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Original article

Chemical composition and protective effect of *Juniperus sabina* L. essential oil against CCl_4 induced hepatotoxicity

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ABSTRACT

The hepatoprotective activity of the total extract of *Juniperus sabina* L. against CCl_4 induced toxicity in experimental animals was previously reported and indicated promising results. Essential oil of *J. Sabina* was prepared by hydrodistillation method. Components of the oil were identified by comparison of GC-MS and retention indexes with reported data. The hepatoprotective effect of the essential oil against CCl_4 induced toxicity was studied using male Wistar rats and silymarin at 10 mg/kg p.o as standard drug. The protective effect was evaluated via serum biochemical parameters such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma glutamyl-transpeptidase (GGT), and total bilirubin as well as tissue parameters including non-protein sulfhydryl groups (NP-SH), malonaldehyde (MDA) and total protein (TP). Histopathological study was applied on the liver tissues using Mayer's hematoxylin stain, Periodic Acid Schiff – Hematoxylin (PAS-H) and Masson trichrome technique on light microscope. Electron microscope images were also obtained for more detailed study.

1. Introduction

Essential oils are highly concentrated mixtures of saturated and unsaturated hydrocarbons, alcohols, aldehydes, esters, ethers, ketones, oxides, phenols and terpenes (Schiller and Schiller, 1994; Wildwood, 1996). Essential oils are of great importance in therapeutic and cosmetic uses (Evans, 2000). Essential oils obtained from different parts or exudates of the plants are the main therapeutic agents in aromatherapy (Dunning, 2013). Few studies were conducted on the chemical composition and biological activity of *Juniperus sabina* L. essential oil. Essential oils obtained from fruits and leaves of *J. sabina* were proved to posses inhibitory activity against protein glycation and oxidative stress (Asgary et al., 2013). Essential oil from berries and branches of *J. sabina* composed mainly of sabinene and α-pinene showed antioxidant (Emami et al., 2009) and weak antimicrobial activities (Asili et al., 2010). Other studies were concerned with the effect of the geographical source on the oil chemical composition (Adams et al., 2006). Study of *J. phoenicea* and *J. procera* for the search of hepatoprotective secondary metabolites resulted in the identification of hinokiflavone, 4-epi-abietol, sugiol as the most active components (Alqasoumi and Abdel-Kader, 2012; Alqasoumi et al., 2013).

We previously reported on the hepatoprotective effect of *J. sabina* total extract of the aerial part against CCl_4 induced toxicity in rats (Abdel-Kader et al., 2016). In the current study we investigated the chemical composition and hepatoprotective effect of the essential oil obtained from the green branches of *J. sabina*.

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2. Materials and methods

2.1. Plant materials

Aerial parts of *Juniperus sabina* L. (Cupressaceae) were described earlier (Abdel-Kader, 2016).

2.2. Preparation of the oil

The dried aerial parts of *J. sabina* (0.5 kg) were subjected to hydrodistillation for 8 h using a Clevenger apparatus with 5 L rounded-bottomed flask. The condensate was extracted with ether. The ether extract was dehydrated over anhydrous sodium sulfate and evaporated to obtain the essential oil. The yield of the oil was 0.5% w/w.

2.3. GC/MS analysis

The GC/MS analyses was carried out on Gas chromatography Mass spectrometer SHIMAZU model 2010 plus equipped with flam ionization detector (FID). Mass spectrometer model MS-2010-Ultra equipped with electron multiplier detector and Quadruple system analyzer. Auto injector model Aoc-20i. GC, injector and detector temperature were set at 220–290 °C respectively. Column temperature was programmed from 60 to 220 °C at a rate of 4 °C/min, lower temperature held for 10 mins, 220 °C to 290 °C at rate of 5 °C/min. Carrier gas: Helium at a flow rate of 1.5 mL/min. Column: Rtx 5MS, crossbond 5% diphenyl, 95% dimethyl polysiloxane. Sample volume 1.0 mL and split ratio was set at 40:1. The mass analyzer was scanned from m/z 35–450 at a scan rate of (3.46) s^{-1}.

The peak identity was confirmed by comparing their mass spectra against commercial (Wiley GC/MS Library, MassFinder 3 Library) (Zaghloul et al., 1989; McLafferty and Stauffer, 1989).

2.4. GC analysis

GC spectrum obtained under same conditions as the above mentioned conditions was used for identification of peaks by comparison of their relative retention index (RRI) to a series of n-alkanes. The quantitative estimation of each compound was carried out based on computerized peak area measurement (Table 1).

Table 1
Composition of the essential oil of *J. sabina*.

