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Phytochemical and biological studies of *Latania verschaffeltii* leaves family (Palmae)

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In Loving Memory of Late Professor Doctor ""Mohamed Refaat Hussein Mahran""

Abstract

This study aimed to identify the chemical constituents of the aqueous methanolic extract from the *Latania verschaffeltii* leaves and evaluate the biological activity. The extract was chromatographed on polyamide S6 column and eluted with H_2O/MeOH mixtures of decreasing polarity, yielding seven phenolic compounds, which were identified using ^1H and ^{13}C NMR. Hepatoprotective activity of extract was evaluated for liver damage caused by CCl_4 in rats by biochemical assays on serum and antioxidant enzyme as well as histopathological studies. Administration of the extract (100 and 200 mg/kg) for 21 days showed that at dose 200 mg showed significant reduction in serum ALT and AST levels to their respective standard value, significant decreases in MDA (1.21 nmol/ml) & NO (45 um/ml) and significant increases in GSH (1.05 µg / ml), TAC (1.6 µg / ml) and SOD (1.92 um/ml). Histopathological studies of liver showed moderate changes in liver histoarchitecture when compared to the CCl_4 group. Furthermore, DPPH study revealing a promising antioxidant activity was observed (IC_{50} = 44.68±1.67 µg / ml) when compared to trolox. The extract of the *L. verschaffeltii* leaves afforded seven phenolic compounds for the first time from this species, with promising hepatoprotective and antioxidant activities. In conclusion, the treatment with the extract of the *L. verschaffeltii* leaves extract could reduce damage induced by CCl_4. The mechanism of protection including the inhibition of lipid peroxidation, increasing the content of GSH, elevating the expression of antioxidant enzymes, all of which result in recuperation of biological parameters.

***Keywords*:** *Latania verschaffeltii*, Hepatoprotctive activity, Antioxidant activity, Phenolic compounds, Flavonoids

Introduction

Herbal medicines have been used for centuries to treat both human and animal diseases around the world, and various pharmacological studies are still validating their uses. Many studies have focused on a range of biological activities, including antioxidants, antibacterial and anti-inflammatory properties, as well as cytotoxic properties. Nowadays, there's a lot of interest in using natural products because they don't have as many side effects as synthetic drugs [1]. Phenolic compounds are known to be safe natural antioxidants, as they reduce the progression of a variety of diseases by blocking free radicals. The liver is widely regarded as the primary organ involved in the detoxification and regulation of metabolic processes in the body, and its associated disorders are numerous and without effective treatments [2]. Despite this, the quest for new medicinal products is ongoing. Many traditional plant-based remedies have been used for centuries to treat liver disorders.

The Palmae is a widely-recognized family of plants, consisting of 181 genera and approximately 2600 species. It is often referred to as the Princess of the Plant Kingdom, and is considered to be the third most significant plant family for human consumption. Palm plants are characterized by their large, evergreen fronds, which are arranged at the apex of a stem without any branches [3]. The Palmae family is renowned for its economic significance human beings, encompassing a wide range of products, such as coconut, oils, and dates, as well as palm, ivory, carnauba, and rattan cane, as well as raffia and palm wood [4].

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The Palmae are known for having a lot of different types of compounds in their roots, leaves, fruits, and seeds, include fatty acids, amino acids, phenolic acids, flavonoids, tannins and alkaloids [5].

The Palmae family has been found to possess a wide range of biological properties, including antimicrobial, especially antifungal and antibacterial, antiviral activity, as well as anti-inflammatory and antioxidant properties, as well as plasmodial, anti-allergy properties, cognitive activity and cytotoxicity properties [6].

The family has been neglected chemically, probably because of the difficulty of collecting fresh material and getting it authenticated [7]. *L. verschaffeltii* no information was available dealing with phytochemical and biological activities. Therefore, this study focuses on the isolation and identification of secondary metabolites in the aqueous methanol extract obtained from the plant leaf. It also aims to determine the total phenolic, flavonoid, *in vitro* antioxidant and *in vivo* hepatoprotective activities of the extract.

Materials and Methods

General experimental procedure

^{1}H NMR and ^{13}C NMR spectra were obtained on Bruker AMX-400, Avance 400, and Avance 300 spectrometers (Bruker, Rheinstetten, Germany) with standard pulse sequences operating at 400, 300 MHz in ^{1}H NMR and 100, 75 MHz in ^{13}CNMR. Chemical shifts are given in δ values (ppm) using DMSO as the internal standard. UV spectra were recorded with Shimadzu UV-1601 (Shimadzu, Tokyo, Japan).

Column chromatography (CC) was carried out on polyamide S6 column for column chromatography (Merck, Germany).and Sephadex LH-20 (Fluka, Pharmazia, Uppsala, Sweden); PC was carried out on Whatman No. 1 and 3 mm paper using the following solvent systems: (1) BAW (*n*- BuOH/HOAc/H2O, 4:1:5); (2) H_2O and (3) AcOH/H2O (15:85). 1, 1- Diphenyl-2-picrylhydrazyl (DPPH), trolox, gallic acid, rutin and other chemicals were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Folin-Ciocalteu solution and 95% ethanol were purchased from Merck Co. (Santa Ana, CA, USA).

Chemicals and biochemical

CCl_4 was purchased from Merck, Germany. Silymarin was acquired from Sedico Pharmaceutical Co, 6th of October City, Egypt. For serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), hepatic reduced glutathione (GSH), total antioxidant capacity (TAC), nitric oxide (NO), malondialdehyde (MDA), superoxide dismutase (SOD), biodiagnostic kits (Biodiagnostic Co., Dokki, Giza, Egypt) were acquired. Solvents and all other used chemicals were of analytical quality.

Plant material

L. verschaffeltii leaves were gathered from El-Abd Garden, Alexandria-Egypt desert road during March 2018. The samples were separately air-dried in the shed, powdered, and kept in tightly sealed round flasks and stored for biological studies. Identification of the plant was confirmed by Prof Dr. Ibrahim El-Garf, Professor of Plant Taxonomy, Department of Botany, Faculty of Science, Cairo University, Egypt. A voucher specimen (No. 13-03-2018-2) has been deposited in the Herbarium, Faculty of Science, Cairo University.

Extraction and isolation

L. verschaffeltii leaves (1.75 kg) were crushed, then the methanolic extract was evaporated under decreased pressure, leaving behind 120 gm of lyophilized material. A sample (100 gm) of the dry extract was fractionated by liquid/liquid fractionation by different solvents with different polarity to give two fraction chloroform and *n*-butanol. Then the *n*-butanol polar fraction. (45 gm) was fractionated by chromatography on polyamide 6 column (45 cm l x 3 cm d, 500 g) and elution was started with water and then with a mixture of water/ methanol with gradual increase of methanol in water till 100% methanol.by 25 % increment.

Similar fractions (500 mL each) were pooled together after investigation by paper chromatography (PC) using n-butanol: acetic acid: water (BAW) (4:1:5) and 15 % acetic acid as solvent system as a developing solvent, and the similar fractions were collected together to give five major fractions (**I-V**).

Five collective fractions were obtained and concentrated under vacuum, fraction III and IV were subjected to further column chromatography for isolation and purification of flavonoids. The main two fractions (**III** and **IV**) were applied on Sephadex column LH-20 CC with MeOH-H_2O (30 %). The first subfraction was purified using Sephadex column LH-20 CC and MeOH-H_2O (10%) as the solvent system, yielding sub fraction (1.3 gm) that were then further purified using Sephadex CC and MeOH HPLC, yielding pure samples of compound 1 and 2 (45 mg) and 3 (25 mg). The second subfraction was purified using Sephadex column LH-20 CC and MeOH-H_2O (30%), resulting in two compounds that were then further purified using Sephadex CC and MeOH -H_2O (30%), yielding pure samples of 4 (22 mg) and 5 (32 mg).

