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Research Article

PHYTOCHEMICAL INVESTIGATION AND HEPATOPROTECTIVE EVALUATION OF *NANNORRHOPS RITCHIANA* IN RATS

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ABSTRACT

The aim of this research was to determine the phenolic and flavonoid compounds present in 70% methanol extract of the leaves of *Nannorrhops ritchiana* using liquid chromatography-tandem mass spectrometry method analysis and to evaluate hepatoprotective activity, not previously studied, for this plant. GC–MS analysis of n-hexane extract of this plant was utilized, to identify the bioactive constituents of long chain hydrocarbons, acids esters, steroids and terpenes. Seven flavonoids and twelve phenolic acids were identified. Five of the flavonoids (Epihesperidin, demethoxycentaureidin7-O-rutinoside, icariin, datiscin, isoschaftoside), and nine of the phenolic acids (Quinic acid, p-hydrobenzoic acid, caftaric acid, caffeoylshikimic acid, malic acid, chlorogenic acid, 2-naphthoxyacetic acid, 3-(2- hydroxyphenyl) propionic acid, D-glucaric), were reported for the first time from this species. Thirty one phytochemical compounds were identified by GC-MS analysis, phytol had the highest peak area of 19.82% and the lowest was stigmasterol showing 0.25%. The methanol extract of the plant was evaluated in D-galactosamine induced hepatotoxicity in albino rats. At the doses of 250 mg/kg and 500 mg/kg, the extract significantly reduced the increased activity of serum enzymes and bilirubin, which were comparable to that of reference drug silymarin and D-galactosamine revealing its hepatoprotective effect. The extract had accelerated the return of the altered levels of the oxidative stress parameters and inflammatory markers to the near normal profile in the dose dependent manner, also, histopathological examination, showed good recovery of necrosis. Hence, hepatoprotective activity of methanol extract may be related to the fact that it contains a combination of different phytochemicals and bioactive constituents.

Keywords: *Nannorrhops ritchiana*, Flavonoids, Phenolics, LC-MS/MS, GC-MS analysis, Hepatoprotective activity, Histopathology.

INTRODUCTION

Arecaceae is known as Palmae or Palmaceae. The Arecaceae comprises 181 genera and 2600 species which are found throughout tropical, and subtropical areas of the world. The genus *Nannorrhops* is one of palm trees belonging to this family. Mazari palm is a common name of *Nannorrhops ritchiana* Griff. species, which is widely present in distinct areas of the world, native to Southwestern Asia, regional to the Pakistan and other countries like Afghanistan and Iran[1]. The Arecaceae is a very large family, of great commercial importance as source of nuts, edible fruits, starchy foods, oils, fiber and woods. Young leaves of *Nannorrhops ritchiana* plant have been used as a purgative in livestock[2]. The fruit is edible and used for the treatment of alimentary tract complaints[3] and in the treatment of other infectious disorders, in Baluchistan, Pakistan[4]. The phytochemical analysis of *Nannorrhops ritchiana* showed the presence of flavonoids, alkaloids, cardiac glycosides, glycosides, resins, saponins, tannins, terpenoids, carbohydrates and amino acids[5]. Antifungal and cytotoxic activities of *Nannorrhops ritchiana* roots extract had been evaluated[6], also antimicrobial activity of plant leaves had been reported[5]. Liver diseases have become one of the major causes of morbidity and mortality all over world. The main scope was to study the bioactive phytochemical constituents of the leaves of *Nannorrhops ritchiana* and to investigate hepatoprotective activity of crude extract for pharmacological benefits.

MATERIALS AND METHODS

Plant material

Leaves of *Nannorrhops ritchiana* were collected from the garden of Manial Palace - El-Manial district- Cairo, Egypt in September 2017. The plant was identified by Mm. Tressa Labib, Taxonomist, at El- Orman Botanical Garden, Giza, Egypt. The plant samples were air-dried, powdered and kept for phytochemical and bioactivity studies.

Apparatus

LC/MS/MS, 4000 Qtrap Applied Biosystems, (Quadrupole / linear ion trap mass spectrometer), and the liquid chromatography (Agilent Technologies, Palo Alto, CA, USA) system coupled with an electrospray ionization-mass spectrometer (ESI-MS/MS) detector were employed to identify phenolic acid and flavonoid compounds. The GC-MS was performed using the GC-MS system (Agilent Technologies), equipped with gas chromatograph (7890B) and mass spectrometer detector (5977A). The GC was equipped with HP-5MS column (30 m x 0.25 mm internal diameter and 0.25 µm film thickness).

Plant extraction

The air-dried leaves (1kg) of *Nannorrhops ritchiana* were extracted with 70% MeOH at room temperature till exhaustion. The methanol extract was evaporated under reduced pressure to

yield 240 g of methanol crude extract. Part was kept for LC/MS/MS analysis of flavonoid and phenolic compounds, and other part was kept for biological study. The residue of the extract was suspended in water (200 ml), then was shaken with n-hexane. The extract was evaporated under reduced pressure to yield 25 g, which was kept for GC-MS analysis.

