract decreased MDA content and increased the levels of SOD, GSH and CAT compared with intoxicated rats. Also, creatinine and urea were improved as a result of the treatment of the extract. The hepatoprotective and antioxidant effect of the extract may be attributed to phenolics, flavonoids or the saponins content of the extract which may be acting as free radical scavenging effect, inhibiting lipidperoxidation and increasing antioxidant activities suggesting that the alcohol extract of C. haematocephala leaves has potential to be explored as valuable hepatoprotective and antioxidant.

Keywords: *Calliandra haematocephala (Hassk.);* sterols; lupeol; fatty acid; total phenolics and flavonoids, Antioxidant; Hepatoprotective activities.

INTRODUCTION

Liver diseases are still a global health problem may be classified as acute or chronic hepatitis (inflammatory liver diseases), hepatosis (non inflammatory diseases) and cirrhosis (degenerative disorder resulting in liver fibrosis). Unfortunately, treatments of choice for liver diseases are controversial because conventional or synthetic drugs for the treatment of these diseases are insufficient and sometimes cause serious side effects[1]. Many of the currently available drugs were derived either directly or indirectly from medicinal plants. Due to their effectiveness, with presumably minimal side effects in terms of treatment as well as relatively low costs.

The reactive oxygen species (ROS) such as superoxide anion radical (O_2); hydrogen peroxide (H_2O_2) and hydroxyl radical (OH) have been implicated in the pathophysiology of various clinical disorders[2]. They play an important role in the inflammation process after intoxication by ethanol, carbon tetrachloride or carrageenan[3]. These radicals and the reactive species derived from them react with the cell membrane, induce lipid peroxidation and are responsible for various deleterious effects in cells and tissues where they are generated[4]. The inhibition of free radical generation can serve as a facile model for evaluating the activity of hepatoprotective agent.

Calliandra haematocephala (Hassk.), family Fabaceae (Pea or Legume)[5] is an evergreen shrub or small trees widely distributed in the tropics[6&7] native to tropical America, Bolivia, cultivated in different regions as Malesia, South Florida, Australia and introduced to Egypt[5]. The leaf of C. haematocephala contains pipecolic acid derivatives. Pipecolic acid is a non protein amino acid, and its derivatives 4,5-dihydroxy-L-pipecolic acid, and 2S,4R-carboxy-2-acetylamino-4-piperidine[8&9]. Six amino acids were isolated from leaves of C. haematocephala and showed insecticidal activity against Spodoptera frugiperda[10]. P-hydroxybenzoic acid, caffeic acid, protocatechuic acid, astilbin, neo-isoastilbin, and catechin-3-O-rhamnoiside were isolated from the EtOAC extract of the bark and showed varied antibacterial activity. In addition to, the non-active hexane fraction gave lupeol and betulinic acid[11]. Three acylated quercetin rhamnosides and 17 known compounds were reported from the leaves and stem of C. haematocephala, the major isolates exhibited moderate to strong radical scavenging properties[12]. Six flavonoids and one phenolic acid were isolated from aerial parts as well as volatile constituents of fresh aerial parts were extracted and analyzed by GC/MS. LD₅₀, analgesic, antipyretic, anticonvulsant, antiulcer, antioxidant and antimicrobial activities of the different extracts were investigated [13]. Butanolic extract of the aerial parts of the plant showed a gastroprotective and immunomodulatory activities[14]. However, the plant possessed antisickling properties[15]. The root bark of C. haematocephala possesses antimalarial activity[16]. Methanolic extract of Calliandra haematocephala leaves represents a potential antiviral for treatment viral myocarditis[17]. Moreover, methanol, extract and chloroform, ethyl acetate, butanol, and aqueous fractions of C. haematocephala leaves possess antiviral activity against rotavirus infection in-vitro[18]. The major compounds of condensed tannin extracts of leaf, twig, and stem bark of C. haematocephala were isolated and identified and exhibited stronger antioxidant activities[19].

This study aims to isolate and identify the major constituents and fatty acids profile of n-hexane fraction of *C*. *haematocephala* leaves in addition, to investigate the alcohol extract for the antioxidant and antihepatotoxic activities. Antioxidant activity was assayed by standard methods, the antihepatotoxic activity was determined by CCl_4 induced liver damage in rats compare with silymarin. The extent of liver damage was assessed by biochemical studies and by histopathological examination.

EXPERIMENTAL SECTION

Chemicals and apparatus

Kits were purchased from Biodiagnostics Company (Cairo, Egypt), Silymarin (a widely used herbal drug from *Silibum* marianum) purchased from Cid Co.; Ltd. (Cairo, Egypt). Folin–Ciocalteu reagent (Sigma Chemical Co.; St. Louis, Mo.; U.S.A.)

All solvents and chemicals were Fisher HPLC grade, (Fisher Scientific, USA).

Silica gel 60 mesh (Merck, Germany) was used for column chromatography. Solvents used in column chromatogram were HPLC of analytical grade (Fisher Scientific, USA). Fractions were monitored by TLC silica gel F_{254} aluminum sheets 20 x 20 cm, and Silica gel F_{254} , HPTLC aluminum sheets 20 x 20 cm, Merck Germany. TLC spots were detected by spraying with *p*-ansialdehyde- H₂SO₄ in EtOH followed by heating.

Nuclear magnetic resonance (NMR) spectra were measured on Bruker AV-400 spectrometer operating at a frequency of 400 MHz using $CDCl_3$ as solvent at room temperature with tetramethylsilane (TMS) as an internal standard.

Electrospray ionization mass spectra (EI-MS) were carried out on a THERMO Scientific Corp.; USA), mass spectrometer 70 eV.

UV.VIS, 2401 spectrophotometer Shimadzue, Inc.; Kyoto, Japan

Gas Chromatography/Mass Spectrometry (GC/MS) analysis for sterols.

GC-MS analysis of sterols was carried out using gas chromatography-mass spectrometry instrument stands with the following specifications

Instrument: a TRACE GC Ultra Gas Chromatographs (THERMO Scientific Corp.; USA), coupled with a thermo mass spectrometer detector (ISQ Single Quadrupole Mass Spectrometer). The GC-MS system was equipped with a TG-5MS column (30 m x 0.25 mm i.d.; 0.25 μ m film thickness). Analyses were carried out using helium as carrier gas at a flow rate of 1.0 ml /min and a split ratio of 1:10 using the following temperature program: 50 °C for 3 min; rising at 5.0 °C/min to 300 °C and held for 20 min. The injector and detector were held at 280 °C. Diluted samples (1:10 hexane, v/v) of 0.2 μ l of the mixtures were always injected. Mass spectra were obtained by electron ionization (EI) at 70 eV, using a spectral range of m/z 40-450. Most of the compounds were identified using two different analytical methods: mass spectra (authentic chemicals, Wiley spectral library collection and NSIT library).