No	Name	RRI	%
1	α-Thujene	928	1.430
2	α-Pinene	940	5.210
3	Sabinene	975	55.820
4	*p*-Cymene	1024	1.160
5	β-Pinene	986	0.540
6	Myrcene	992	1.150
7	Limonene	1029	0.500
8	*Cis*-Sabinene hydrate	1071	0.160
9	Carvone	1089	0.280
10	Linalool	1101	0.420
11	α-Thujone	1108	0.380
12	Verbenone	1120	0.540
13	Limomene oxide	1137	0.520
14	*trans*-Sabinol	1140	0.990
15	Pinocarvone	1161	0.830
16	α-Terpineol	1190	0.340
17	*trans*-Carveol	1205	0.560
18	Geraneol	1229	0.530
19	Citronellol	1232	1.040
20	Linalyl acetate	1235	2.210
21	*trans*-Sabinene hydrate acetate	1256	0.350
22	α-terpinyl acetate	1335	1.280
23	Elemene	1393	0.680
24	*trans*-Caryophyllene	1421	0.750
25	α-Humulene	1454	0.45
26	α-Muurolene	1477	1.070
27	Cadinene	1515	0.420
28	*cis*-Calamenene	1546	7.900
29	α-Calacorene	1563	0.220
30	Dodecanoic acid	1569	1.070
31	Caryophyllene oxide	1582	1.480
32	Humulene oxide	1610	1.070
33	*epi*-Cubenol	1644	2.970
34	Cadalene	1673	1.110
35	Calamenene-10 β-ol	1689	0.850
36	Calamenene-10 α-ol	1697	0.790
	Total%	97.07	

2.5. Animals

Male Wistar albino rats (160–180 g) of similar age (8–10 weeks) and Swiss albino mice of either sex (25–30 g), provided by the Experimental Animal Care Center, College of Pharmacy, Prince Sattam Bin Abdulaziz University, Al-Kharj, KSA, were used. The animals were kept under controlled temperature (22 ± 2 °C), humidity (55%) and light/dark conditions (12/12 h). The animals were provided with Purina chow and free access to drinking water *ad libitum* (Alqasoumi et al., 2009). The experimental and procedures were approved by the Ethical Committee at Prince Sattam Bin Abdulaziz University.

2.6. LD_{50} determination

Acute oral toxicity of the essential oil was evaluated in in Swiss albino mice. Thirty animals were equally divided into five groups (n = 6) as per sex. Group I was kept as control, treated with 1% Tween 80. Other groups received the oil as suspension in Tween 80 at doses of 0.8, 1.6, 3.2 and 6.4 mL/kg by intraperitoneal injection. Animals were observed for symptoms of toxicity for 24 h and at the end of the experiment. The number of mortality in each group were counted. LD_{50} were calculated using Karber's method (1931).

2.7. Hepatoprotective activity

Rats were divided into four groups and four subgroups five animals each. *Group I* received 1% Tween 80 in normal saline and was kept as a control. *Groups II- IX* received 1.25 mL of CCl_4 in liquid paraffin (1:1) per 1 Kg body weight intraperitoneally. *Group II* received only CCl_4 treatment. *Group III* was treated with 10 mg/kg p.o. (20.7 μmole/kg) of silymarin (Sigma-Aldrich, St. Louis, MO, USA) (Abdel-Kader et al., 2016). Groups *IV* was divided into four sub groups *IV*a- *IV*d treated with 50, 100, 150 and 200 mg/kg of *J. sabina* essential oil. Treatment started 5 days prior to CCl_4 administration and continued till the end of the experiment. After 48 h, following $CC1_4$ administration the animals were sacrificed under ether anesthesia. Blood samples were obtained by heart puncture and the serum was separated for biochemical parameters measurements. The livers were immediately removed and representative pieces were immersed in10% formalin for fixation necessary for histopathological study.

2.7.1. Determination of enzyme levels

Serum glutamate oxaloacetate transaminase Aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma glutamyltranspeptidase (GGT), (ALP) and total bilirubin were determined following the reported methods

(Edwards and Bouchier, 1991). The enzyme activities were measured by Reflotron® diagnostic strips (Roche, Basel, Switzerland) and Reflotron® Plus instrument (Roche) (Table 2).