Applying fraction IV to Sephadex column LH-20 CC with MeOH-H2O (30 %) as the solvent system resulted in two subfractions, the first sub fraction was purified using Sephadex column LH-20 CC and MeOH-H2O (30%), resulting in one compounds which was then further purified on Sephadex LH-20 CC using MeOH-H2O (10%) to produce the pure compound of 6 (17 mg). while on purification of the second subfraction (110 mg) using MeOH-H_2O (30 %). resulted in the formation of one compounds that were then further purified on Sephadex LH-20 CC using MeOH-H_2O (10%) to produce the pure compound of 7 (15 mg).

Quantification of total phenolic content (TPC)

TPC was carried out using the Folin-Ciocalteu colorimetric method described by [8]. Five mg of MeOH extract dissolved in 5 ml of 50% MeOH to prepare a solution of final concentration, 1 mg/ml. An aliquot (2 ml) of the extract and standard solution of gallic acid (8-100 µg/ml) was added to 25 ml volumetric flask containing 1.5 ml of Folin-Ciocalteu reagent and 4ml of 20% Na_2CO_3 solution, then the solution was diluted to 25 ml with distilled water. The absorbance was measured, after 30 min at 765 nm using spectrophotometer, against a blank prepared at the same time using 2 ml of distilled water instead of the standard solution. All concentrations were carried out in triplicate. (TPC) were expressed as gallic acid equivalent (GAE).

Quantification of total flavonoid content (TFC)

Total flavonoid concentration of plant extract and fractions was determined according to the reported procedure by [9]. 100 µl of plant extract (10 mg/ml) in methanol was mixed with 100 µl of 20 % $AlCl_3$ in methanol and a drop of acetic acid and then diluted to 5 ml with methanol. The absorbance was measured at 415 nm after 40 min against the blank. The blank consisted of all reagents and solvent without $AlCl_3$. All determinations were carried out in triplicate. The total flavonoid concentration was expressed as rutin equivalents.

Determination of *in-vitro* antioxidant activity

The free radical scavenging activity of the extracts, based on the scavenging activity of the stable 1,1-diphenyl-2- picrylhydrazyl (DPPH) free radical, was determined by the method described by [10].

A methanolic solution of DPPH (400 µM), the tested extracts dissolved in methanol were added at different concentrations (0.1-100 µg/ml). After 20 min the decrease in the absorbance of test mixture (due to quenching of DPPH free radical) was measured at 517 nm. Methanol was used as a blank, the methanol and DPPH solution as a baseline control (A0) and trolox as positive control.

The capability of scavenging DPPH radical was then calculated by using the following equation:

Scavenging effect (%) = $(A_0 - A_1) \times 100 / A_0$

Where A_0 is the absorbance of the control reaction and A_1 is the absorbance in the presence of the sample of the tested extracts. The IC_{50} was calculated as the amount of antioxidant present in the sample necessary to decrease the initial DPPH concentration by 50%. The least IC_{50} the highest the effectiveness [11].

The IC_{50} was calculated graphically using a calibration curve in the linear range by plotting the extract concentration versus the corresponding scavenging effect.

Determination of hepato-protective Activity

Toxicity study

The LD_{50} of the aqueous methanolic extract of *L. verschaffeltii* was estimated according to karber's procedure (1931). Male albino mice (100-120 gm) were divided into groups, each of six animals. Preliminary experiments were carried out to determine the minimal dose that kills all animals (LD_{100}) and the maximal dose that fails to kill any animal. Several doses at equal logarithmic intervals were chosen in between these two doses; each dose was injected in a group of six animals. The number of dead animals in each Biologically guided study group, 24 hrs after injection was determined and the median lethal dose (LD_{50}) was calculated

Determination of *in-vivo* hepatoprotective activity

Animals

Adult male Sprague-Dawley rats (100–150 g) were used in this study. They were purchased from the Animal House, National Research Center, Egypt. All animals were kept in a controlled air environment (57% ± 2% moisture and 12:12 hours light and dark photo-cycle) and 22°C ± 2°C temperature with access to water and ad libitum diet. All experimental methods followed the ethical rules of the Misr University for Science and Technology Ethics Committee in Egypt (PG-1). The animals were observed daily to guarantee that at any point of the investigations the animals did not tolerate. CCl_4-induced hepatotoxicity study was performed as stated by [12]**.**

Experimental design

Forty-two were distributed into seven groups, 6 rats in each as follows:

Group I: rats were administrated saline orally (1 ml/ kg b. wt.) for 21 days as normal control.

Groups II and III: rats were orally administrated *L. verschaffeltii* extract at two different doses (100 and 200 mg/kg b. wt., respectively), alone for 21 days.

Group IV: rats were orally administrated saline for 15 days followed by a single dose of CCl_4 (1.5 mg/kg b.wt.) twice weekly until day 21.

Group V: the reference drug silymarin (25 mg/kg b.wt.) was administrated orally for 15 days and CCl_4 (1.5 mg/kg b.wt.) was conducted twice a week until day 21.

Groups VI and VII: *L. verschaffeltii* extract (100 and 200 mg/kg b.wt., respectively) was administrated orally for 15 days, followed by CCl_4 (1.5 mg/kg, b.wt.) twice a week until day 21.

All experimental methods followed the ethical rules of the Misr University for Science and Technology Ethics Committee in Egypt. The animals were observed daily to guarantee that at any point of the investigations the animals did not tolerate

Biochemical assays

A test-reagent kit developed according to the method described by [13] was used to colorimetrically measure the activity of the serum enzymes (AST and ALT). The superoxide dismutase (SOD) activity in rat liver cytosol was measured as the degree of inhibition of auto-oxidation of pyrogallol at an alkaline pH according to the method described by [14]. The method used by [15] preserved hepatic nitric oxide level. According to [16] approach, the MDA level estimated. The method used by

[17] to estimate TAC level. The level of reduced glutathione (GSH) was determined in rat liver homogenate according to the method described by [18].

Blood sample preparation

By the end of the experiment, blood samples were obtained from the retro-orbital vein plexuses, under ether anesthesia. In clean, dry test tubes, sera were extracted from the acquired blood samples by centrifuging at 1,500 rpm (4°C) for 10 minutes while allowing the blood to coagulate. For additional analyses of the levels of liver enzymes and the evaluation of hepatic oxidative stress markers, the clear supernatant serum was maintained at 20°C. Additionally, liver tissue was isolated for histological analysis.

Histopathological studies

Liver sections were cut out and washed quickly with normal saline, then handled thoroughly for histopathological observation. First, the materials were placed in 10% buffered neutral formalin and paraffin, then layers of 5 *μm* thickness were sorted out in alcohol-xylene series and stained with alum hematoxylin and eosin. For histopathology transformation, the liver sections were examined through light electric microscope [19].

Statistical analysis

All information in each group was shown as mean ± SD of six rats. Among several other procedures, we implanted the one-way variance assessment analysis of variance (ANOVA) for statistical analysis, followed by the least significant difference at $p < 0.05$.

RE SULTS AND DISSCUSSION

Phytochemical results

Seven known phenolic compounds as shown in figure 1 were isolated for the first time from *L. verschaffeltii* leaves from the aqueous methanolic extract. The compounds were identified as; Apigenin-7-***O***- *β*-D-glucopyranoside (**1**), Luteolin-6-*C*- *β*-D glucopyranoside (Isoorientin**)** (**2**) apigenin-8-*C*-*β*-D-glucopyranoside (Vitexin) (**3**), apigenin-6-*C*-*β*-D-glucopyranoside (isovitexin) (**4**), luteoline-8-*C*-*β*-D glucopyranoside (Orientin) (**5**), their aglycone; apigenin (**6**), and luteolin (**7**). by using different spectroscopic analyses and comparing with previously reported data.