Gas chromatographic-mass spectra (GC-MS) analysis for fatty acid methyl esters

GC-MS analysis of the fatty acid methyl esters matter was carried out using gas chromatography-mass spectrometry instrument stands with the following specifications. Instrument: a TRACE GC Ultra Gas Chromatographs (THERMO Scientific Corp.; USA), coupled with a THERMO mass spectrometer detector (ISQ Single Quadrupole Mass Spectrometer). The GCMS system was equipped with a PR5 MS column (30 m x 0.25 mm i.d.; 0.25 m film thickness). Analyses were carried out using helium as the carrier gas at a flow rate of 1.0 ml/min at a split ratio of 1:10 and the following temperature program: 50 °C for 3 min; rising at 4.0 °C/min to 260 °C and held for 6 min; rising at 6 °C/min to300 °C and held for 1 min. The injector and detector were held at 200 and 200 °C, respectively. Diluted samples (1:10 hexane, v/v) of 0.2 μ l of the mixtures were always injected. Mass spectra were obtained by electron ionization (EI) at 70 eV, using a spectral range of m/z 40-450. Most of the compounds were identified using two different analytical methods: mass spectra (authentic chemicals, Wiley spectral library collection and NSIT library).

Plant materials

Calliandra haematocephala (Hassk.), were collected from the National Research Centre Botanical Garden during the period May and June 2012, and was kindly identified by, Mrs. Tersea Labib, taxonomist at Orman Botanical garden, Giza and Dr. Mona Marzok, researcher in at the Herbarium of National Research Centre (NRC), Cairo, Egypt. A voucher specimen was deposited at the Herbarium of the NRC, Cairo, Egypt.

Extraction and Isolation of Compound

Air-dried, powdered (2kg) of *Calliandra haematocephala* (Hassk.), leaves were extracted with methanol at room temperature and concentrated under reduced pressure at 40 °C, the extract yield was **13.84%**. The residue of methanolic extract was suspended in water and sequentially fractionated with chloroform (**12.04 %**), ethyl acetate (**1.84 %**), n-butanol (**0.78 %**) and aqueous fraction (**3.35 %**). The obtained extracts were stored at -20 °C until used.

Isolation and identification of sterols and triterpenes

20 gm of methanol extract was dissolved in 80% methanol HPLC grade using ultrasonic water bath as assistant, the residue was partitioned with n-hexane several times, the combined hexane layers were dried over anhydrous Na₂SO₄ and concentrated under reduced pressure. Hexane fraction was subjected to silica gel (mesh 60) column chromatography (CC) and was successively eluted with a mixture of solvents of increasing polarity made up hexane: ethyl acetate (100, 99:1, 95:5, 90:10, 85:15, 80:20, 75:25 and 70:30) yielding 58 fractions (100 ml) and was monitored by TLC silica gel F_{254} aluminum sheets (Merck) using different solvent systems such as toluene:ethyl acetate (8:2 v/v) and n-hexane: ethyl acetate (70:30 v/v), (visualization: *p*-anisaldehyde -sulphuric acid reagent heated at 110°C). Similar fractions were pooled together, fractions 15 to 22 were fatty acids and lipids, F23 to F28 corresponding to the *n*-hexane:ethyl acetate (90:10 v/v) were found to contain similar spot, further purification was carried out using HPTLC silica gel F_{254} aluminum sheets (Merck). Spots were scraped and eluted by chloroform afforded pure compound (I), a yellow powder (15 mg), gave a positive test with Liebermann-Burchard reagent (violet color) and Salkowski test and subjected to MS and ¹HNMR analysis and was identified by comparison with previous data.

Fractions 29 to 33 corresponding to the *n*-hexane:ethyl acetate (80:20 v/v) were found to contain similar spot, further purification on HPTLC afforded a mixture of sterols (2) and was identified and characterized by GC/MS.

Investigation of Fatty Acids

About 2g of n-hexane fraction was saponified with alcoholic (ethanol 95%) KOH 10% and the residue was fractionated into unsaponifiable and saponifiable matters according to the method[20] as well as, the fatty acids were liberated by acidification of the saponifiable matter, extracted with ether, dried over anhydrous Na_2So_4 and concentrated under reduced pressure. The fatty acid methyl esters were prepared according to the method adopting[21] and analyzed by GC/MS. The fatty acid methyl esters were identified on the basis of fragmentation pattern of mass spectra data and a library database [Wiley (Wiley Institute, Los Angeles, CA) and NIST (National Institute of Technology, Los Angeles, CA)]. Quantitative determination was carried out on the basis of peak area measurements of the GC/MS chromatograms.

Determination of total phenolics and total flavonoids and in the alcohol extract:

Reagents: Aluminum chloride was prepared by dissolving 2 g of aluminum chloride (Sigma) in 100 ml pure methanol. Foline reagent, saturated solution of sodium carbonate (20 gm /100 ml dist. Water).

Preparation of total phenolics and total flavonoids compounds:

For determination of the total phenolic and flavonoid compounds in the extracts: known weight of the extract was dissolved in 80% methanol in measuring flask 100 ml. and completed to 100 ml.

Estimation of total phenolics

The total phenolics content (TPC) was determined by Folin–Ciocalteu according to the method described by [22&23] with some modification. Briefly, 1.0 ml. of the sample extract was added to 7.5 ml. of redistilled water and 0.5 ml. of Folin–Ciocalteu reagent (Sigma Chemical Co.; St. Louis, Mo.; U.S.A.) was added. After 15-min equilibration, the mixture was neutralized with 1.0 ml. of 20% Na₂CO₃, mixed well by a vortex. After a 30-min reaction, the absorbance of the mixture was measured at 760 nm with a (UV–VIS spectrophotometer). Chlorogenic acid was used a standard curve, the mean of three readings were used and results were expressed as milligrams of chlorogenic acid equivalent per gram of the extract (mg /1 g extract).