LC/MS/MS analysis of phenolic and flavonoid compounds

The 70% methanol extract was analysed by LC/MS/MS, 4000 Qtrap Applied Biosystems, (Quadrupole) /linear ion trap mass spectrometer) was used. Liquid samples (5µl) were directly injected to the liquid chromatography (Agilent Technologies, Palo Alto, CA, USA) system coupled with an electrospray ionization-mass spectrometer (ESI-MS/MS) detector. The chromatographic separation was achieved with a Phenomenex synergy Hydro-RP (2.1 mm i.d. × 150 mm, phenomenex, Torrance, CA, USA) followed by 0.2 ml/min of flow under 400 psi at 30^0C. The gradient elution composed of 0.1% formic acid (A) in water and 0.1% formic acid acetonitrile (B), 5% B linear gradient (min 0-1); 5-100% B linear gradient (min 1-10); 100% B (min 10-11); 100-5% B linear gradient (min 11-14); 5% B (min 14-20). The column was re-conditioned for 15 min before the next run[7].The compounds were identified by comparing MS/MS spectra data (MS/MS fragment spectra) in a data-dependent scan mode, with those stored in MS/MS spectra library[8].

Gas chromatography–mass spectrometry analysis (GC-MS)

The GC-MS system (Agilent Technologies) was equipped with gas chromatograph (7890B) and mass spectrometer detector (5977A) at Central Laboratories Network, National Research Centre, Cairo, Egypt. The GC was equipped with HP-5MS column (30 m x 0.25 mm internal diameter and 0.25 µm film thickness). Analysis was carried out using helium as the carrier gas at a flow rate of 1 ml/min at a split-less mode, injection volume of 1 µl and the following temperature program: 60°C for 2 min; rising at 5°C /min to 300°C and held for 10 min. The injector and detector were held at 280°C and 300°C, respectively. Mass spectra were obtained by electron ionization voltage (EI) at 70 eV and using a spectral range of m/z 50-550 and solvent delay 3 min. Identification of different constituents was determined by comparing the spectrum fragmentation pattern with those stored in Wiley and NIST Mass Spectral Library data.

Animals

In this study, albino rats (100-120 g) were obtained from the animal house, National Research Centre. Rats were grouped and housed in polyacrylic cages (38 x 23 x 10 cm) with not more than six animals per cage and were maintained under standard laboratory conditions (temperature 25 ± 2ºC) with dark and light cycle (12/12h). The animals were fed with the standard pellet diet and fresh water ad libitum. All animals were acclimatized to laboratory conditions for a week before commencement of experiment. All procedures were described reviewed and approved by the National Research Centre Ethical Committee.

Biological assay

The acute toxicity study was conducted in accordance with Lorke's method[9]. No lethality or any toxic symptoms were observed for the extract. After estimation of LD_{50} of the extract, the doses were selected for the hepatoprotective investigations as we used 10% and 20 % of the highest dose examined for the LD_{50}, which it was 5000 mg/kg b.w. So we used low dose (250 mg/kg) and the high dose (500 mg/kg) of the extract.

Study design

For each extract, total number of 30 rats were divided into 5 groups of 6 animals each as follows:
Group I: Normal Control: The animals received distilled water (D.W.) 5 ml/kg b.w; p.o. for 8 days.
Group II: D-galactosamine Group: Also received distilled water 5 ml/kg b.w; p.o. for 8 days. A single dose of D-Gal N in D.W. 200 mg/kg b.w. was given i.p. after one hour of vehicle on the 8th day.
Group III: Standard Silymarin Group: The animals received 100 mg/kg b.w; p.o. was given for 8 days. The animals received a single dose of D-Gal N in D.W. 200 mg/kg b.w; i.p. after 1 hour of vehicle on the 8th day.
Group IV: D-Gal N + low Dose of *Nannorrhops ritchiana* extract Group: low dose of extract (250 mg/kg) was administered p.o. for 8 days. A single dose of D-Gal N in D.W. 200 mg/kg b.w. was given i.p. after 1hour of vehicle on the 8th day.
Group V: D-Gal N + high Dose of *Nannorrhops ritchiana* extract Group: high dose of extract (500 mg/kg) was administered p.o. for 8 days. A single dose of D-Gal N in D.W. 200 mg/kg b.w. was given i.p. after 1hour of vehicle on the 8th day [10].

Biochemical studies

At the end of the experiment, the animals were kept fasting for 12 hours before urine and blood sampling. Blood was withdrawn from the retro-orbital venous plexus of the eye using a capillary tube under light anesthesia by diethyl ether, according to the method of[11]. Blood was collected in tubes and livers were removed quickly on ice and homogenized for estimation of liver parameters.