Characterization and identification of the isolated compounds

Apigenin-7-*O*- *β*-D-glucopyranoside (1)

yellow amorphous powder, R_f values (x100) 21 (HOAc-15), 52 (BAW). UV Spectral Data λmax (nm): MeOH: 265, 333; NaOMe: 270, 389; +NaOAc: 267, 355, 385;+NaOAc /H_3BO_3: 268, 337; +$AlCl_3$): 275, 302, 345, 385; +$AlCl_3$/ HCl: 277, 299, 341. **^{1}H NMR** (400 MHz, DMSO-d6) δ (ppm): 7.95 (d, J = 8 Hz, H-2', 6'), 6.95 (d, J = 8 Hz, H-3', 5'), 6.85 (s, H-3), 6.83 (d, J = 2.5 Hz, H-8), 6.45 (d, J = 2.5 Hz, H-6). 5.05 (d, J = 7.5 Hz, H-1"), 3.15-3.58 (m, rest of glucose protons.**^{13}C NMR** (100 MHz, DMSO-d6) δ (ppm): 164.8 (C-2), 103.4 (C-3), 182.4 (C-4), 162.3 (C-5), 100.0 (C-6), 163.4 (C-7), 95.3 (C-8), 157.4 (C-9), 105.8 (C-10), 121.2 (C-1'), 129.1 (C-2'), 116.6 (C-3'), 161.6 (C-4'), 116.6 (C-5'), 129.1 (C-6').100.4 (C-1"), 73.6 (C-2"), 76.9 (C-3"), 70.1 (C-4"), 77.7 (C-5"), 61.1 (C-6").

Luteolin-6-*C*- *β*-D glucopyranoside (Isoorientin) (2)

yellow amorphous powder, R_f values (x100) 13 in (HoAC-15%), 17 in (BAW).; UV Spectral Data λmax (nm): MeOH: 242sh, 255, 271, 349; +NaOMe :267,278sh,337sh,406; +NaOAc: 276, 323, 393; +NaOAc /H_3BO_3: 265, 377, 429; +$AlCl_3$): 265, 279, 296sh, 361,384. **^{1}H NMR** (400 MHz, DMSO-d6) δ (ppm): 7.43 (1H, dd, *J* = 2Hz & 8.1Hz, H-6′), 7.40(1H, d, *J* = 2Hz, H-2′), 6.90 (1H, d, *J* = 8.1Hz, H-5′), 6.67 (1H, *s*, H-3), 6.48 (1H, s, H-8), 4.59 (1H, d, *J*=9.8, H-1″), 3.10-3.93 (5H, m, H-2″— H-6″). **^{13}C NMR** (100 MHz, DMSO-d6) δ (ppm): 164.07 (C-2), 103.20 (C-3), 182.28 (C-4), 161.10 (C-5), 109.34 (C-6), 164.00 (C-7), 93.98 (C-8), 93.98 (C-8), 156.67 (C-9), 103.75(C-10), 121.80 (C-1′), 113.70 (C-2′), 146.25 (C-3′), 150.28 (C-4′), 116.51 (C-5′), 119.41 (C-6′),73.52 (C-1″),71.07 (C-2″), 79.42 (C-3″),70.64 (C-4″), 82.03 (C-5″), 61.94 (C-6″).

Apigenin-8-*C*-*β*-D-glucopyranoside (Vitexin) (3)

yellow amorphous powder, R_f values (x100) 0.25 in (HOAc-15%), 0.28 in BAW; UV Spectral Data λmax (nm): MeOH: 271, 332, 364,+NaOMe: 280, 394,+NaOAc: 277, 384,+NaOAc/H3BO3: 273, 335,+AlCl3: 278, 348,+AlCl3/HCl: 278, 348; **^{1}H NMR** (400 MHz, DMSO-d6) δ (ppm): 13.135 (s, OH-5), 10.80(s, OH-7), 7.97 (d, J=8Hz, H-2', 6'), 6.92 (d, J=8Hz, H-3', 5'), 6.69 (s, H-3), 6.24 (s, H-6), 5.01 (d, J=9.6Hz, H-1"), 4.73 (s, OH-2"), 4.57 (s, OH-3"), 3.86 (s, OH-4"), 3.73 (d, J=8.4Hz, Ha-6"), 3.17-3.54 (5H, m, H-2", H-3", H-4", H-5", H-6b"), (overlapped the rest sugar protons). **^{13}C NMR** (100 MHz, DMSO-d6) δ (ppm): 164.01 (C-2), 102.65 (C-3), 182.19 (C-4), 161.80 (C-5), 98.85 (C-6), 167.53 (C-7), 105.10 (C-8), 156.49 (C-9), 104.16 (C-10), 122.01 (C-1'), 129.23 (C-2'), 116.40 (C-3'), 160.82 (C-4'), 116.40 (C-5'), 129.323(C-6'), 71.39 (C-1"), 71.06 (C-2"), 79.17 (C-3"), 70.19 (C-4"), 82.14 (C-5"), 62.21 (C-6").

Apigenin-6-*C*-*β*-D-glucopyranoside (isovitexin) (4)

yellow amorphous powder, R_f values (x100) 0.50 in (HOAc-15%), 0.60 in BAW; UV Spectral Data λmax (nm): MeOH: 332, 272; +NaOMe: 339, 332 sh, 281;, +NaOAc: 391, 305 sh, 281; +NaOAc/H_3BO_3: 350, 400 sh, 278; +$AlCl_3$: 340, 385, 305, 278; +$AlCl_3$/HCl: 340, 385, 365, 278; **^{1}H NMR** (400 MHz, DMSO-d6) δ (ppm): 7.92 (2H, d, J = 8.8 Hz, H-2′/6′), 6.94 (2H, d, J = 8.8 Hz, H-3′/5′), 6.74 (1H, s, H-3), 6.50 (1H, s, H-8), 4.61 (1H, brs, H-1″), 3.17-3.70 (5H, m, H-2″-H-6″). **^{13}C NMR** (100 MHz, DMSO-d6) δ (ppm): 182.30 (C-4), 164.90 (C-2), 164.40 (C-7), 163.96 (C-5), 161.54 (C-4′), 156.96 (C-9), 128.71 (C-2′/6′), 121.70 (C-1′), 116.47 (C-3′/5′), 109.47 (C-6), 103.74, (C-10),103.30 (C-3), 94.58(C-8), 81.66 (C-5″), 79.44 (C-3″), 73.33 (C-1″), 71.15 (C-2″), 7o.72 (C-4″), 61.74 (C-6″).

Luteoline-8-*C*-*β*-D glucopyranoside (Orientin) (5)

yellow amorphous powder, R_f values (x100) 0.17 in (HOAc-15%)) and 0.35 in (BAW); UV Spectral Data λmax (nm): MeOH: 255, 267, 293(sh),346, +NaOMe: 268, 278sh, 334sh, 405, +NaOAc: 287, 325, 386, +NaOAc/H_3BO_3: 264, 375, 430(sh), +$AlCl_3$: 276, 302sh, 329, 429, +AlCl3/HCl: 265, 276, 296sh, 357, 384; **^{1}H NMR** (400 MHz, DMSO-d6) δ (ppm):7.92 (1H, dd, J = 8.3Hz & 2Hz, H-6′),7.41 (1H, d, J = 2Hz, H- 2′), 6.91 (1H, d, J=8.3Hz, H-5′), 6.66 (1H, s, H-3), 6.53 (1H, s, H-6), 4.70 (1H, d, J = 9.1Hz, H-1″), 3.17- 3.99 (5H, m, H-2′- H-6′). **^{13}C NMR** (100 MHz, DMSO-d6) δ (ppm): 182.36 (C-4), 164.23 (C-2), 163.89 (C-7), 161.01 (C-5), 156.77 (C-9), 150.20 (C-4′), 146.15 (C-3′), 121.81 (C-1′), 119.47 (C-6′), 116.56 (C-5′), 113.60 (C-2′), 109.07 (C-10), 103.74(C-3), 103.13 (C-6), 94.10 (C-8), 81.76 (C-5″), 79.25 (C-3″), 73.45 (C-1″), 70.89 (C-2″), 70.61 (C-4″), 61.81 (C-6″).