Estimation of total flavonoid:

The total flavonoids content (TFC) was determined according to method as adopted [24&23]. Briefly, 3 ml. of the extract was mixed with 3 ml. of AlCl₃ (2 % in methanol) solution and the mixture was allowed to stand for 30 min. The absorbance was measured at 415 nm. with a (UV–VIS spectrophotometer). Rutin was used as a standard curve and the mean of three readings were recorded, the total flavonoids was expressed in mg as rutin equivalent per 1g of the extract (mg /1g extract).

Acute toxicity:

Acute Oral Toxicity Study

Healthy male Swiss albino rats (8 weeks) used for the acute oral toxicity study were bred and reared at the Animal House. The animals were housed in polypropylene cages with stainless steel grill tops and provided with bedding of clean paddy husk. The animals were acclimatized to laboratory conditions for 1-week prior to treatment. The temperature in the animal room was maintained between 25 ± 2 °C with a relative humidity of $65\pm 10\%$, and illumination cycle set to 12 h light and 12 h dark. The rats were fed with standard laboratory pelleted feed.

Treatment

All rats were fasted overnight before treatment and were given food one hour after treatment. A single high dose, as recommended by Organization for Economic Co-operation and Development (*OECD* 1992)[25] guidelines of 2,000 mg/kg. Methanol extract dissolved in water, at dose 100, 250,500,750 and 1000 mg/kg bw. and was administered by gavage to five groups each 10 male rats weighing between 150 and 200 g, and water was given to 10 male and as a control group. After a single administration, signs of possible toxicity were observed every hour for the first six hours and every day for 14 days. Surviving animals were observed for any signs or symptoms of toxicity and for mortality for up to 14 days as described previously[26&27]. The visual observations included changes in the skin and fur, eyes and mucous membranes, and behavioral pattern.

Experimental animals

Male healthy albino rats Wistar strain (120-150 g) were obtained from Animal House of National Research Center (NRC), Dokki, Giza, Egypt were kept in conventional cages with free access to water ad libitum and standard rat feed with rodent pellet diet at 27 ± 2 °C, humidity $65\pm 10\%$, 12 hrs light/dark cycle, respectively. They were kept for

two weeks to acclimatize to the laboratory conditions. Silymarin (a widely used herbal drug from *Silibum marianum*) was used for comparison at 100 mg/kg bw.)[28]. All the experiments were performed in accordance with the guide for the care and use of laboratory animals, as adopted by the National Research Center.

Hepatotoxicity induced by carbon tetrachloride

liver injury was induced by intraperitoneal injection of 0.5 ml / kg bw. of CCI_4 , (Sigma–Aldrich Co.; USA) diluted 1: 9 (v/v) in olive oil twice a week for two consecutive weeks [29&30].

Hepatoprotective activity (Prophylactic test)

The rats were divided randomly into groups of 6 rats, the extract was suspended in distilled water and administered orally through an intragastric tube at the dose of 100 and 200 mg/kg b.w. after 1 h of CCl_4 injection. Control group was administrated with normal saline instead of the plant extract.

Group I: Controls received the vehicle of normal saline (0.5 ml/kg bw.).

Group II: Received CCl₄ (0.5 ml/kg bw. i.p.) twice-weekly for two consecutive weeks.

Grou III: Received silymarin 100 mg/kg bw. daily for two consecutive weeks and simultaneously administered CCl_4 (0.5 ml/kg bw) twice-weekly for two consecutive weeks.

Group IV: Received alcohol extract of *C. haematocephala* leaves 100 mg/kg bw. daily for two consecutive weeks and simultaneously administered CCl_4 (0.5 ml/kg bw.) twice-weekly for two consecutive weeks.

GroupV: Received alcohol extract of *C. haematocephala* leaves 200 mg/kg bw. daily for two consecutive weeks and simultaneously administered CCl_4 (0.5 ml/kg bw.) twice-weekly for two consecutive weeks.

Blood sampling and serum preparation

At the end of experimental period, all the animals were sacrificed by cervical decapitation under mild anesthesia and blood samples were collected, allowed to stand for 30 min to coagulate at room temperature and were centrifuged at 4000 rpm for 20 min at 4 °C to obtain serum for the determination of various biochemical parameters such as ALT, AST, GGT, albumin, bilirubin, urea and creatinine.

Preparation of liver homogenate

The liver tissues were isolated, washed immediately with ice-cold saline (0.9% NaCl). The liver tissue samples were cut into two pieces. One small piece was fixed in formalin for histopathological examination. The other piece was utilized for the biochemical analyses. The liver was homogenized in chilled Tris-HCl buffer (0.025 M, pH 7.4) using a homogenizer. The homogenates were centrifuged at 4000 rpm for 20 min at 4°C. The supernatants were collected and stored at - 20 °C for subsequent determinations.

Biochemical analysis

Determination of aspartate aminotransferase (AST) and alanine aminotransferase (ALT). Aspartate and alanine aminotransferases (AST &ALT) were estimated by the method[31].

Estimation of hepatic gamma glutamyl transferase.

Gamma glutamyl transferase (γ -GT) was estimated by the method[32-34].

Determination of serum total bilrubin.

Serum total bilirubin was determined according to the method[35].

Determination of serum albumin.

Albumin was measured in blood serum according to the method [36].

Estimation of serum urea.

Urea was estimated according to the method[37].

Estimation of serum creatinine.

Creatinine was estimated according to the method[38&39].

Estimation of hepatic superoxide dismutase activity.

The activity of superoxide dismutase (SOD) was estimated according to the method[40].

Estimation of catalase (CAT) enzyme in liver.

Catalase activity was assayed in tissue liver homogenate according to the method [41&42].

Determination of hepatic glutathione (GSH) content.

Reduced glutathione (GSH) was colorimetrically determined according to the method [43].

Determination of lipid peroxidation products (Malondialdehyde).

Malondialdehyde (MAD) was determined in tissue liver homogenate according to the method [44&45].

Histopathological study

After blood samples were obtained, rats were decapitated and slices of liver were removed out and fixed instantaneously in normal saline for 24 hours, the specimens were washed in tap water, tissues were fixed in 10% buffered formalin. After fixation, the tissues were dehydrated in a graded series of alcohol, cleared in xylene, and embedded in paraffin wax. Multiple 6 um sections from each block were mounted on slides and stained with hematoxylin and eosin following the standard procedure described[46]. The prepared sections of liver were examined by light microscope. Changes of any in the cytoarchitecture were noticed.