2.7.2. Determination of tissue parameters

Non-protein sulfhydryl groups (NP-SH) were measured following Sedlak and Lindsay method (1968). Livers were cooled in ice bath. Weight of 200 mg of liver tissues was homogenized in 8 mL of 0.02 *M* ethylenediaminetetraacetic acid (EDTA). Aliquots of 5 mL of the homogenate were mixed in 15 mL test tubes with 4 mL of distilled water and 1 mL of 50% trichloroacetic acid (TCA). To precipitate protein, the tubes were shaken for 10–15 min at intervals and centrifuged at 3000 rpm for 15 min. Two mL of the resulted supernatants were mixed with 4 mL of 0.4 *M* Tris buffer, pH 8.9 and 0.1 mL of 0.01 *M* DTNB [5,5′-dithio-bis-(2-nitrobenzoic acid)] and the mixtures were shaken. Five minutes after the addition of DTNB, the absorbance values were measured at 412 nm and compared with blank with no homogenate (Table 3).

For the measurement of MDA level, aliquots from liver homogenate were incubated with shaking at 37 °C for 3 h, mixed with 1 mL of 10% aqueous TCA and centrifuged at 800 rpm for 10 min. From the supernatants, 1 mL from each were mixed with 1 mL aqueous solution of 0.67% 2-thiobarbituric and heated for 10 min on boiling water bath. Mixtures were cooled, mixed with 1 mL distilled water and the absorbance values were measured at 535 nm. The content of MDA (Table 3) (nmol/g wet tissue) were estimated from the calibration curve of MDA solution (Utley et al., 1967).

For the determination of the TP, portions of the homogenate were mixed with 0.7 mL Lowry's solution and kept at room temperature for 20 min in dark then 0.1 mL of diluted Folin's reagent were added. Mixtures were kept for 30 min at room temperature away from light. The absorbance values then measured at 750 nm (Table 3) (Lowry et al., 1951).

2.8. Statistical Analysis

Analysis of variance (ANOVA) test was used to judge whether the difference between groups is significant or not. Non paired samples such as control and CCl_4-treated group were compared for significance using Dunnette's test (Woolson and Clarke, 2002). All the reported values are presented as mean ± S.E.

2.9. Histopathology

The livers samples were dehydrated, cleared and infiltrated by immersion in increasing concentrations of ethanol (70–100%), xylene (3 times, 1 h each) followed by paraffin wax (4 times, 1 h each). The tissues were oriented by hot forceps in moulds and then chilled on cold plates and excess waxes were removed. Thin sections (3 µm) were made using rotary microtome (*Leitz* 1512) and placed onto clean slides. The slides were drained vertically for several minutes and placed onto a warming table at 37–40 °C (Prophet et al., 1994).

Table 3
Effect of *J. sabina* oil on MDA, NP-SH and Total protein in liver tissue of CCl_4-intoxicated rats.

Treatment	MDA (nmol/g)	NP-SH (nmol/g)	TP (g/l)
Control	1.11 ± 0.05	4.80 ± 0.11	114.37 ± 2.82
CCl_4	6.95 ± 0.59***[a]	2.19 ± 0.20***[a]	52.09 ± 2.65***[a]
Silymarin	2.26 ± 0.18***[b]	4.14 ± 0.15***[b]	99.99 ± 3.70***[b]
50 mg/kg	5.69 ± 0.24[b]	2.35 ± 0.20[b]	57.48 ± 2.18[b]
100 mg/kg	4.17 ± 0.04**[b]	2.85 ± 0.20[b]	67.66 ± 2.99**[b]
150 mg/kg	3.09 ± 0.09***[b]	3.31 ± 0.13**[b]	78.44 ± 3.43**[b]
200 mg/kg	1.79 ± 0.27***[b]	4.67 ± 0.43***[b]	83.83 ± 4.88***[b]

All values represent mean ± SEM. *p < 0.05; **p < 0.01; ***p < 0.001; ANOVA, followed by Dunnett's multiple comparison test.
[a] As compared with Control group.
[b] As compared with CCl_4 only group.

2.9.1. Mayer's hematoxylin stain

The slides were stained in Mayer's hematoxylin solution for 15 min after deparaffinization and hydration. The slides were then washed in lukewarm running tap water for 15 min then immersed in 80% ethyl alcohol for two minutes and counterstained in eosin-phloxine solution for 2 min. The slides were then washed with 95% ethyl alcohol, absolute ethyl alcohol, and xylene (2 min each) and finally mounted in resinous medium.

2.9.2. Periodic acid Schiff – Hematoxylin (PAS-H) to study PAS-positive materials

Deparaffinized liver sections were immersed in 1% periodic acid for 10 min, washed with distilled water for 2 min, immersed in Schiff reagent (Product 191203S, BDH Laboratory Supplies, Poole, England) for 10 min, and then washed under running tap water for 10 min. The nuclei were counterstained with Harris's hematoxylin for 2 min, differentiated in acid alcohol 2 dips, rinsed with tap water 2 dips, and blued in running tap water for 10 min, dehydrated, cleared, and a coverslip mounted with DPX (Product 03600, Loba Chemie Pvt. Ltd., Mumbai, India) (Hamad and Ahmed, 2018).