Apigenin (6)

yellow amorphous powder, R_f values (x100) 0.12 in (HOAc-15%); UV Spectral Data λmax (nm): MeOH: 267, 296sh, 336, +NaOMe: 275, 324, 392, +NaOAc: 274, 301,376, +NaOAc/H3BO3: 268, 302sh, 338, +AlCl3: 276, 301, 348, 384, +AlCl3/HCl: 276, 299, 340, 381; **^{1}H NMR** (400 MHz, DMSO-d6) δ (ppm): 7.92 (2H, d, J=8.5, H-2′, H-6′), 6.91 (2H, d, J=8.5, H-3′, H-5′), 6.75 (1H, s, H-3), 6.44 (1H, d, J=2.5, H-8), 6.14 (1H, d, J=2.5, H-6). **^{13}C NMR** (100 MHz, DMSO-d6) δ (ppm): 170.32 (C-2), 103.60 (C-3), 183.69 (C-4), 163.15 (C-5), 100.93 (C-6), 166.08 (C-7), 95.65 (C-8), 163.93 (C-9), 104.65 (C-10), 123.25 (C-1′), 129.42 (C-2′, C-6′), 117.14 (C-3′, C-5′), 159.65 (C-4′).

Luteolin (7)

R_f values (x100) 0.28 in (BAW); UV Spectral Data λmax (nm): MeOH: 252, 265, 290sh, 347, +NaOMe: 266sh, 329sh, 401., +NaOAc: 269, 326sh, 384, 281, +NaOAc/H3BO3: 259, 301sh, 370, 430sh, +AlCl3: 274, 300sh, 328, 426, +AlCl3/HCl: 266sh, 275, 294sh, 355, 385; **^{1}H NMR**: δ (400 MHz, DMSO), δ (ppm): 7.40 (d, J = 2.1 Hz, H-2'), 7.39 (dd, J = 8.5 Hz and J = 2.1 Hz, H-6'), 6.85 (d, J = 8.5 Hz, H-5'), 6.60 (s, H-3), 6.4 (d, J = 2.5 Hz, H-8), 6.15 (d, J = 2.5 Hz, H-6). **^{13}C NMR** (100 MHz, DMSO-d6) δ (ppm): 163.80 (C-2), 102.80 (C-3), 181.60 (C-4), 157.30 (C-5), 98.90 (C-6), 164.40 (C-7), 93.80 (C-8), 161.50 (C-9), 103.60 (C-10), 121.40 (C-1'), 113.30 (C-2'), 145.80 (C-3'), 149.80 (C-4'), 116.00 (C-5'), 118.90 (C-6').

Quantification of total phenolic content (TPC)

The TPC in the methanolic extract of leaves *L. verschaffeltii* was calculated as gallic acid equivalent (GAE) with reference to gallic acid standard curve (Y=0.003x - 0.113, R^2= 0.994) was 43.76± 2.4 mg. GAE per gram of extract.

Quantification of total flavonoid content (TFC)

The TFC in the methanolic extract of the leaves *L. verschaffeltii* was calculated as rutin equivalent (RE) with reference to rutin standard curve (Y=0.003x + 0.054, R^2 = 0.988) was 22.05± 0.42 mg. RE per gram of extract.

Biological activity

***In-vitro* antioxidant activity of the methanolic extracts from the leaves of *L. verschaffeltii* using DPPH scavenging activity method.**

The methanolic extract of the leaves from *L. verschaffeltii* showed (44.68 µg/ml) with the high antioxidant activity as shown in Table (1). It is evident from the present study that the aqueous methanol extracts of the leaves from *L. verschaffeltii* could be used as good sources of natural antioxidants in the pharmaceutical industry. Thus, further investigations are required to make optimal use of these plants. However, the compounds responsible for the antioxidant activities need to be isolated.

Table (1): In vitro antioxidant activity of the methanolic extracts from the leaves of *L. verschaffeltii* using DPPH scavenging activity method.

Extracts	Conc. (µg/ml)	% scavenging activity	IC_{50} (µg/ml)
L. verschaffeltie	**10**	**20.08 ± 7.21**	**44.68 ± 1.67**
	50	**54.36 ± 10.44**	
	100	**84.54 ± 7.29**	
Trolox			**56.82±0.87**

Determination of hepatoprotective activity

Toxicity study

Methanolic extract of *L. verschaffeltii* showed LD_{50} = 5 g/kg b.wt. and from this result it could be concluded that the methanolic extract was safe, non-toxic and had wide therapeutic index [20].

***In-vivo* hepatoprotective activity**

The outcomes achieved indicated that pretreatment with *L. verschaffeltii*. extract at 100 and 200 mg/kg (groups II and III) resulted in normal, healthy parameters that demonstrated the potency hepatoprotectivity of *L. verschaffeltii* (Table 2, Fig 3), while oral CCl_4 administration (group IV) showed increased ALT and AST serum concentrations in comparison to the control (group I). The hepatic cellular injury, leakage, and liver injury were justified by the improved effects of antioxidant enzymes such as GSH, SOD and TAC. These enzymes' level was significantly reduced after CCL_4 treatment (group IV) in comparison to the control group (group I), although NO and MDA levels were significantly high (Table 3 & Fig. 4,5, 6). The explanation of the hepatotoxic effect of CCl4, occurs as a results of its metabolic precursor CCl_3•, which occasionally attacks polyunsaturated fatty acids and produces lipid peroxides, that in turn alkylates cellular proteins and other macromolecules [21].

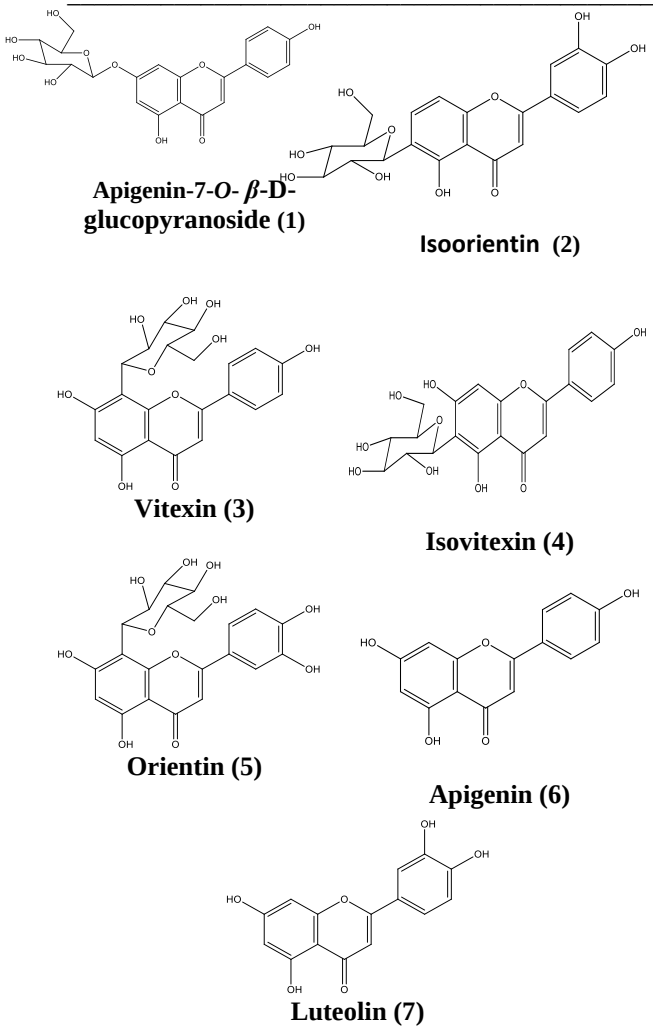

Fig.: (1): chemical structures of isolated compounds

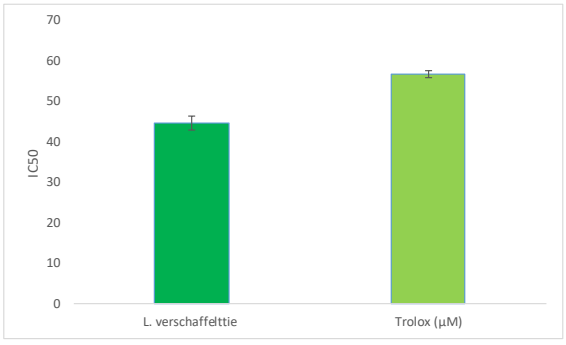

Fig. (2): IC_{50} of the methanolic extract of the leaves from *L. verschaffeltii* using DPPH scavenging activity