Statistical analysis

Statistical Analysis. All data were expressed as mean \pm SD of six rats in each group. Statistical analysis was carried out by one-way analysis of variance (ANOVA), Costat Software Computer Program:

RESULTS AND DISCUSSION

Chemical profiles of hexane extract

Two compounds were isolated and purified through silica gel column, and preparative HPTLC from hexane fraction, among them, compounds 1 and 2, which was obtained in the largest amount.

Compound I obtained from column chromatography (CC) successively eluted with a mixture of solvents of increasing polarity made up *n*-hexane:ethyl acetate. F23 to F28 corresponding to the *n*-hexane:ethyl acetate (90:10 v/v) monitored by TLC silica gel and were found to contain similar major spots. Further purification carried out using HPTLC silica gel F_{254} afforded compound I a yellow powder (15 mg). Compound I was checked on TLC silica gel developed with *n*-hexane:ethyl acetate (70:30 v/v), gave one spot, a violet color with *p*-anisaldehyde - H_2SO_4 reagent (R_F 0.59), a positive test with Liebermann-Burchard reagent (violet color) and Salkowski test. Compound I subjected to MS and ¹HNMR analysis and was identified as lupeol, by comparison with previous reported ¹H and MS spectral data.

Compound 1 : Lupeol (3β-Lup-20(29)-en-3-ol) (15 mg): yellow powder, gave a positive test with Liebermann-Burchard reagent (violet color) and Salkowski test. The structure of the compound was elucidated on the basis of the spectra analysis (MS and ¹H-NMR) and published data. ¹H NMR (400 MHz, CDCl₃): exhibited seven signals as singlet to δ (ppm) 0.78 (3H, s, H-24), 0.82 (3H, s, H-28), 0.84 (3H, s, H-25), 0.93 (3H, s, H-27), 0.95 (3H, s, H-23), 1.03 (3H, s, H-26) and at lower fields other to δ 1.67 (3H, s, H-30) assignable to a methyl group on double bond, a multiplet at δ 1.94 (2H, m, H-21); a double doublet to δ 2.28 (1H, m, H-19),) to be assigned to a methynic proton; a double doublet to δ 3.20 (1H, dd, J=11.6, 5.03 Hz, H-3) assignable to a geminal proton of a secondary alcohol, two doublets for proton to an exocyclic methylene to δ 4.55 (2H, d, J=2.0 Hz, H-29) and 4.67 (2H, d, J=2.0 Hz, H-29) which indicated the presence of an iosprenyl side chain proton (H a -29 and H b -29) This suggested its relation with lupane-type of triterpenoid by comparing the ¹H NMR data of compound 1 with previously published values[47]. EIMS of this compound showed the molecular ion, (M+) at m/z: 426 (calcd. 426 for C₃₀H₅₀O); EI/MS: m/z (relative intensity,%): 426(24), 411(5), 393(2), 357(2), 302(4), 257(16), 229(26), 218(100), 207(43), 203(37), 189(57), 175 (13) ,161(22), 147 (25), 121(51); 107 (56), 95 (63), 81 (94), 69 (69), 41 (81). Mass spectrum of compound I was confirmed by comparison with published data [48], and it was characterized as lupeol which was isolated previously from this species[11].



Lupeol

Table (1): GC/MS of the band 2 isolated from hexan fraction by column and TLC chromatography

Sterol	RT	Percentage Molecular formula		MW
Cholestanol	63.01	3.05	C27H48O	388
campesterol	63.78	1.49	$C_{28}H_{48}O$	400
Stigmasterol	64.16	76.87	$C_{29}H_{48}O$	412
Stigmastanol	64.34	2.01	C29H52O	416
β- Sitosterol	65.13	16.58	$C_{29}H_{50}O$	414

Hexane fraction was subjected to silica gel (mesh 60) column chromatography (CC) and was successively eluted with a mixture of solvents of increasing polarity made up hexane: ethyl acetate. Fractions 28 to 33 corresponding to the *n*-hexane:ethyl acetate (80:20 v/v) were found to contain similar spot further purification was carried out using HPTLC silica gel F_{254} afforded pure compound, band **2** a white powder (22 mg), gave a positive test with Liebermann-Burchard reagent (violet color) and Salkowski test. TLC silica gel developed with *n*-hexane:ethyl acetate (70:30 v/v) gave a violet color with the *p*-anisaldehyde -sulphuric acid reagent (R_F 0.47), indicating their steroidal nature, it gave one spot on TLC. Chromatogram of GC /MS of spot 2 led to the identification of number of sterols as shown in Table 1. These mixture showed five sterols and were identified through mass spectrometry attached with GC using two different analytical methods and published data. The sterols mixture consisted mainly of cholestanol, campesterol, stigmasterol, stigmastanol and β -sitosterol of which stigmasterol was the most predominant one represented 76.87 % of the total followed by β -sitosterol (16.58%). and was identified and characterized by GC/MS.

Fragmentation pattern of the first peak revealed the presence of a molecular ion peak at m/z 388, calculated for the molecular formula $C_{27}H_{48}O$ in addition to the characteristic peaks at m/z 373, 355, 233, 215, 165,121, 95, and 81 and was identified through mass spectrometry attached with GC and published data as cholestanol[49].

Fragmentation pattern of the second peak showed a molecular ion peak at m/z 400 calculated for the molecular formula $C_{28}H_{48}O$, in addition to the following characteristic peaks at 382, 367,342,289, 273, 255, 231, 213, 207, 145, 121, 91, 79, 57 and 43(100) which was identified through mass spectrometry attached with GC and published data as campesterol[50].

For stigmasterol, mass spectrum revealed the presence of a molecular ion peak at m/z 412 calculated for the molecular formula $C_{29}H_{48}O$, in addition to the following characteristic peaks at 351, 314, 300, 271, 229, 213, 107, 81 and 55(100%). The data were in agreement with previous mass spectra[51].

Mass spectrum of peak No. 4 displayed molecular ion peak at m/z 416 calculated for the molecular formula $C_{29}H_{52}O$ in addition to the characteristic peaks at m/z 401, 283, 290, 271, 233, 215, 201, 107, 95, 81 and 57, the sterol was identified through GC/MS using two different analytical methods and published data as stigmastanol [52].