2.9.3. Masson trichrome technique for connective tissue fibers demonstration (mainly collagen)

Deparaffinized liver sections were stained with Weigert's iron hematoxylin for 10 min, washed with water, stained in an acid fuchsin solution for 5 min, rinsed rapidly in water, differentiated in 1% phosphomolybdic acid for about 5 min, drained and counterstained with methyl blue, dehydrated, cleared and mounted sections in DPX (Hamad and Ahmed, 2016).

Table 2
Effect of *J. Sabina* oil on the serum levels of liver injury markers in CCl_4-intoxicated rats.

Treatment	AST (U/L)		ALT (U/L)		GGT(U/L)		ALP(U/L)		Bilirubin(mg/dl)	
	Mean ± S.E	% Change	Mean ± S.E	% Change	Mean ± S.E	% Change	Mean ± S.E	% Change	Mean ± S.E	% Change
Control	113.00 ± 4.08		31.45 ± 2.05		4.10 ± 0.17		357.00 ± 18.49		0.55 ± 0.02	
CCl_4	296.75 ± 7.72***[a]		225.25 ± 12.45***[a]		15.02 ± 0.33***[a]		596.50 ± 11.54***[a]		2.93 ± 0.07***[a]	
Silymarin	139.00 ± 7.16***[b]	53.15	77.62 ± 16.46***[b]	65.53	6.50 ± 0.30***[b]	56.73	411.50 ± 25.10***[b]	31.01	1.04 ± 0.12***[b]	64.27
50 mg/kg	283.75 ± 7.49[b]	4.38	218.00 ± 8.79[b]	3.12	14.22 ± 0.42[b]	5.32	542.00 ± 7.22* [b]	9.13	2.62 ± 0.11[b]	10.65
100 mg/kg	280.25 ± 6.12[b]	5.56	184.75 ± 8.75*[b]	17.98	13.40 ± 0.31*[b]	10.81	533.25 ± 9.10**[b]	10.60	2.44 ± 0.02***[b]	16.53
150 mg/kg	237.50 ± 8.30***[b]	19.96	152.75 ± 6.53***[b]	32.18	11.30 ± 0.31***[b]	24.79	485.00 ± 7.71***[b]	18.69	2.00 ± 0.19**[b]	31.79
200 mg/kg	188.00 ± 9.03**[b]	36.64	129.75 ± 4.78***[b]	43.39	10.45 ± 0.31***[b]	30.44	446.75 ± 7.57***[b]	25.10	1.37 ± 0.06***[b]	53.11

All values represent mean ± SEM. *p < 0.05; **p < 0.01; ***p < 0.001; ANOVA, followed by Dunnett's multiple comparison test.
[a] As compared with Control group.
[b] As compared with CCl_4 only group.

2.10. Electron microscopy

Tissue specimens of about 2–3 mm thick were fixed in 4% glutaraldehyde (Product 16210, Electron Microscopy Sciences (EMS), Hatfield, PA, USA) for 2 h followed by 1% osmium tetroxide (Product 19100, EMS) for 1 h. Then, dehydrated using 70% ethanol for 10 min, 100% ethanol for 10 min, and 100% ethanol for 15 min, 100% propylene oxide (Product 8.07027.1001, Merck KGaA, Darmstadt, Germany) for 15 min × 2. Samples were subjected to infiltration using mixture of EMbed 812 one-step single mix formula composed of 20 mL of EMbed 812 (Product 14900, EMS), 16 mL of Dodecenyl Succinic Anhydride (DDSA) (Product 13710, EMS), 8 mL of Methyl-5-Norbornene-2,3-Dicarboxylic Anhydride (NMA) (Product 19000, EMS) and 0.66–0.88 mL of 2,4,6-Tri (dimethylaminomethyl) phenol (DMP-30) (Product 13600, EMS). Tissues were drained of most of the propylene oxide. Then tissues were soaked in 1:1 solution of propylene oxide: embedding medium for 1 h at room temperature followed by 2:1 embedding medium to propylene oxide at RT overnight. Finally, the mixture was replaced with 100% embedding medium for 2 h at RT. Specimen rotator (Product 15920D, Thermo Fisher Scientific, Carlsbad, CA, USA) was used during fixation, dehydration and infiltration. Embedding was finalized by transferring tissues in EMS embedding capsules (Product 69910-05, EMS) then filled with the embedding medium. Capsules were then incubated at 60 °C in oven for 24 h to make blocks. Ultramicrotome (Product PT-PC #75,840, RMC Boeckeler Instruments, Inc., Tucson, AZ, USA) were used to obtain ultra thin section of 100–200 nm after cooling to room temperature. Sections were loaded on grid (Product G200-Cu, EMS) and stained manually in 1% uranyl acetate (Product 93-2840, STREM CHEMICALS, Newburyport, MA, USA) for 15 min away from light, rinsed with normal saline for 6 times followed by 0.5% lead citrate (Product 17810, EMS) beside several pellets of sodium hydroxide then rinsed in distilled water. Tissues were then dried and examined under transmission electron microscope (TEM) (Product FEI TECNAI 12, Thermo Fisher Scientific, Hillsboro, Oregon, USA) (Luft, 1961, Woods and Stirling, 2013).