Preparation of liver homogenate

Livers were removed quickly and placed in iced normal saline, per fused with the same solution to remove blood cells, blotted on filter paper and frozen at -80ºC. The frozen tissues were cut into small pieces and were homogenized in 5 ml cold buffer 0.5 g of Na_2HPO_4 and 0.7 g of NaH_2PO_4 per 500 ml deionized water pH=7.4 then were centrifuged at 4000 rpm for 15 minutes at 4ºC and the supernatant was removed for parameters estimation[12].

Biochemical investigations

Serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), alkaline phosphatase (ALP), total protein and total bilirubin using spectrophotometer. Oxidative stress markers [paraoxinase1 (PON1), nitric oxide (NO), malondialdehyde (MDA) and reduced glutathione] in liver tissue homogenate. Urinary 8-hydroxyguanosine by HPLC as a marker of DNA damage. Tissue inhibitor of metalloproteinase-1 (TIMP-1) and hyaluronic acid as indicators for liver extracellular matrix deposition and degradation.

Histopathology study

The animals were sacrificed by decapitation method and the abdomen was cut open to remove the liver. The liver samples were washed with normal saline. Initially the liver was fixed in 10% buffered neutral formalin for 24 hr, then embedded in paraffin wax, cut into 5 µm thick section and was stained using hematoxylin-eosin dye to study the liver histological structure of the control and treated rats. The sections were then observed under microscope for histopathological changes in liver architecture and their photomicrographs were taken for the evaluation of changes.

Table 1: Flavonoid compounds detected in *Nannorrhops ritchiana* using LC/MS/MS method

Compound	Molecular Weight	Molecular Formula	Experimental Mass	Mass Fragments MS/MS(*m/z*)
Demethoxycentaureidin 7-O-rutinoside	638	$C_{29}H_{34}O_{16}$	637.50$[M-H]^-$	329.40, 488.94, 637.50
Rutin	610	$C_{27}H_{30}O_{16}$	609.40$[M-H]^-$	267, 300, 609.40
Epihesperidin	610	$C_{28}H_{34}O_{15}$	611.00$[M+H]^+$	431.30, 449.30, 611.30
Icariin	676	$C_{33}H_{33}O_{15}$	677.30$[M+H]^+$	347.40, 369.13, 677.30
Datiscin	594	$C_{27}H_{30}O_{15}$	593.30$[M-H]^-$	267.03,285.03, 593.30
Isoschaftoside	564	$C_{26}H_{28}O_{14}$	563.10$[M-H]^-$	191.00, 353.30, 563.10
Hesperetin	302	$C_{16}H_{14}O_6$	303.30$[M+H]^+$	111.00,285.20, 303.30

Table 2: Phenolic compounds detected in *Nannorrhops ritchiana* using LC/MS/MS method

Compound	Molecular weight	Experimental mass (MS_1)	Mass Fragments MS/MS(*m/z*)
Quinic acid	192	190.90$[M-H]^-$	93.00, 85.00
P-hydrobenzoic acid	138	136.80$[M-H]^-$	92.90, 65.00
Caffeic acid	180	179.00$[M-H]^-$	135.10, 133.90
Catechin	290	288.90$[M-H]^-$	245.00, 205.20,179.10
Caftaric acid	312	311.30$[M-H]^-$	183.10,149.00
Caffeoylshikimic acid	336	335.00$[M-H]^-$	289.20,178.80,161.20
Malic acid	134	132.90$[M-H]^-$	114.90, 88.90,70.80
Chlorogenic acid	354	353.20$[M-H]^-$	190.90,178.80,173.10
2-Naphthoxyacetic acid	202	203.10$[M+H]^+$	143.00, 157.10, 203.10
Benzoic acid	122	121.00$[M-H]^-$	92.20, 92.80, 121.00
3-(2- Hydroxyphenyl) propionic acid	166	164.90$[M-H]^-$	86.80, 104.90, 164.90
D-Glucaric acid	210	209.00$[M-H]^-$	88.90, 128.80, 209.00

Table 3: Compounds identified in n-hexane extract of *Nannorrhops ritchiana* by GC-MS