For β -sitosterol mass spectrum revealed the presence of a molecular ion peak at m/z 414 calculated for the molecular formula C₂₉H₅₀O in addition to the characteristic peaks at m/z 399, 396, 329, 273, 255, 231 and 213. β -sitosterol was identified through mass spectrometry attached with GC and published data [53]. The sterols were detected in this species for the first time.



Table (2): Fatty acids composition of Calliandra haematocephala leaves

Fatty acids		Relative Percentage
Dodecanoic acid, methyl ester lauric C12:0	25.41	4.27
Tetradecanoic acid, methyl ester Myristic C14:0	30.89	4.57
Hexadecanoic acid, methyl ester Palmitic acid C16:0	30.02	36.85
Octadecanoic acid, methyl ester Stearic acid C18:0	41.72	7.59
9-Octadecenoic acid Oleic acid C18:1	45.37	15.6
9,12-Octadecadienoic acid methyl ester Linoleic acid C18:2	46.66	14.57
9,12,15-Octadecatrienoic acid, methyl ester, Linolenic acid, C18:3	48.37	16.55

Fatty acids composition of *Calliandra haematocephala* leaves are presented in (Table 2). The leaves are comprised a much higher proportion of palmitic (36.85%), linolenic acid (16.55%), oleic (15.6%) and linoleic (14.57%) which composed 83.57% of the total fatty acids while they had a markedly lower concentration of lauric (4.27%), myristic (4.57%) and stearic acid (7.51%). Palmitic acid was found the predominated fatty acids. Regarding to the proportion between the saturated and unsaturated fatty acids the data showed that the concentration of saturated fatty acids accounted 53.28% whereas the unsaturated fatty acids represented 46.72%. In addition to, the unsaturated fatty acids were found approximately in equal level. Abou Zeid, *et al.*, 2006 [13] analyzed the fatty acid of *Calliandra haematocephala* leaves by GC using authentic samples and identified ten saturated and four unsaturated fatty acids (54.736% and 26.31% respectively) in addition to the unidentified ones (18.954%).The major fatty acids are palmitic (17.445%), linolenic (14.653%) and lauric (13.772%) while caprylic (0.965%) and pelargonic acids (1.378%) are minor.

Total phenolics and flavonids

Table (3): Percentage of total phenolic and total flavonoids in the alcohol extract of Calliandra haematocephala leaves

Extract	Percentage of total	Percentage of total	Percentage of
	phenolic	flavonoids	non flavonoids
Calliandra haematocephala total alcohol extract	31.01	4.7	26.31

Total phenolics content was determined from crude extract of *Calliandra haematocephala* leaves using Folin Ciocalteu reagent. The results are presented in (Table 3). The results obtained showed the extract contains high percentage of total phenolics (31.01 %). The percentage of flavonoids (4.7%) which represented 15.15% of total phenolics, here the percentage of total flavonoids was found in appreciable amount of total phenolics.

Acute toxicity

The alcoholic extract of *C. haematocephala* was found to be nontoxic up to the dose of 200 mg/kg bw and did not cause any behavioral changes or death of tested animals.

Antihepatotoxicity

 Table (4): Hepatoprotective effect of alcohol extracts Calliandra haematocephala leaves on the levels of serum albumin, total bilirubin,

 ALT, AST and GGT in CCl4 treated rats

Prophylactic Groups	Albumin (g/dl)	Total bilirubin (mg/dl)	ALT (unit/ml)	AST (unit/ml)	GGT (U/L)
Control	4.96 ± 0.44^{a}	$0.48 \pm 0.046^{\circ}$	37.36 ± 1.92 ^e	33.89 ± 2.11 ^d	8.80 ± 1.18 ^e
Control (++) CCl ₄	3.23 ± 0.37°	1.18 ± 0.17^{a}	70.35 ± 3.86^{a}	62.25 ± 3.60^{a}	44.31 ± 4.80^{a}
Silymarin at 100mg/kg b.w	4.62 ± 0.41^{a}	$\begin{array}{c} 0.80 \pm \\ 0.09^{\mathrm{b}} \end{array}$	55.52 ± 3.01^{b}	50.04 ± 2.65°	23.70 ± 2.65 ^{bc}
<i>C. haematocephala</i> extract at 100 mg/kg b.w	3.58 ± 0.3^{bc}	0.74 ± 0.063^{b}	48.66 ± 2.94 ^{bc}	48.09 ± 2.50^{b}	21.58 ± 2.07 ^b
<i>C. haematocephala</i> extract at 200 mg/kg b.w	3.91 ± 0.37^{b}	$0.52 \pm 0.051^{\circ}$	42.80 ± 2.36^{d}	45.51 ± 2.11 ^b	12.01± 1.42 ^c

Data are mean \pm SD of four rats in each group.

Unshared letters between groups are the significance values at P < 0.05 for all parameters. Statistical analysis is carried out using one way analysis of variance (ANOVA) coStat computer program.

In the present study, significant hepatotoxicity was observed after rats were administered with CCl_4 at dose 0.5 ml/kg bw. as indicated by significant (P < 0 .05) increase in the liver serum marker enzymes, AST, ALT, GGT, and bilirubin while decrease in the albumin level (Table 4). Intoxicated rats treated with silymarin, as standard drug at 100 mg/kg bw. significantly (P < 0 .05) reduced the levels of liver enzymes, and bilirubin while elevated the albumin titer indicating the prophylactic effect of silymarin drug. This was confirmed by histopathological examination that may be due to regeneration of hepatocytes with no evidence of inflammatory infiltration.

Regarding to the effect of alcoholic extract of *C. haematocephala* at 100 mg and 200 mg/kg bw. on the intoxicated rats, (Table 4 and figures 1,2,3) significantly (P < 0.05) decreased the levels of liver enzymes AST, ALT, GGT and bilirubin level, and significantly (P < 0.05) elevated the albumin level as compared with CCl₄ treated rats. The results of the treatments were similar to silymarin standard drug. From the results obtained the higher dose of extract 200 mg/kg bw. was found to be statistically significantly more efficiency than the low dose 100 mg/kg bw. as compared with CCl₄ treated rats. However, the 200 mg/kg dose showed restorative effects on liver enzymes values, bilirubin, albumin and histology of the liver examination further confirmed the reduction in hepatic injury.