3. Results and discussion

The facts that total extract of *J. sabina* showed marked hepatoprotective activity (Abdekl-Kader, 2016) and that essential oils are highly concentrated mixtures of low molecular weight diverse secondary metabolites (Schiller and Schiller, 1994; Wildwood, 1996) initiate the study of the plant essential oil chemical composition and hepatoprotective effect.

Combination of GC-MS and GC analyses of the essential oil of *J. sabina* enable the identification of 36 components representing 97.07% of the oil components. Hydrocarbons represent the major components of the oil as their percentage was 77.14. Sabinene represents 55.820% of the oil components followed by α -pinene (5.210%). Total alcohols represent 9.01% of the oil with the major alcohol citronellol 1.040%. Acetate esters represents 3.84% out of them linalyl acetate represents 2.21%. Caryophyllene oxide represents 1.48% while the total oxides representing 3.07% of the oil. Ketones represent the least component in the oil with total percentage of 2.03 of which pinocarvone represents 0.83% of the oil. Monoterpenes derivatives were the major components of the oil representing 76.24% while sesquiterpenes derivatives represents 19.76%. Only one fatty acid; dodecanoic acid was detected in the oil (Table 1).

The induction of hepatoxicity using CCl_4 cause severe disturbances of calcium homeostasis leading to necrotic cell death (Weber et al., 2003). Significant increase of transaminases (AST and ALT) and alkaline phosphatase (ALP) levels was due to hepatocytes damage (Zafar and Ali, 1998). Severe jaundice was diagnosed via elevated levels of serum bilirubin (Table 2) (Lin et al., 1997).

The standard drug silymarin at a dose of 10 mg/ kg (20.7 μmol/ kg) provides protective effect mediated via; scavenging the free radicals, increase the intracellular concentration of GSH, enhancement of the cellular membrane permeability, stimulation of protein synthesis leading to regeneration of liver cells (Saller et al., 2007; Dehmlow et al., 1996). These effects resulted in normalization of the biochemical and tissue parameters (Tables 2 and 3).

The safety of the oil was accessed via LD_{50} determination following Karber's method (1931). The LD_{50} of the oil was 3.4325 mg/kg. For the hepatoprotective effect four doses were used 50, 100, 150 and 200 mg/kg. The essential oil showed dose dependent hepatoprotective effect. The effect was comparable with that of silymarin at the highest dose used at 200 mg/kg. The protective effect was accessed via the measurement of biochemical and tissue parameters as well as histopathological study. The effect of *J. sabina* essential oil at 200 mg/kg on reducing the levels of aspartate amino transferase (AST), alanine aminotransferase (ALT), gamma glutamyl transpeptidase (GGT), alkaline phosphatase (ALP) and total bilirubin (36.64, 43.39, 30.44, 25.10, 53.11 % respectively) was less than the effect observed in the group of animals treated with silymarin (53.15, 65.53, 56.73, 31.01 and 64.27 % respectively) (Table 2). Tissue parameters such as MDA, NP-SH and total proteins were also measured as a sign for hepatocytes recovery. MDA is the final product of unsaturated fatty acid peroxidation and is markedly increased in the group treated with CCl_4. Treatment with *J. sabina* essential oil at 200 mg/kg resulted in better decrease in the level of MDA (1.79 ± 0.27 nmol/g) toward the normal level (1.11 ± 0.05 nmol/g) than silymarin (2.26 ± 0.18 nmol/g). The level of NP-SH groups in the normal control group was 4.80 ± 0.11 nmol/g and significantly decreased to 2.19 ± 0.20 nmol/g by CCl_4. Treatment with 200 mg/kg of *J. sabina* essential oil significantly ($p < 0.001$) restored the level of NP-SH (4.67 ± 0.43 nmol/g) closer to the normal level than silymarin (4.14 ± 0.15 nmol/g).