Peak	Compound Name	Molecular Formula	RT	Area %
1	Decanoic acid	C10H20O2	5.44	0.32
2	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-, (R)-	C11H16O2	8.061	1.05
3	Dodecanoic acid	C12H24O2	8.439	1.9
4	2-Cyclohexen-1-one, 4-(3-hydroxybutyl)-3,5,5-trimethyl-	C13H22O2	10.602	0.89
5	Tetradecanoic acid	C14H28O2	11.362	0.37
6	Pentadecanoic acid, methyl ester	C16H32O2	12.227	0.71
7	2-Pentadecanone, 6,10,14-trimethyl-	C18H36O	12.524	0.28
8	Pentadecanoic acid	C15H30O2	12.845	0.56
9	9-Octadecenoic acid (Z)-	C18H34O2	13.6	0.38
10	Hexadecanoic acid, methyl ester	C17H34O2	13.68	7.66
11	n-Hexadecanoic acid	C16H32O2	14.458	10.14
12	Heptadecanoic acid, methyl ester	C18H36O2	15.036	1.1
13	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	C19H34O2	15.957	1.88
14	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	C19H32O2	16.06	7.33
15	Phytol	C20H40O	16.289	19.82
16	Octadecanoic acid, methyl ester	C19H38O2	16.375	0.72
17	9,12-Octadecadienoic acid (Z,Z)-	C18H32O2	16.724	6.83
18	9,12-Octadecadienoic acid (Z,Z)-	C18H32O2	16.833	12.84
19	Methyl 6-cis,9-cis,11-trans-octadecatrienoate	C19H32O2	18.887	0.97
20	cis-5,8,11,14,17-Eicosapentaenoic acid	C20H30O2	18.956	0.38
21	Diisooctyl phthalate	C24H38O4	21.376	2.54
22	unidentified	----------	24.567	1.55
23	3-[3-Bromophenyl]-7-chloro-3,4-dihydro-10-hydroxy-1,9(2H,10H)-acridinedione	C19H13BrClNO3	24.769	1.84
24	1-Heptatriacotanol	C37H76O	25.296	0.39
25	Vitamin E	C29H50O2	27.213	2.39
26	.alpha.-Tocopherolquinone	C29H50O3	27.281	3.51
27	Stigmasterol	C29H48O	28.54	0.25
28	.gamma.-Sitosterol	C29H50O	29.21	1.68
29	Betulin	C30H50O2	29.667	1.09
30	Arundoin	C31H52O	30.451	3.03
31	Lanosterol	C30H50O	31.784	3.84
32	Ethyl iso-allocholate	C26H44O5	32.305	1.75

Table 4: Liver functions tests in different studied groups

Groups	Parameters				
	GOT (U/L)	GPT (U/L)	T. Protein (g/dL)	ALP (U/L)	T. Bili (mg/dL)
Control	87.95 ±6.17	30.04±1.91	5.5±0.27	114.15±4.73	0.74±0.03
Galactosamine	215.65±12.21*	81.64±3.66*	8.16±0.21*	217.05±8.88	1.31±0.08*
Silymarin	84.39±18.81•	31.08±2.54•	6.05±0.18•	111.16±4.79	0.81±0.03•
***Nannorrhops ritchiana* low dose**	175.35±7.99•	46.7±0.90•	6.76±0.19•	184.86±2.10	0.88±0.03•
***Nannorrhops ritchiana* high dose**	147.15±7.00•	30.71±1.33•	5.2±0.21•	127.1±4.28	0.78±0.03•

Data presented as mean ± SE, *Significant at $P< 0.05$ compared to control group, •Significant at $P< 0.05$ compared to Galactosamine group. NS Non significant compared to Galactosamine group

Table 5: Oxidant / antioxidant parameters in different studied groups

Groups	Parameters		
	NO (µmol/L)	**MDA** (nanomol/mL)	**PON 1** (mg/dL)
Control	16.72±0.36	97.39±3.53	91.61±3.89
Galactosamine	41.39±1.06*	241.3±5.83*	50.16±4.29*
Silymarin	17.25±0.49•	110.81±2.05•	92.2±4.37•
***Nannorrhops ritchiana* low dose**	26.16±1.01•	178.8±2.87•	70.35±2.03•
***Nannorrhops ritchiana* high dose**	20.68±0.67•	161.85±5.17•	56.8±4.17 **NS**

Data presented as mean ± SE, *Significant at *P*< 0.05 compared to control group, •Significant at *P*< 0.05 compared to Galactosamine group. NS Non significant compared to galactosamine group

Table 6: Inflammatory markers in different studied groups

Groups	Parameters	
	HA (U/L)	**MMP 1** (ng/ml)
Control	22.69±0.85	430.03±8.41
Galactosamine	40.88±2.12*	874.11±53.93*
Silymarin	21.90±0.58•	461.81±17.23•
***Nannorrhops ritchiana* (D3) low dose**	31.36±0.94•	947.71±28.11 **NS**
***Nannorrhops ritchiana* (D3) high dose**	27.34±0.80•	749.99±38.17 **NS**

Data presented as mean ± SE, *Significant at *P*< 0.05 compared to control group, •Significant at *P*< 0.05 compared to Galactosamine group. NS Non significant compared to Galactosamine group.