AST, ALT, and ALP used as serum liver marker enzymes and were characterized by balanced activities with high concentrations in cytoplasm of liver cells, due to the hepatic intoxication as the treatment by CCl_4 , the lysosomal instability leakage these enzymes into blood stream. On the other hand, an elevation of plasma AST and ALT activities could be regarded as a sign of damage to the liver cell membrane[54].

Serum bilirubin is considered test of liver function, provide useful information about how the liver is functioning and employed in the diagnosis of hepatic diseases. It is the main bile pigment which is formed from the breakdown of hemoglobin in red blood cells. The broken down hemoglobin travels to the liver, and conjugated with glucuronic acid in hepatocytes to increase its water solubility. Inhibition of the conjugation reaction, consequent release of bilirubin itself from damaged hepatocytes into blood stream as the result of liver damage[55].

 CCl_4 metabolism by the action of the mixed function of the cytochrome P_{450} oxygenase system into the trichloromethyl free radical (CCl_3^{\bullet}) This free radical, reacts very rapidly with oxygen to yield a highly reactive trichloromethyl peroxy radical (CCl_3OO^{\bullet}). Both radicals are capable of binding to proteins or lipids, or abstracting a hydrogen atom from an unsaturated lipid, the chemical products of this oxidation are known as lipidoxidation[56&57]. Peroxidation reactions are dangerous for the viability of cells, even tissues. Enzymatic (catalase, superoxide dismutasse) and nonenzymatic (vitamins A and E), natural antioxidant defense may be inhibiting lipid peroxidation to take place. Therefore, numerous studies reported that CCl_4 is widely used to induce liver damage[58].

In the present study, the hepatoprotective activity of *Calliandra haematocephala* alcohol extract in an experimental model using CCl₄-induced liver toxicity was confirmed by histopathologic analysis, This hepatocellular injury led to an elevation in the level of blood hepatic serum markers AST, AST, GGT and decrease of albumin which are established as indicators for liver injury. These results are in agreement with previous studies[59-62]. The level of total bilirubin in the present study was also elevated in the CCl₄-induced treated group as the result of leakage from damaged hepatic cells and is used also as a marker of liver injury, the data obtained are in agreement with previous studies[63].

Administration of *C. haematocephala* alcohol extract at dose of 100 or 200 mg/kg bw. decreased the hepatic serum markers level AST, AST, GGT and elevated the albumin level reflecting a liver recovery. Besides, elevation in serum liver markers and bilirubin associated with CCL_4 toxicity was reduced by the extract (100 and 200 mg/kg), in a dose-dependent manner. Previous studies, proved that plant extracts have strong protective effects against the hepatotoxic activity supposed that plant extract components might act as antioxidant activities [66-68].

Effect of C. haematocephala leaves alcohol extract on kidney function:

Table (5): Hepatoprotective effects of alcohol extract of *Calliandra haematocephala* leaves on the level of urea and creatinine of CCl₄ induced hepatotoxicity rats.

Treatments	Urea (mg/dl)	Creatinine (mg/dl)		
Control	22.07 ± 2.48^{d}	$0.41 \pm 0.05^{\circ}$		
Control (++) CCl ₄	63.64 ± 7.04^{a}	0.91 ± 0.097^{a}		
Silymarin at 100 mg/kg b.w	$42.28\pm4.55^{\rm c}$	0.74 ± 0.079^{b}		
C. haematocephala extract at 100 mg/kg b.w	53.45 ± 4.81^{b}	0.75 ± 0.081^{b}		
C. haematocephala extract at 200 mg/kg b.w	52.74 ± 4.72^{b}	0.70 ± 0.062^{b}		
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Data are mean $\pm SD$ of four rats in each group.

Unshared letter between groups are the significance values at P = 0.05 for all parameters. Statistical analysis is carried out using one way analysis of variance (ANOVA) coStat computer program.

The present study showed that administration of CCl₄ to rats caused significant increments in serum creatinine and urea levels compared with the control group. However, administration of *C.haematocephala* leaves alcohol extract at doses of 100 and 200 mg/ kg bw. to the CCl_4 intoxicated rats caused a significant decrease in serum creatinine and urea level compared with control (Table 5 and figures 4,5). Silymarin drug at level 100 mg/kg bw. showed significant effect represented by reduction in the level of urea and creatinine comparing to CCl_4 intoxicated animals. Liver is a vital organ that regulates most of the metabolites, liver disorder (intoxication, damage, injury) resulting in loss of its normal functions that affects other organs such as kidneys. Kidney play an important role of the body by reabsorbing important material and excreting waste products. Creatinine is a waste product that is synthesized in the liver, urea is the main end product of protein catabolism that take place in liver by deamination of amino acids also urea cycle, where ammonia is converted into urea in liver and excreted through urine. Renal diseases as a results of hepatotoxicity which diminished the glomerular filtration that lead to urea retention[62]. Otherwise, administration of CC1₄ causes nephrotoxicity due to liver injury as indicated by significant elevation in serum level of urea and creatinine[69&70]. The present prophylactic experimental studies (Table 5and Figures 4,5) showed that rats administrated by $CC1_4$ causes nephroxicity this was confirmed by significant (P < 0.05) elevation of creatinine and urea levels compared to normal control, this could be attributed to the damage of nephron structural integrity[59]. Administration of *C. haematocephala* alcohol extract in rats treated with CCl₄ caused a recovery from renal injury, as evidenced by significant decreased in the amounts of creatinine and urea in serum within two weeks of treatment, revealing the antioxidant and renal protective effects against injuries induced by CCl₄.

Antioxidant effect of the extract

Prophylactic groups	MDA (µmol/g tissue)	GSH (mmol/g tissue)	CAT (U/g tissue)	SOD (U/mg tissue)
Control	0.83 ± 0.072^{d}	2.96 ± 0.24^{a}	170.72 ± 16.61^{a}	63.96 ± 5.11 ^a
Control (++) CCl ₄	1.83 ± 0.10^{a}	0.92 ± 0.077^{e}	55.20 ± 4.65^{d}	36.17 ± 3.22^{d}
Silymarin at 100 mg/kg b.w	$0.99 \pm 0.088^{\circ}$	1.61 ± 0.088^{bc}	87.94 ± 7.41 ^c	54.96 ± 4.40^{bc}
<i>C. haematocephala</i> extract at 100 mg/kg b.w	1.12 ± 0.088^{b}	1.45 ± 0.12^{cd}	88.28 ± 9.32 ^c	54.11 ± 4.34 ^{bc}
<i>C. haematocephala</i> extract at 200 mg/kg b.w	$0.97 \pm 0.098^{\circ}$	1.72 ± 0.12^{b}	126.16 ± 11.08^{b}	56.68 ± 4.43^{b}

 Table (6): Protective effect of the alcohol extract of Calliandra haematocephala leaves on the level of hepatic antioxidant biomarkers (CAT, SOD, GSH) and malondialdehyde(MDA) of CCl₄ treated rats

Data are mean \pm SD of four rats in each group.