Histopathologyical study was conducted using Mayer's hematoxylin stain, Masson trichrome technique and Periodic Acid Schiff – Hematoxylin (PAS-H) on light microscope (Fig. 1). Masson trichrome stain collagen with blue colour and give an indication about fibrosis due to liver injury. Using PAS-H confirms the presence of glycogen in the liver cells (Krishna, 2013). Tissue specimens were also examined by electron microscopy (Fig. 2). Liver cells of the CCl_4 treated group showed degenerative liver tissue, necrosis with complete occlusion of the blood vessels (b-i), large amount of collagen fibers with the blue colour (b-ii), and absence of PAS positive materials indicating injured poor functioning capacity of hepatocytes (b-iii). Electron microscope pictures (Fig. 2) showed marked degeneration, necrosis and vacuolization of hepatocytes (b-i). The nuclei showed clumps of chromatin materials (b-ii) and abnormal pattern of dissociated Golgi apparatus (b-iii). Treatment with Silymarin prior to CCl_4 provided clear evidence of healing and regaining microanatomical architecture of liver tissue (Fig. 1). However, some veins still suffering from hyperemia and partial occlusion (c-i), reduced amount of collagen fibers (c-ii) and very few amounts of PAS positive materials were observed (c-iii). Electron microscopy (Fig. 2) showed restored cellular and nuclear membranes with decreased vacuolization and presence of fat droplets (c-i), regaining many normal nuclei without clumps (c-ii), normal cytoplasm with normal Golgi apparatus and very few small fat droplets are present in the cytoplasm (c-iii).

Liver cells of CCl_4 and 150 mg/kg *J. sabina* essential oil treated group (Fig. 1) showed partial healing of central veins (d-i), moderate amount of collagen fibers (d-ii), and moderate amount of PAS