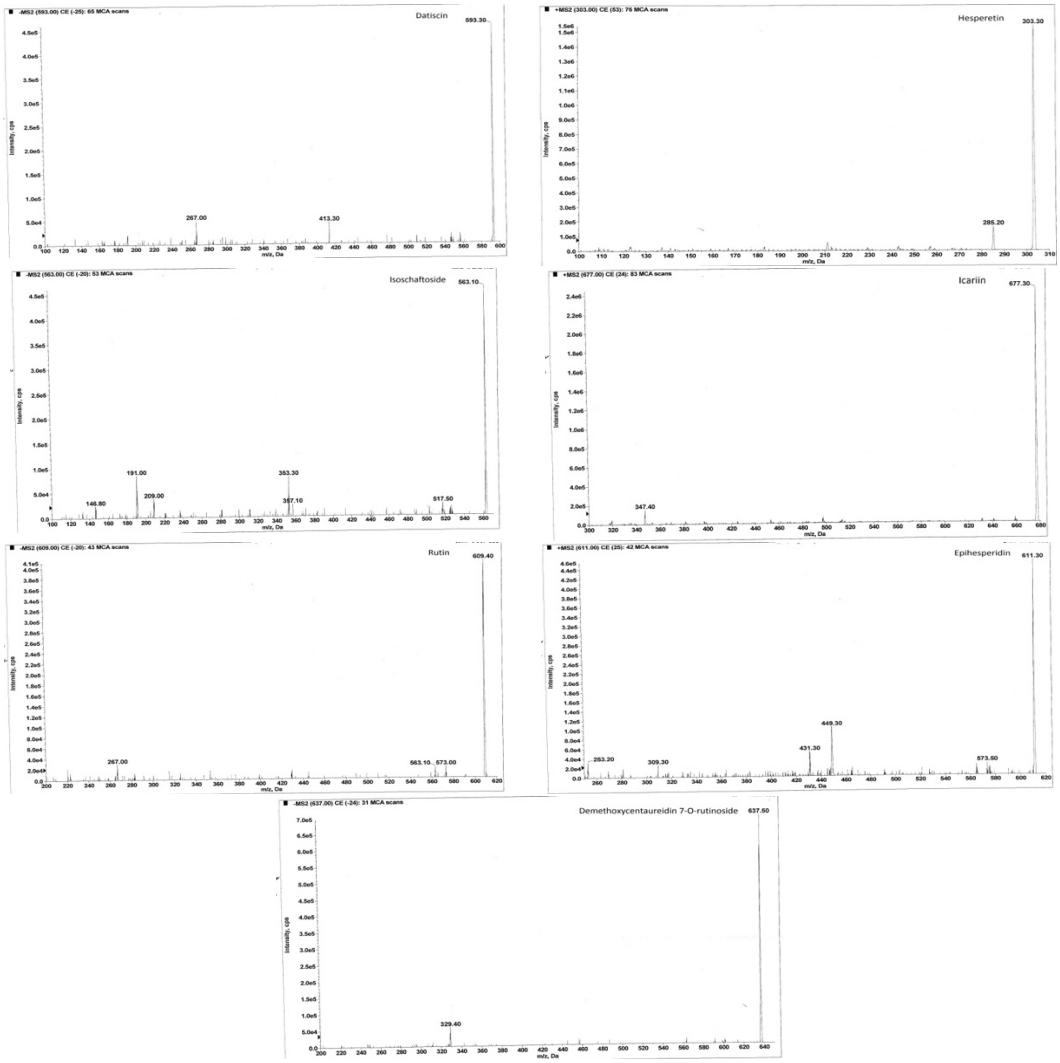

Fig 1: Flavonoid compounds of *Nannorrhops ritchiana* using LC/MS/MS

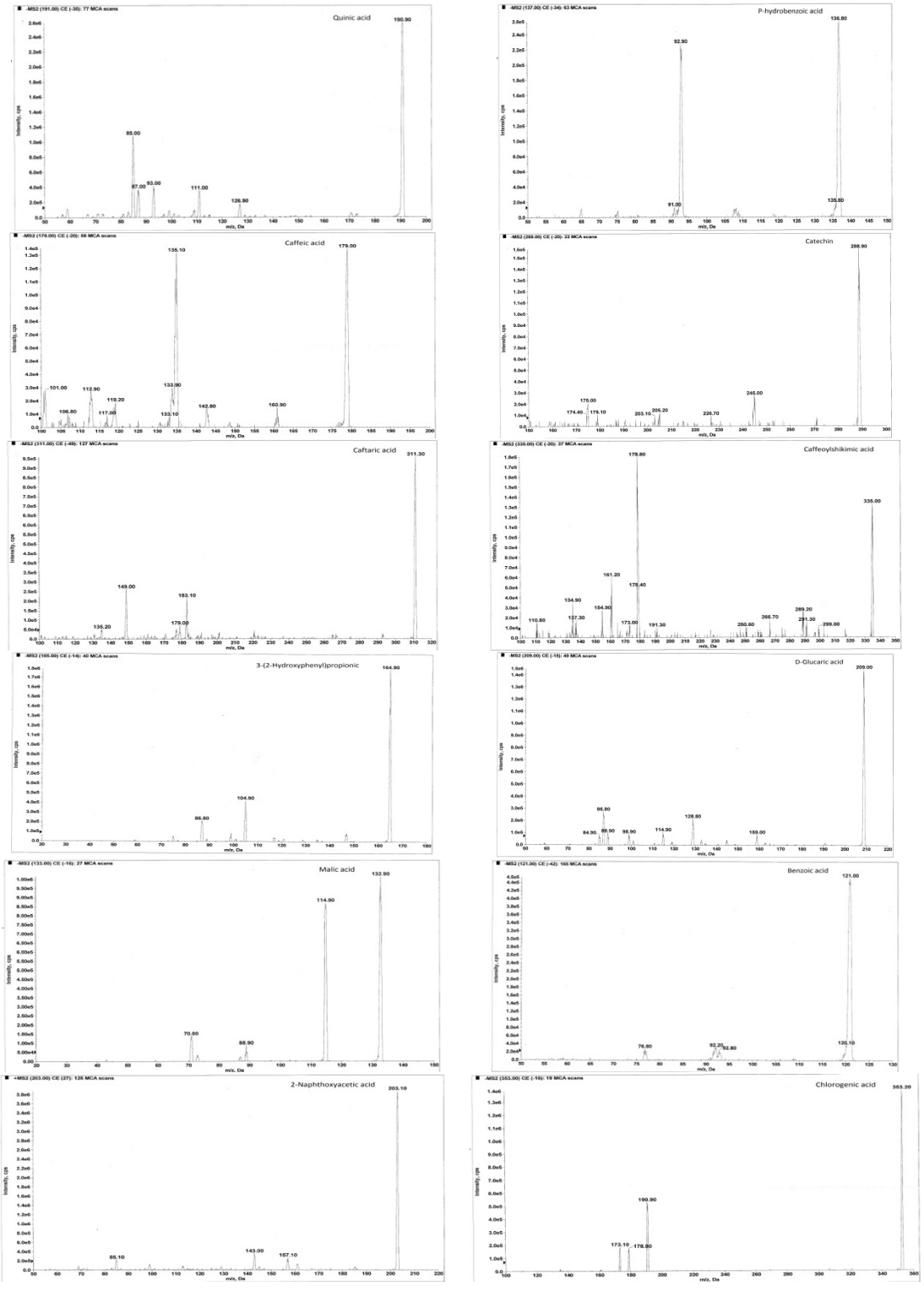

Fig 2: Phenolic compounds of *Nannorrhops ritchiana* using LC/MS/MS

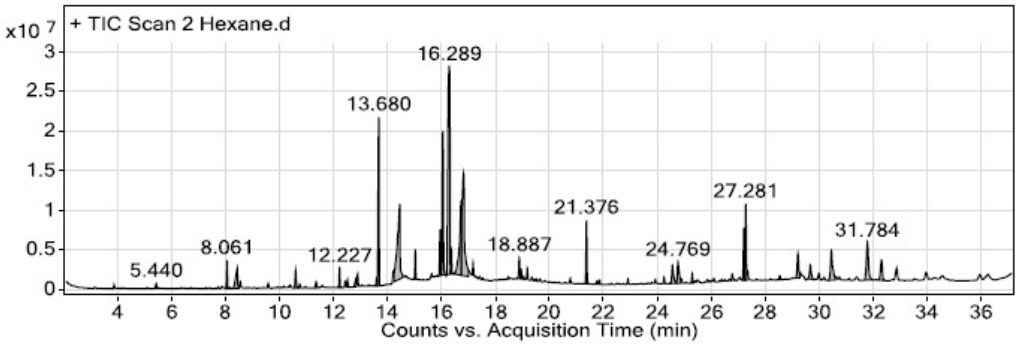

Figure 3: GC- MS chromatogram of the compounds identified in n-hexane extract of *Nannorrhops ritchiana* leaves