Unshared letter between groups are the significance values at P < 0.05 for all parameters. Statistical analysis is carried out using one way analysis of variance (ANOVA) coStat computer program

Table 6 and figures 6,7 summarize the hepatoprotective effects of alcohol extract of *C.haematocephala* leaves at 100 and 200 mg/kg.bw on the concentrations of MDA, GSH, CAT and SOD of CCl₄ induced intoxication rats. In general, the MDA concentration (table 6, Figures 6,7) was significantly higher in the liver tissue of the intoxicated rats as compared to either the normal rats or those received the alcohol extract, the extract at both doses showed significant reduction in the level of MDA. The hepatotoxicity in CCl₄ rats in terms of antioxidant enzymes GSH, SOD and CAT was significantly (P < 0.05) decreased as compared to the normal control group. Whereas, oral administration of the extracts at 100 and 200 mg/kg.b.w. in rats treated with CCl₄ showed recovery of the activities of antioxidant enzymes and elevation of the concentrations of antioxidant enzymes comparing to CCl₄ induced hepatotoxicity rats, the extract showed significant dose-dependent restoration of antioxidant levels. The results showed that the extract possesses protective effect comparable with silymarin (standard drug).

Effect of the extract on oxidative stress:

Free radical scavengers protects cells against the toxic effect of ROS, when ROS generation exceeds the cellular capacity it causes cellular injury. Direct elimination of ROS by chain reaction terminators such as SOD, CAT and GSH[71]. SOD removes superoxide radicals by converting them into H₂O₂ by CAT and GSH and hence diminishes the toxic effects due free radicals derived from secondary reaction[72&73]. CAT is antioxidative defense which catalyzes the reduction of hydrogen peroxides [74] and involved in detoxification of H_2O_2 concentrations [72]. Glutathione, (GSH) is an important antioxidant[75], decreased glutathione levels is an indicator of increased oxidative stress[76&77]. Exposure liver to CCl₄ causes a series effects that deregulate cell functions and affect the levels of the endogenous enzymatic antioxidant system (CAT, SOD, and GPx). CCl₄ administration causes acute hepatotoxicity and severe oxidative damage in- vivo to cell membranes associated with an increase in lipoperoxidation, as evidenced by an increase in MDA levels and a decrease in the reduced GSH content. The data showed that CCl_4 treated rats resulted in significant decreases in the levels of the hepatic oxidative stress markers CAT, SOD, GSH and increase in the level of MDA compared with control group (Table 6 Figures 6,7). Oral administration of C. haematocephala leaves alcohol extract at dose 100 and 200 mg/kg bw. to intoxicated rats significantly rescued the decreases of CAT, SOD and GSH activities relative to CCl₄ control and silymarin treated groups, while, significantly decreased the MDA formation down to the levels similar to those observed in the control group and recovery toward normality. On the other hand, the extract proved equal efficiency to silymarin (Table 6). The results described here of the extract at two doses enhanced and restored the endogenous antioxidant defense system, and reversed lipid peroxidation in the liver, thereby increasing transcription of CAT and GSH and reducing cell damage which may play an important role in liver protection. Similar results of antihepatotoxic effects described in other plants, under similar condition [78]. Many studies reported that herbal extracts or natural products have marked protective effects against oxidative stress by enhancing antioxidant enzyme activities to prevent CCl₄ induced hepatotoxicity[79-82]. Catalase activity was found to be significantly decreased after a toxic paracetamol dose[83]. These results further support our finding that C. haematocephala leaves alcohol extract at dose 100 and 200 mg/kg bw. reduced hepatic injury in CCl₄-treated rats, a coordinated action of this antioxidant enzyme system being essential for ROS detoxification[84]. Furthermore, the histopathological observations substantiated the

biochemical analysis. CCl_4 caused damage to the hepaticarchitecture and produced histological changes such as vacuole formation, infiltration, necrosis associated with inflammatory of hepatocytes and fatty changes. Treatment with the *C. haematocephala* alcohol leaves extract at doses 100 and 200 mg/kg bw. significantly improved the structure of hepatic cells.

Phytochemical investigation of Calliandra haematocephala alcohol leaves extract revealed the presences of phenolics, flavonoids, saponins, sterols or / triterpenes, alkaloids, and lipoidal matter. Total alcohol extract was found to contain high percentage of total phenolics (31.01 %) and flavonoids (4.7%). From the results obtained it could be explained that the mechanism of liver protection may be due to the presence of phenolic, flavonoids or saponins matter and may be owing to their antioxidant activity. It has been reported that flavonoids of C.haematocephala are responsible for their antioxidant properties [13&12]. Flavonoids represent a beneficial group of naturally occurring compounds with hepatoprotective potentials. The hepatoprotective potential of some flavonoids may be owing to their antioxidant activity and capability of normalization of impaired membrane function activity[85]. Silymarin is a natural extract with hepatoprotective properties composed mainly of flavonolignans, with silibinin being its principal constituent[86]. The antioxidant effect of the extract either in 100 mg/kg bw. or 200 mg/kg bw. may be related to the phenolic, flavonoids content of the extract which play an important role as potent antioxidant activity or in plasma membrane stabilization, as well as in repairing liver damage caused by CCl₄. The results obtained are in agreement with other studies that have reported hepatoprotective and antioxidant effects of extracts containing flavonoids and other phenolics compounds [82&87-89]. Many studies have shown that polyphenols, such as hyperin and rotenone, significantly induce the gene expression of SOD, CAT, and GPx[90] and may also regulate GSH synthesis[91]. In this regard, several studies have reported synergistic physiological effects of mixtures of polyphenols in plant extracts, as well as antioxidant and ROS scavenger effects of these plants. This can be supported by scientific findings on phenolic acids mainly present in plants, the flavonoid rich alcoholic extract of Thuja occidentalis was found to possess good hepatoprotective property against carbon tetrachloride induced liver damage[92]. Total flavonoids isolated from Juglandis mandshuricae cortex extract[93], Rosa laevigata Michx fruit [94,95], Bidens bipinnata[96] showed antioxidant and significant hepatoprotective activities compared to the standard silymarin. As well, as some phenolic compounds can prevent CCl₄-induced hepatic damage owing to their antioxidant properties [96&97]. Apple polyphenols have significant protective effect against acute hepatotoxicity induced by CCl₄ in mice, which may be due to its free radical scavenging effect, inhibition of lipid peroxidation, and its ability to increase antioxidant activity[98].