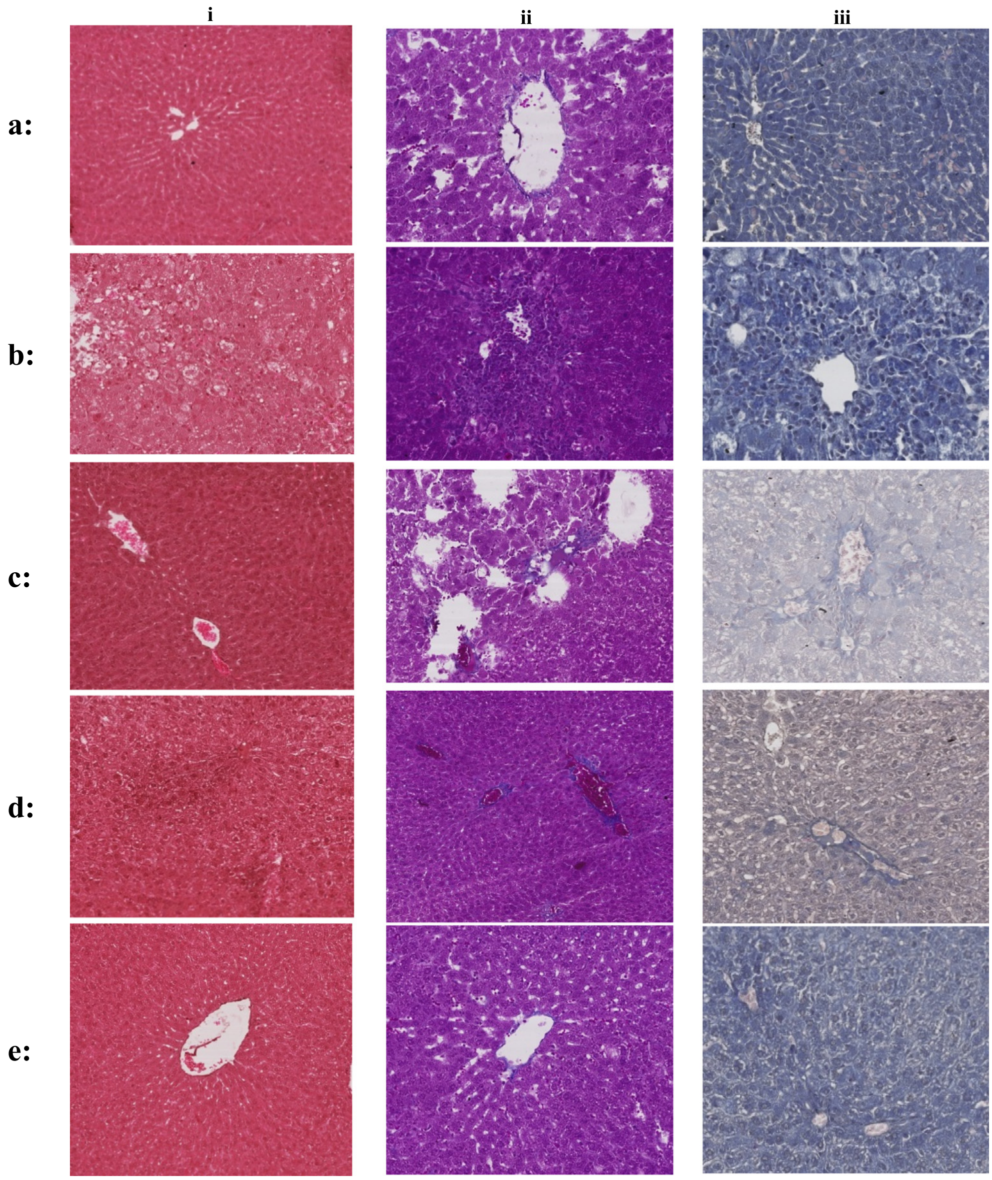

Fig. 1. Light microscope pictures liver tissues stained with **i** Mayer's hematoxylin stain **ii:** Masson trichrome technique **iii:** Periodic Acid Schiff – Hematoxylin (PAS-H), (**a**) Liver cells of normal control group. (**b**) Liver cells of CCl_4 treated group **i:** Degenerative liver tissue, necrosis accompanied by occlusion of blood. **ii:** Large amount of collagen fibers taking the blue colour. **iii:** Absence of PAS positive materials which indicates illed hepatocyte with very low functioning capacity. (**c**) Liver cells of CCl_4 & silymarin treated group **i:** Clear evidence of healing and regaining microanatomical architecture of liver tissue. Hyperemia still appears in some central veins without complete occlusion **ii:** Improvement by lowering the amount of collagen fiber but not completely disappeared **iii:** Very few amount near to absence of PAS positive materials. (**d**) Liver cells of CCl_4 & 150 mg treated group **i:** Healing process start but still degeneration and necrosis and hyperemia in central veins **ii:** Moderate amount of collagen fibers **iii:** Moderate amount of PAS positive materials which indicates healing starts but cells did not completely recovery. (**e**) Liver cells of CCl_4 & 200 mg treated group **i: ii: iii:** All shows complete recovery and almost normal cells appearance.

positive materials (d-iii) indicating partial healing. Treatment with CCl_4 and 200 mg/kg *J. sabina* essential oil resulted in complete recovery of liver cells (e). Electron microscopy for liver specimen of animals treated with of CCl_4 and 150 mg/kg *J. sabina* essential oil (Fig. 2) showed partial recovery indicated by some degeneration, necroses (d-i), regaining intact nuclear membrane but still some uneven distribution of chromatin material in the nucleus (d-ii), partial degenerated pattern of Golgi apparatus, weak cytoplasmic production and very few fat materials but no fat droplets (d-iii). Liver cells of CCl_4 and 200 mg/kg *J. sabina* essential oil treated group showed almost normal appearance of nucleus, functioning cytoplasm and fat materials (e-i), very high improvement and healing activity by regaining intact nuclear membrane and even distribution of chromatin material in the nucleus (d-ii). The cytoplasm shows normal pattern of Golgi apparatus and very few fat materials with complete absence of fat droplets.

4. Conclusion

The yield of the essential oil from the aerial parts of *J. sabina* was 0.5% w/w. GC-MS study of the oil enable the identification of 36 components representing 97.07% of the oil components. Sabinene was the major components of the oil representing 55.820% followed by α -pinene (5.210%). The LD_{50} of the oil found to be 3.4325 mg/kg. The essential oil showed dose dependent hepatoprotective effect. The effect was comparable with that of silymarin at the highest dose used 200 mg/ml. The effect of the oil on