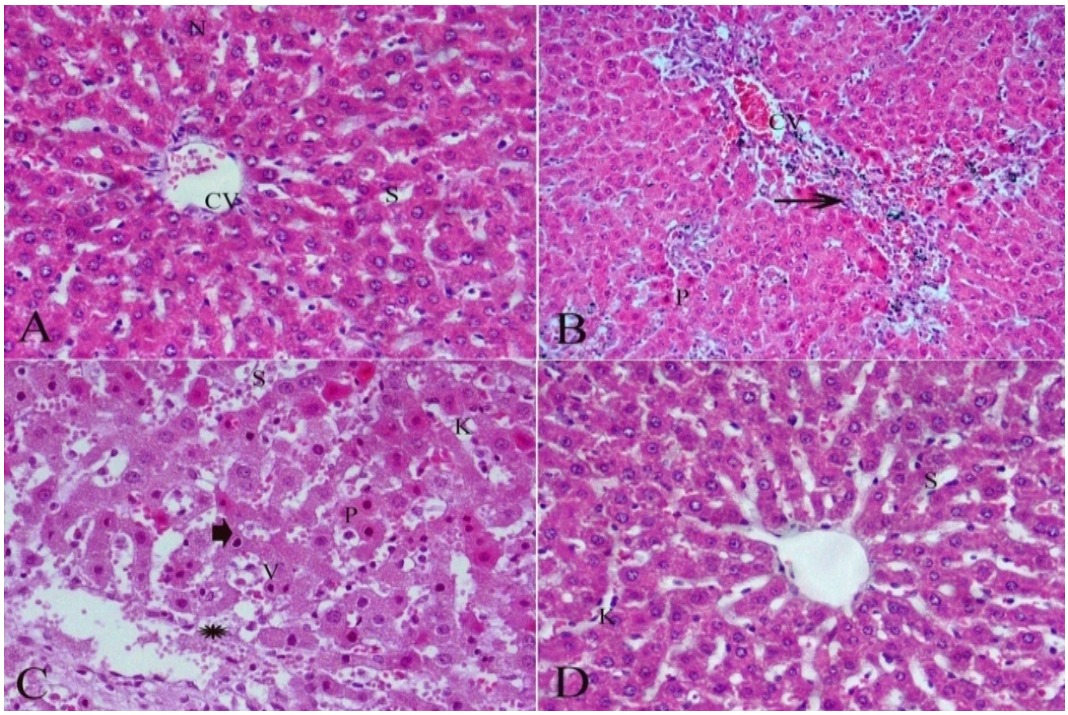

Fig 4. Photomicrographs of liver sections stained with haematoxylin and eosin: A) control group showing normal hepatic lobule. Central vein (CV), hepatocytes with prominent nuclei (N) and hepatic sinusoids (S) are seen, B) galactosamine group showing distorted liver architecture and intense inflammatory cells infiltrating around central vein, hepatocellular necrosis (arrowhead) with hemorrhage between hepatocytes (H). Activated kupffer cells (K) and pyknotic nuclei (P) were also observed, C) galactosamine group showing distorted liver architecture, degenerated of hepatocytes (star), dilated and hemorrhage of blood sinusoids (S). Activated kupffer cells (K), pyknotic nuclei (P) and apoptotic nuclei (arrowhead) were also observed, D) galactosamine and silymarin group showing hepatocytes mostly had normal appearance with minimal dilated of blood sinusoids (S) and activated kupffer cells (K) (H & EX 400).