On the other hand, administration of *L. verschaffeltii* extract at both dosages (100 and 200 mg/kg) (VI and VII) caused marked improvements in the antioxidant's enzyme level (Tables 2 and 3). Besides, silymarin treated rats (group V) showed an increase in the amount of GSH, SOD, and TAC in the liver tissues. This increase may be due to GSH regeneration or the creation de novo GSH, SOD, and TAC (Tables 3) [22]. CCl_4 administration showed increased ALT and AST serum concentrations of the group (IV) relative to the control group (I). These enzymes increased impacts justified the hepatic cellular damage, leakage, and liver injury. In comparison to the control group, the oxidative stress mediators' levels GSH, SOD and TAC were significantly decreased, while the NO and MDA levels were remarkably increased in group (V) (Table 3). This result was explained as the increased MDA levels in the liver in group IV enhanced lipid peroxidation cause destruction of the antioxidant defense mechanisms that remove accumulated free radicals and tissue damage [23,24].

L. verschaffeltii extract showed a hepatoprotective impact in the current study, which was resisting to CCl_3• cell damage. The antioxidant system associated with SOD, TAC, and GSH has also been improved, which is an evidence of hepatic tissue repair and plasma membrane maintenance from the deficiency induced by CCl_4, particularly in the high dose group (VII), all resulting in the rehabilitation of biological standards. However, the presence of bioactive components in the plant extract, particularly flavonoids and other polyphenolic compounds, could be accountable for the preventive, therapeutic, and antioxidant properties of *L. verschaffeltii* [25].

Histopathological findings

The histopathological results of the liver confirmed our antioxidant markers analysis verifying the hepatoprotective capability of the *L. verschaffeltii* extract. The control animal's liver section (Fig. 7) exhibited normal hepatic cells with normal morphological features.

The liver section pretreated with *L. verschaffeltii* extract (100 mg/kg) showed normal photomicrograph with ordinary liver tissue and dilation of hepatic sinusoids (Fig.8, 9). On the other hands, histopathological changes were noticed in rats after administration of CCl_4 (group IV) revealed marked congestion and inflammatory infiltration around the central vein and hepatocytes appeared with large vesiculated nuclei with necrosis and fatty changes (Fig. 10). This was in agreement with previous studies which states that CCl_4 causes cell death [26], infiltration of mononuclear cell, steatosis, and hepatocytes damage, and accelerate the mitotic process in liver [27]. Silymarin pre-treated rats (group V) before CCL_4 showed recovery of almost the normal structure of liver with dilated hepatic sinusoids (Fig. 11). This in agreement with [28] who stated that Silymarin has many pharmacologic actions including antioxidant, anti-inflammatory, antifibrotic, and insulin resistance modulation.

Marked improvements were seen in rats that had been pre-medicated with *L. verschaffeltii* extract at both doses (100 and 200 mg/kg) (VI and VII) in comparison with the

CCl_4-treated group with restoration of the normal architecture of the liver (Fig. 12, 13).

The liver section of *L. verschaffeltii* extract handled in a dose-dependent manner, totally reversed all CCl_4-induced deteriorations, with mild necrotic hepatocytes and congested central vein (Figure 13) at 100 mg/kg dose, while the extract at 200 mg/kg dose showed improved the histoarchitecture and well-arranged hepatic cord (Fig. 13). These observations recommended the treatment with greater extract dose of *L. verschaffeltii.* (200 mg/kg) after CCL_4 deteriorations.

To confirm their antioxidant properties are linked to their function in sustaining the health of the liver, more research is required on the variation in the enzymatic antioxidant action as well as the effectiveness and interactions of this plant's pure complexes.

Table (2): Effect of oral administration of the methanol extract from the leaves of *L. verschaffeltii* (100 & 200 mg/kg) on AST and ALT serum activity in CCl_4 induced hepatotoxicity in rats.

	Serum ALT (U/L)		**Serum AST (U/L)**	
	Mean ± SE	**% change**	**Mean ± SE**	**% change**
Control (saline) Group I	25 ± 2		19 ± 3	
100 mg/ Kg *L.verschaffeltii* Group II	28.5^{b} ± 1.5	14	24.5^{b} ± 1.5	28.9
200 mg/ Kg *L.verschaffeltii* Group III	19.5^{b} ± 2.5	22	18.5^{bc} ± 3.5	2.6
Liver damaged group Group IV	111^{a} ± 6	344	90.5^{a} ± 3.5	376.3
Silymarin+ CCL_4 Group V	35^{b} ± 3	40	39.5^{ab} ± 2.5	107.9
100 mg/ Kg *L.verschaffeltii* + CCL_4 Group VI	79^{abc} ± 7	216	65^{abc} ± 2	242.1
200 mg/ Kg *L.*verschaffeltii+ CCL_4 Group VII	50^{ab} ± 2	100	53^{ab} ± 3	178.9
F (p)	71.836^{*} ($<0.001^{*}$)		92.73^{*} ($<0.001^{*}$)	

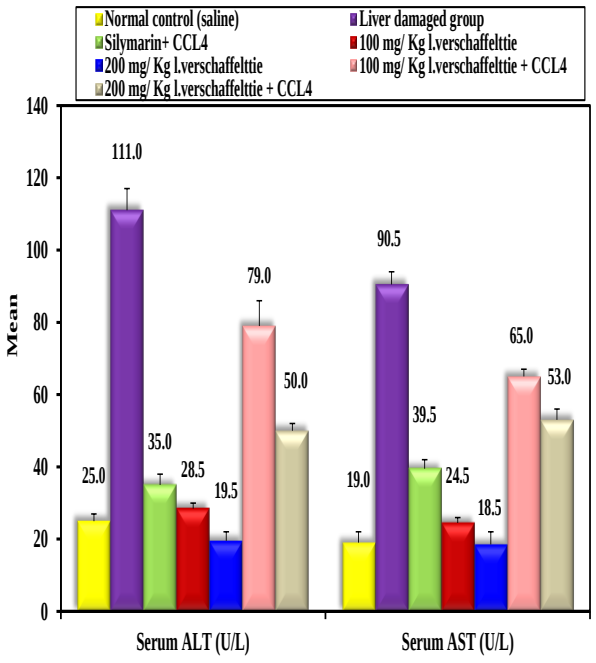

Figure (3): Effect of oral administration of the methanol extract from the leaves of *L.verschaffeltii* (100 & 200 mg/kg) on AST and ALT serum activity in CCl_4 induced hepatotoxicity in rats.

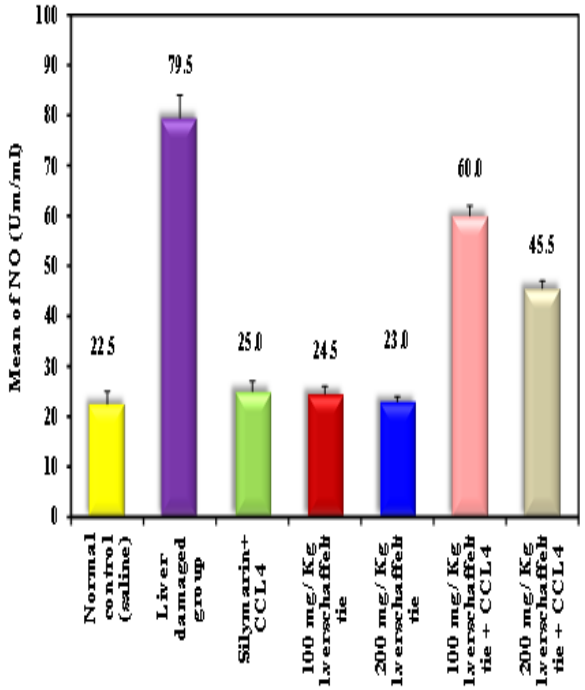

Figure (4): Effect of oral administration of *L. verschaffeltii* (100 & 200mg/kg) on NO in CCl_4 induced hepatotoxicity in rats.