Methanol extract of *Clitoria ternatea* L. (Family: *Fabaceae*) flower exert antioxidant and hepatoprotective activities against acetaminophen-induced liver toxicity. The hepatoprotective activity may be due to its free radical scavenging and antioxidant activity, resulting from the presence of phenolic compounds in the flower extract[99]. Methanol and aqueous extracts of *Phaseolus trilobus* seeds possess hepatoprotective property and are effective in oxidative stress induced cholestatic hepatic injury[100]. The protective action of *P.trilobus* extracts may be attributed to the presence of the flavonoids[101] which provides maximum conjugation with free radical species generated on bile duct ligation thereby reducing the number of free radicals and extent of cellular damage by decreasing lipid peroxidation, scavenging super oxide radicals and maintaining level of GSH. Both the extracts may enhance the resistance to the hepatocyte membrane damage mediated by bile duct ligation induced liver injury. Saponins plays an important role as antihepatotoxic and therapeutic activities, in this occasion, the protective effect of *C. haematocephala* alcohol leaves extract may be due to saponins content, previous studies demonstrated that total saponins or the pure saponins compounds exhibited potential antihepatotoxic [81&102-106].





Fig.(1) Effect of alcohol extract of *Calliandra* haematocephala leaves on albumin level of CCl₄ treated rats



Fig.(3) Effect of alcohol extract of *Calliandra* haematocephala leaves on total billirubin (mg/dl) of CCl4 treated rats



Fig.(5) Effects of alcohol extract of *Calliandra* haematocephala leaves on the level of urea of CCl4 treated rats

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Fig.(2) Effect of alcohol extract of *Calliandra* haematocephala leaves on the levels of serum ALT, AST and GGT of CCl₄ treated rats



Fig(4) Effect of alcohol extract of *Calliandra* haematocephala leaves on the level of creatinine (mg/dl) of CCl4 treated rats



Fig.(6) Effect of the alcohol extract of *Calliandra* haematocephala leaves on the level of GSH and MDA of CCl4 treated rats



Fig.(7) Effect of the alcohol extract of *Calliandra haematocephala* leaves on the level of CAT and SOD of CCl4 treated rats

Histopathological studies

Histopathological examination of rat's liver of the control group, showed the normal structure of the hepatic lobules (the structural units of the liver); each is formed of cords of hepatocytes and blood sinusoids in between (**Figure 8**). Examination of liver of rat given CCl_4 twice-weekly for two consecutive weeks (**Figure 9**) showed disturbance of the structure of the hepatic lobule, congestion of the central vein , necrosis associated with inflammatory infiltration around the vein, fatty changes were also seen.

Prophylactic effect

Oral administration of silymarin at a dose of 100 mg/kg p.o. daily for two consecutive weeks and simultaneously administered CCl_4 (0.5 ml/kg bw.) twice-weekly for two consecutive weeks (**Figure 10**) revealed that the hepatocytes of the treated rats appeared more or less as normal. In some rats, slight disturbance of the structure of the hepatic lobule, hydropic degeneration was noticed.

Examination of liver of rats given oral alcohol extract of 100 mg/kg bw. daily for two consecutive weeks and simultaneously administered CCl_4 (0.5 ml/kg bw.) twice-weekly for two consecutive weeks (**Figure 11**) showed disturbance of the structure of the hepatic lobule. The hydropic degeneration and a few of foci of necrotic cells were shown. In rats received alcohol extract 200 mg/kg bw. daily for two consecutive weeks and simultaneously administered CCl_4 (0.5 ml/kg bw.) twice-weekly for two consecutive weeks and simultaneously administered CCl_4 (0.5 ml/kg bw.) twice-weekly for two consecutive weeks, liver showed the structure of the hepatic lobule that appeared more or less like normal (**Figure 12**).

Effect of the alcohol extract of *Calliandra haematocephala* leaves at 100 and 200 mg / kg bw. on the histopathological changes in the liver of CCl₄-intoxicated rats



Figure (8): Section in liver of control rat shows the normal structure of the hepatic lobule (H&E, Scale bar: 20 µm).



Figure (9): Section in liver of rat given CCl₄ shows disturbance of the structure of the hepatic lobule. Notice the congestion of the central vein (arrow), necrosis associated with inflammatory infiltration (arrow head) around the vein. Fatty changes (red arrow) are also seen (H&E, Scale bar: 20 µm).



Figure (10): Section in liver of rat co administered with CCl₄ and silymarin (100 mg/kg b.w.) shows disturbance of the structure of the hepatic lobule. Notice the hydropic degeneration (arrows) (H&E, Scale bar: 20 μm).



Figure (11): Section in liver of rat co administered with CCl₄ and *Calliandra* extract (100 mg/kg b.w.) shows disturbance of the structure of the hepatic lobule. Notice the hydropic degeneration (arrows) and foci of necrotic cells (arrow heads) (H&E, Scale bar: 20 μ m).



Figure (12): Section in liver of rat co administered with CCl₄ and *Calliandra* extract (200 mg/kg b.w.) shows the structure of the hepatic lobule that appear more or less like normal (H&E, Scale bar: 20 µm).

CONCLUSION

Sterols, triterpene (lupeol) and fatty acid methyl esters were evaluated for the first time. The results of this study demonstrate that *Calliandra haematocephala* alcohol leaves extract was effective for the prevention of CCl₄-induced hepatic damage in rats and may be partially responsible for the pharmacological effect of hepatoprotection. Moreover, the possible mechanisms of hepatoprotective effect may be attributed to the plant comprised high content phenolic and flavonoids which act as free radical scavenging effect, inhibition of lipid peroxidation, and increased antioxidant activity and this was confirmed by histpathological studies. The results revealed that *Calliandra haematocephala* alcohol leaves extract could be used as hepatoprotective agent.

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Conflict of Interest

The authors declared that there is no conflict of interests.

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