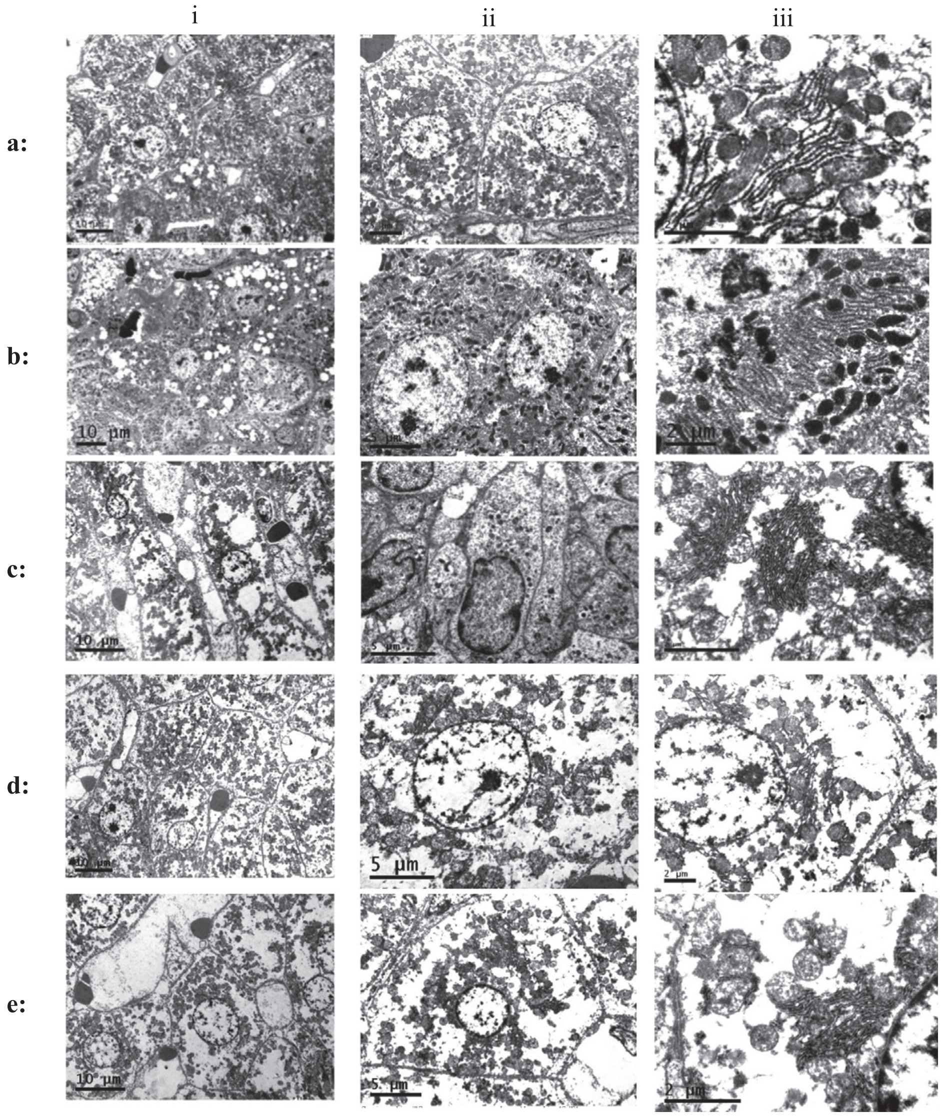

Fig. 2. Electron microscope pictures of liver tissues **i:** General **ii:** Nuclear study **iii:** Cytoplasmic study. (**a**) Liver cells of normal control group. (**b**) Liver cells of CCl_4 treated group **i:** Degeneration and necrosis of hepatocytes. Also increased presence of vacuoles **ii:** Unusual multiple clumps of chromatin materials **iii:** Abnormal pattern of dissolved Golgi apparatus. (**c**) Liver cells of CCl_4 & silymarin treated group **i:** Intact cellular and nuclear membrane associated with decreased amount of cytoplasmic vacuolization and normal presence of fat droplets **ii:** High healing activity by regaining many normal nuclei without clumps **iii:** Normal cytoplasm with normal Golgi apparatus and very few small fat droplets are present in cytoplasm. (**d**) Liver cells of CCl_4 & 150 mg treated group **i:** Normal cells with moderate regaining of functioning cytoplasm. Also, shows cells with remaining effects of degeneration and necrosis. **ii:** Moderate improvement and healing activity by regaining intact nuclear membrane but still uneven distribution of chromatin material in the nucleus (i.e. some clumps remains) **iii:** Cytoplasm shows degenerated pattern of Golgi apparatus, weak cytoplasmic production and very few fat materials but no fat droplets. (**e**) Liver cells of CCl_4 & 200 mg treated group **i:** Almost normal appearance of nucleus, functioning cytoplasm and fat droplets **ii:** Very high improvement and healing activity by regaining intact nuclear membrane and even distribution of chromatin material in the nucleus. **iii:** Cytoplasm shows normal pattern of Golgi apparatus, normal production of cytoplasm and very few fat materials but no fat droplets.

reducing the level of MDA toward the normal level and restoring NP-SH groups was superior to the effect observed with silymarin treatment. Light microscope pictures showed complete recovery of cells in the group treated with CCl_4 and 200 mg/kg *J. sabina* essential oil. Similarly, electron microscope study revealed very high level of protection for the same group which received CCl_4 and 200 mg/kg *J. sabina* essential oil. The fact that the LD_{50} is fifteen fold higher than the effective dose gives an indication about the safety of the essential oil for use.

Declaration of Competing Interest

The authors declare that; there is no conflict of interest.

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