Table (3): Effect of oral administration of the methanol extract from the leaves of *L. verschaffeltii* (100 & 200mg/kg) on NO, MDA, GSH, SOD and TAC in CCl_4 induced hepatotoxicity in rats.

	NO (Um/ml)		MDA (nmol/ml)		GSH (µg/ml)		SOD (U/ml)		TAC (ng/ml)	
	Mean ± SE	% change	Mean ± SE	% change	Mean ± SE	% change	Mean ± SE	% change	Mean ± SE	% change
Control (saline) Group I	22.5 ± 2.5		0.48 ± 0.05		1.55 ± 0.15		0.73 ± 0.04		2.35 ± 0.15	
100 mg/Kg *L. verschaffeltii* Group II	24.5^{b} ± 1.5	8.9	0.47^{bc} ±0.09	2.1	1.4^{b} ± 0.10	↓9.7	0.63^{b} ± 0.04	13.7	2.45^{b} ± 0.15	4.3
200 mg/Kg *L.verschaffeltii*Group III	23^{b} ± 1	2.2	0.43^{bc} ±0.04	10.4	1.65^{b} ± 0.15	↑6.5	0.71^{b} ± 0.04	2.7	2.3^{b} ± 0.20	2.1
Liver damaged group Group IV	79.5^{a} ± 4.5	253.3	2.78^{a} ± 0.07	479.2	0.46^{a} ± 0.01	↓70.3	0.84^{a} ± 0.10	297.3	0.81^{a} ± 0.04	65.5
Silymarin+ CCL_4 Group V	25^{b} ± 2	11.1	0.81^{ab} ±0.02	68.8	1.3^{b} ± 0.10	↓16.1	2.9^{b} ± 0.20	15.1	2.1^{b} ± 0.10	10.6
100 mg/Kg *L.verschaffeltii* +CCL_4 Group VI	60^{abc} ± 2	166.7	2.21^{abc}±0.05	360.4	0.81^{abc}±0.02	↓47.7	1.1^{abc} ± 0.05	163	1.15^{abc}±0.05	51.1
200 mg/Kg *L.verschaffeltii* +CCL_4 Group VII	45.5^{abc} ± 1.5	102.2	1.21^{abc}±0.06	152.1	1.05^{abc}±0.02	↓32.3	1.92^{abc}±0.02	50.7	1.6^{abc} ± 0.10	31.9
F (p)	88.988* (<0.001*)		266.773* (<0.001*)		19.323* (<0.001*)		87.295* (<0.001*)		26.776* (<0.001*)	

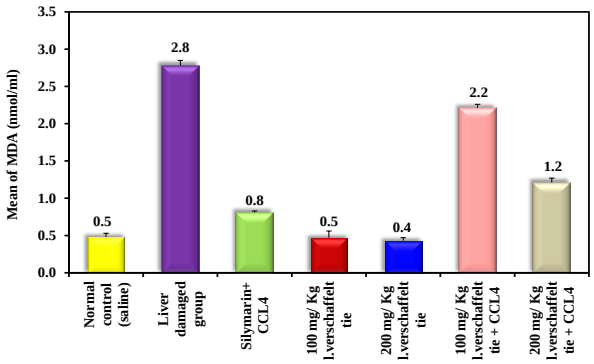

Figure (5): Effect of oral administration of the methanol extract from the leaves of *L. verschaffeltii* (100 & 200mg/kg) on MDA in CCl_4 induced hepatotoxicity in rats.

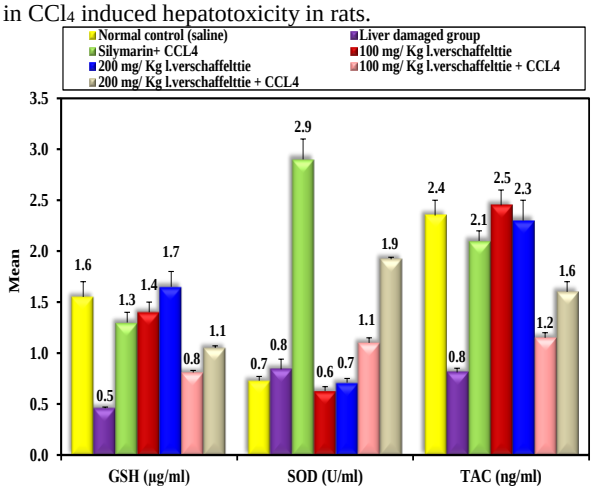

Figure (6): Effect of oral administration of the methanol extract from the leaves of *L. verschaffeltii* (100 & 200mg/kg) on GSH, SOD and TAC in CCl_4 induced hepatotoxicity in rats

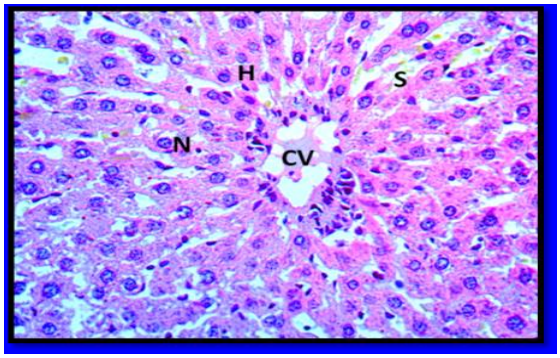

Fig. (7): Photomicrograph of a section from liver of control rats (Group I) showing a normal central vein (CV) surrounded by radiating hepatocytes (H) having round dark nuclei (N) and homogenous cytoplasm. Mild sinusoids (S) studded by kuffer's cell were seen. (H&E stains X400 Mag).

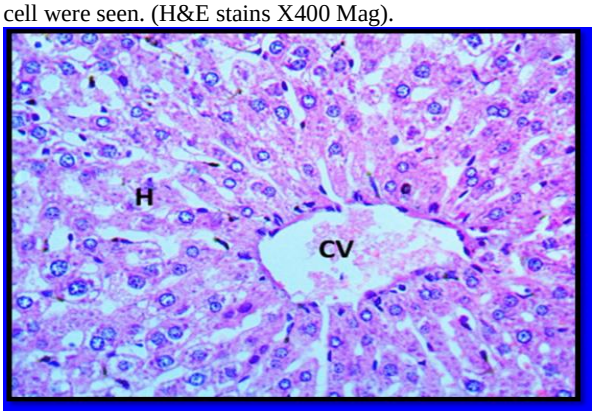

Fig. (8): Photomicrograph of section from liver of rats treated with methanolic extract from the leaves of *L. verschaffeltii* (100 mg/kg) (Group II) showing normal structure of liver with dilated central vein (CV) surrounded by normal hepatocytes (H). (H&E stains X400 Mag).

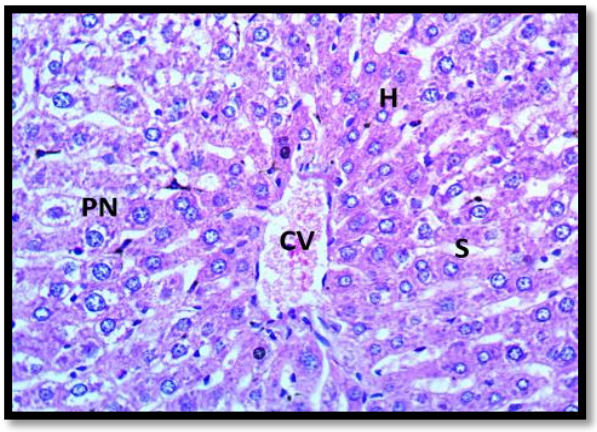

Fig. (9): Photomicrograph of section from liver of rats treated with methanolic extract from the leaves of *L. verschaffeltii* extract (200 mg/kg) showing normal structure of liver with dilated of hepatic sinusoids (S).

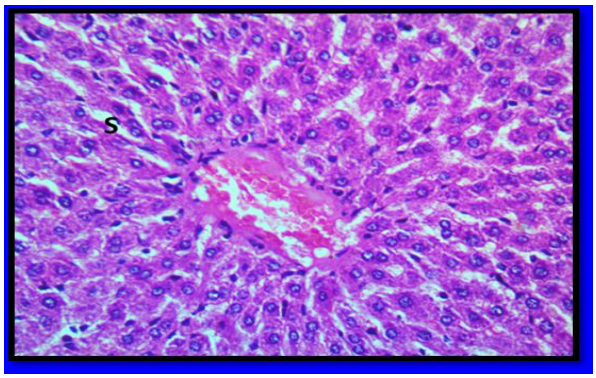

Fig. (10): Photomicrograph of section from liver of treated with Silymarin (Group IV) showing normal structure of liver with dilated of hepatic sinusoids (S). (H&E stains X400 Mag).

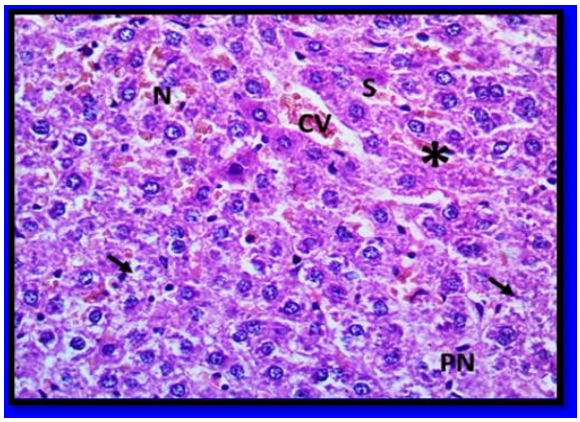

Fig. (11): Photomicrograph of a section of rat liver treated with CCl_4 (Group V) showing congested central vein (CV) and sinusoid (star), hepatocytes with large vesiculated nuclei (N). Fatty changes (arrow) and some hepatocytes with pyknotic (PN) was seen. (H&E stains X400 Mag).

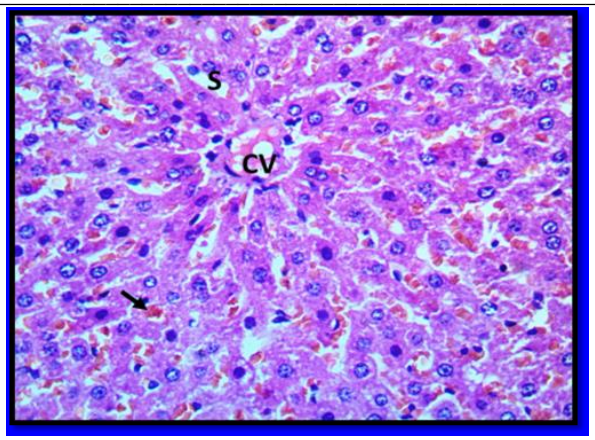

Fig. (12): Photomicrograph of section from liver of rats treated with methanolic extract from the leaves of *L. verschaffeltii* extract (100 mg/kg) and CCl_4 (Group VI) showing normal structure of liver with congestion of central vein (CV) and blood sinusoids (S) with congestions (arrow). (H&E stains X400 Mag).

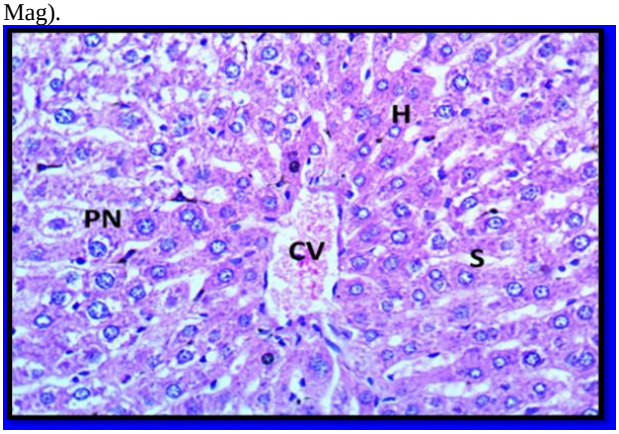

Fig. (13): Photomicrograph of section from liver of rats treated with methanolic extract from the leaves of *L. verschaffeltii* extract (200 mg/kg) and CCl_4 (Group VII) showing restoration of the normal structure of liver with the central vein (CV) surrounded by normal hepatocytes (H) and sinusoid (S). Mild pyknotic hepatocytes (PN) can be seen. (H&E stains X400 Mag).

Conclusion

The aqueous methanolic extract from the leaves of *L. verschaffeltie* provided seven phenolic compounds (**1-7**), which are isolated from this species for the first time. Additionally, this is the first record of an estimation of the total phenolic, total flavonoid contents and the *in vitro* antioxidant and *in vivo* hepatoprotective activity of *L. verschaffeltii* demonstrate that extract was effective for the prevention of CCl_4-induced hepatic damage in rats. Our results showed that the hepatoprotective effects of *L. verschaffeltii* extract may be due to both an increase in the activity of the antioxidant-defense system and an inhibition of lipid peroxidation. However, the protective, curative and antioxidant qualities of *L. verschaffeltii*may be attributed to the presence of active principles in the

plant extract, particularly flavonoids and other polyphenolic compounds.

Authors' contribution:

All authors contributed to conducting laboratory experiments, interpreting results, writing, editing and reviewing the manuscript

Conflicts of interest

The authors declare that they have no competing interests that could influence the work reported in this paper.

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References

1) Aktay, Göknur, Didem Deliorman, Ender Ergun, Fatma Ergun, Erdem Yeşilada, and Cemal Çevik. 2000. "Hepatoprotective Effects of Turkish Folk Remedies on Experimental Liver Injury." *Journal of Ethnopharmacology* 73 (1–2): 121–29. https://doi.org/10.1016/S0378-8741(00)00286-5.
2) Gutiérrez, Rosa Martha Pérez, Sylvia Mitchell, and Rosario Vargas Solis. 2008. "Psidium Guajava: A Review of Its Traditional Uses, Phytochemistry and Pharmacology." *Journal of Ethnopharmacology* 117 (1): 1–27. https://doi.org/10.1016/j.jep.2008.01.025.
3) Christenhusz, Maarten J.M., and James W. Byng. 2016. "The Number of Known Plants Species in the World and Its Annual Increase." *Phytotaxa* 261 (3): 201–17. https://doi.org/10.11646/phytotaxa.261.3.1.
4) Zandi, Peiman. 2014. "Arecaceae : The Majestic Family of Palms Arecaceae : The Majestic Family of Palms Palms Are Most Conspicuous in Coastal Areas in Tropical and Sub-Tropical Ecological Zones as Well as in The," no. September.
5) Silva, Ricardo Sérgio da, Paulo Henrique Oliveira de Miranda, Luciclaudio Cassimiro de Amorim, Paulo Henrique Eloi Fernandes, Elias Vinícius Ferreira do Amaral, Bruno Oliveira de Veras, Fernanda Granja da Silva Oliveira, Maria Tereza dos Santos Correia, Jackson Roberto Guedes da Silva Almeida, and Márcia Vanusa da Silva. 2021. "Emerging Source of Bioactive Compounds from Arecaceae Family: A Systematic Review." *Research, Society and Development* 10 (10): e426101018994. https://doi.org/10.33448/rsd-v10i10.18994.
6) Shukla, Abha, Gurukula Kangri Vishwavidyalaya, Supriya Dubey, and Gurukula Kangri Vishwavidyalaya. 2022. "A Review: Traditionally Used Medicinal Plants of Family Arecaceae With Phytoconstituents and Therapeutic Applications." *International Journal of Biology, Pharmacy and Allied Sciences* 11 (12). https://doi.org/10.31032/ijbpas/2022/11.12.6655.
7) Ibrahim, Haitham, Fathia Elshaarawy, and Eman Haggag. 2018. "Investigation of Livistona Decipiens Leaf Methanol Extract and Evaluation of Antioxidant, Antimicrobial and Cytotoxic Activities." *Journal of Advanced Pharmacy Research* 0 (0): 0–0. https://doi.org/10.21608/aprh.2018.5173.1063.
8) Attard, Everaldo. 2013. "A Rapid Microtitre Plate Folin-Ciocalteu Method for the Assessment of Polyphenols" 8 (1). https://doi.org/10.2478/s11535-012-0107-3.
9) Kiranmai M,Mahendra Kumar CB, and Ibrahim Mohammed. 2011. "Comparison of Total Flavanoid Content of Azadirachta Indica Root Bark Extracts Prepared by Different Methods of Extraction." *Research Journal of Pharmaceutical , Biological and Chemical Sciences* 2 (3): 254–61.
10) Boly R, Lamkami T, Lompo M, DuboisJ, and Guissou I P. 2016. "DPPH Free Radical Scavenging Activity of Two Extracts From." *Intern International Journal of Toxicological and Pharmacological Research* 8 (1): 29–34.
11) Olugbami, J O, M A Gbadegesin, and O A Odunola. 2014. "In Vitro Evaluation of the Antioxidant Potential, Phenolic and Flavonoid Contents of the Stem Bark Ethanol Extract of Anogeissus Leiocarpus." *African Journal of Medicine and Medical Sciences* 43 (Suppl 1): 101–9. http://www.ncbi.nlm.nih.gov/pubmed/26681826%0Ahttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC4679201.
12) Obi, Frederick Otunuya, Ighofimoni Afonughe Usenu, and Julie Osaretin Osayande. 1998. "Prevention of Carbon Tetrachloride-Induced Hepatotoxicity in the Rat by H. Rosasinensis Anthocyanin Extract Administered in Ethanol." *Toxicology* 131 (2–3): 93–98. https://doi.org/10.1016/S0300-483X(98)00119-X.
13) Reitman, S., and S. Frankel. 1957. "A Colorimetric Method for the Determination of Serum Glutamic Oxalacetic and Glutamic Pyruvic Transaminases." *American Journal of Clinical Pathology* 28 (1): 56–63. https://doi.org/10.1093/ajcp/28.1.56.
14) Marklund, Stefan, and Gudrun Marklund. 1974. "Involvement of the Superoxide Anion Radical in the Autoxidation of Pyrogallol and a Convenient Assay for Superoxide Dismutase." *European Journal of Biochemistry* 47 (3): 469–74. https://doi.org/10.1111/j.1432-

1033.1974.tb03714.x.

15) Fraihat, Safwan M. 2017. "Green Methods for the Determination of Nitrite in Water Samples Based on a Novel Diazo Coupling Reaction." *Green Processing and Synthesis* 6 (2): 245–48. https://doi.org/10.1515/gps-2016-0005.
16) Pradeep, Kannampalli, Chandrasekaran Victor Raj Mohan, Kuppanan Gobianand, and Sivanesan Karthikeyan. 2007. "Silymarin Modulates the Oxidant-Antioxidant Imbalance during Diethylnitrosamine Induced Oxidative Stress in Rats." *European Journal of Pharmacology* 560 (2–3): 110–16. https://doi.org/10.1016/j.ejphar.2006.12.023.
17) Lorente, Leonardo, Sergio T. Rodriguez, Pablo Sanz, Antonia Pérez-Cejas, Pedro Abreu-González, Javier Padilla, Dácil Díaz, et al. 2018. "Serum Total Antioxidant Capacity Prior to Liver Transplantation for Hepatocellular Carcinoma Is Associated with 1-Year Liver Transplantation Survival." *Journal of International Medical Research* 46 (7): 2641–49. https://doi.org/10.1177/0300060518768150.
18) Curello, S., C. Ceconi, A. Cargnoni, A. Cornacchiari, R. Ferrari, and A. Albertini. 1987. "Improved Procedure for Determining Glutathione in Plasma as an Index of Myocardial Oxidative Stress." *Clinical Chemistry* 33 (8): 1448–49. https://doi.org/10.1093/clinchem/33.8.1448.
19) Obeid, Raneem F., and Hesham S. Abdelmoneim and Radwa T. Elsharkawy. 2020. "Histological Evaluation of the Antioxidant Effect OF Vitamin E On Reversing The negative impact of tartrazine on extraction socket healing. (randomized controlled trial)" 66: 285–92.
20) El Kady, Wafaa M. El, Abeer A.A. Salama, Samar Y. Desoukey, Eman G. Hagag, Siham M. El-Shenawy, and M.A. El-Shanawany. 2015. "Comparative DNA Profiling, Botanical Identification and Biological Evaluation of Gazania Longiscapa DC and Gazania Rigens L." *Bulletin of Faculty of Pharmacy, Cairo University* 53 (2): 129–45. https://doi.org/10.1016/j.bfopcu.2015.07.001.
21) Unsal, Velid, Mustafa Cicek, and İlhan Sabancilar. 2021. "Toxicity of Carbon Tetrachloride, Free Radicals and Role of Antioxidants." *Reviews on Environmental Health* 36 (2): 279–95. https://doi.org/10.1515/reveh-2020-0048.
22) Wilk, Aleksandra, Dagmara Szypulska-Koziarska, Karolina Kędzierska-Kapuza, Jerzy Sieńko, Agnieszka Kolasa-Wołosiuk, Kazimierz Ciechanowski, and Barbara Wiszniewska. 2019. "The Comparison of Parameters of Oxidative Stress in Native Rat Livers between Different Immunosuppressive Regimens." *Medical Science Monitor* 25: 8242–47. https://doi.org/10.12659/MSM.915230.
23) Ashok Shenoy K., S. N. Somayaji, K. L. bairy. 2001. "Hepatoprotective Effects of Ginkgo biloba against carbon tetrachloride induced hepatic injury in rats." *Ndian Journal of Pharmacology* 33: 260–66.
24) Khattab, Hala A.H. 2012. "Effect of Ginkgo Biloba Leaves Aqueous Extract on Carbon Tetrachloride Induced Acute Hepatotoxicity in Rats." *The Egyptian Journal of Hospital Medicine* 48 (1): 483–95. https://doi.org/10.21608/ejhm.2012.16250.
25) Ullah, Asad, Sidra Munir, Syed Lal Badshah, Noreen Khan, Lubna Ghani, Benjamin Gabriel Poulson, Abdul-hamid Emwas, and Mariusz Jaremko. 2020. "Important Flavonoids and Their Role as a Therapeutic Agent." *Molecules* 25 (5243): 1–39.
26) Badawi, Marwa Sayed. 2022. "A Study on the Antioxidant Activity of Rosmarinic Acid Against Carbon Tetrachloride-Induced Liver Toxicity in Adult Male Albino Rats." *International Journal of Morphology* 40 (1): 157–67. https://doi.org/10.4067/S0717-95022022000100157.
27) Abu-Gharbieh, Eman, and Naglaa G. Ahmed. 2016. "Bioactive Content, Hepatoprotective and Antioxidant Activities of Whole Plant Extract of Micromeria Fruticosa (L) Druce Ssp Serpyllifolia F Lamiaceae against Carbon Tetrachloride-Induced Hepatotoxicity in Mice." *Tropical Journal of Pharmaceutical Research* 15 (10): 2099–2106. https://doi.org/10.4314/tjpr.v15i10.7.
28) Gillessen, Anton, and Hartmut H.J. Schmidt. 2020. "Silymarin as Supportive Treatment in Liver Diseases: A Narrative Review." *Advances in Therapy* 37 (4): 1279–1301. https://doi.org/10.1007/s12325-020-01251.