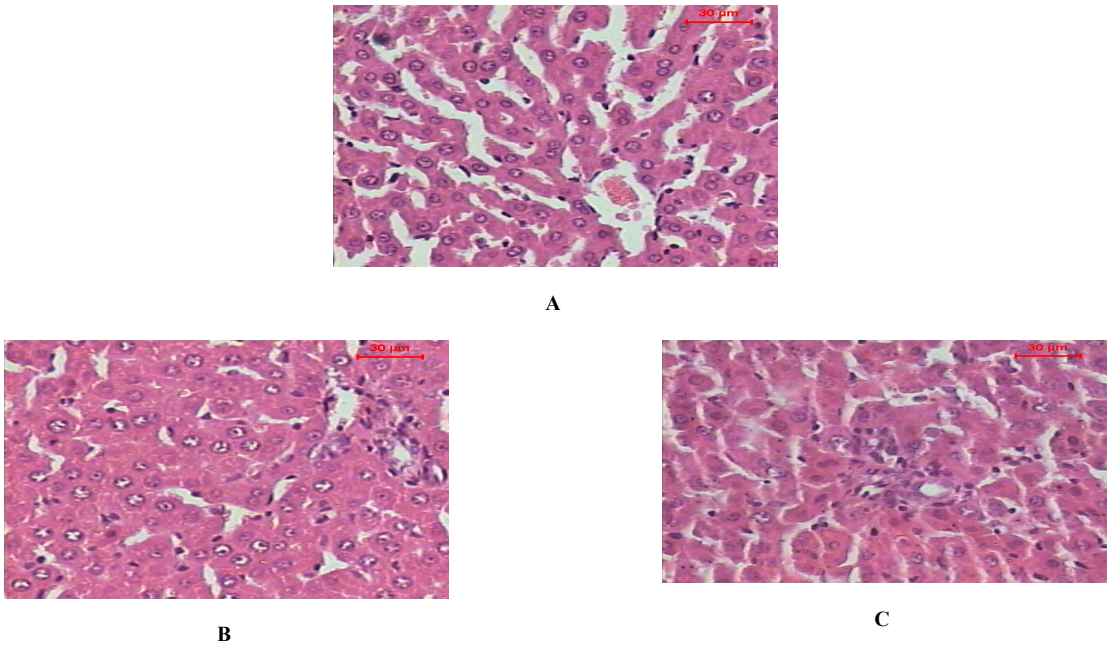

Fig 5. Photomicrographs of liver sections stained with haematoxylin and eosin:A) Low dose and galactosamine showing moderately improved and dilated blood sinusoids (S) with activated kupffer cells (K) and pyknotic nuclei (P).B) Low dose and galactosamine showing moderately improved.C) High dose and galactosamine showing resorted of most hepatocytes architecture, with minimal dilated blood sinusoids (S) with activated kupffer cells (K) (H & E X 400).

RESULTS AND DISCUSSION

Identification of phenols from different sources is becoming increasingly important due to their potential application for treating diseases. In *Nannorrhops ritchiana* identification of phenolic acids and flavonoids using LC/MS/MS method, based on comparing MS/MS spectra data with those stored in MS/MS spectra library[8]. Seven flavonoid compounds were identified, in this study, five of these flavonoids were reported for the first time from this species as: Epihesperidin**,** demethoxycentaureidin7-O-rutinoside, icariin, datiscin, isoschaftoside, along with, rutin, and hesperetin, previously identified (Table 1), (Fig 1). Also, twelve phenolic acid compounds were identified in this plant species, nine compounds reported for the first time: Quinic acid, p-hydrobenzoic acid, caftaric acid, caffeoylshikimic acid, malic acid, chlorogenic acid, 2-naphthoxyacetic acid, 3-(2-hydroxyphenyl) propionic acid, D-glucaric acid, along with, catechin, caffeic acid and benzoic acid, previously identified (Table 2), (Fig 2). GC-MS analysis of n-hexane extract of plant showed the presence of thirty one phytochemical compounds (Table 3) (Fig 3). The identification of compounds was based on the peak area, retention time and molecular formula. Of the 31 compounds eluted from the extract, phytol had the highest peak area of 19.82% and the lowest was stigmasterol showing 0.25%.

Hepatoprotective activity

In this study, D-galactosamine significantly increased the mean value levels of liver function tests compared to control group. It was observed that levels of SGOT, SGPT, ALP, TP and bilirubin were elevated in galactosamine group as it caused severe liver injury. DGa1N could be taken as an index value of liver damage, that might be due to the fact that its administration disturbed plasma membrane permeability causing leakage of liver enzymes from the cell, which led to elevation in serum enzymes levels[13].The values of liver enzymes were significantly restored by high dose (500 mg/kg) followed by low dose (250 mg/kg) of plant extract (Table 4). The reduction in the levels of enzymes and bilirubin towards the normal value was an indication of regeneration process and hepatoprotective activity of plant methanol extract. Also, DGa1N administration induced liver injury, was generally attributed to the formation of the highly reactive hydroxyl radical (OH·) which led to severe oxidative damage of the liver cells' components like lipids, proteins and DNA[14], and increasing the secretion of proinflammatory cytokines such as TNF-α and interkulin-1α (IL-1α)[13].Thus, the current results appeared a significant increase in oxidative stress parameters (NO and MDA) along with a significant reduction in PON1, the antioxidant enzyme, in DGa1N group compared to control one. The MDA and NO levels were significantly decreased in treated groups compared to DGa1N group. Surprisingly, the antioxidant enzyme (PON1) was significantly increased only in the low dose of the plant (Table 5). In addition, hyaluronic acid (HA) was significantly increased in DGa1N group compared to control one indicating liver injury, thus several liver diseases were associated with elevation of HA levels[15]. Also there was significant increase of matrix metallopeptidase1 (MMP1) [which were important markers for diagnosis of inflammatory process], in DGa1N group compared to control group (Table 6). No significant difference in the elevation of MMP1 levels was observed in low and high doses treatment of the plant. On the other hand, HA levels were significantly decreased in the treated groups compared to DGa1N group (Table 6). These results indicating the ability of the used plant in attenuating liver inflammation and injury, maintaining liver health and functions confirming its hepatoprotective activity.

Histopathological results

The light microscopy examination of control rat liver revealed normal hepatocytes that appeared to radiate from the central vein, blood sinusoids with prominent nucleus (Fig 4 A). The sections from galactosamine group indicated disordered of hepatic structure that associated with clear apparent and intense centrilobular necrosis altogether with centrilobular area infiltration of mononuclear cells especially near the central vein and portal area. Hepatocytes of these areas showed vacuolation. In addition, there were dilation, hemorrhage of sinusoids, and activated kupffer cell. Apoptotic and pyknotic nuclei were also

observed (Fig 4B-C). In silymarin group, histopathological investigation showed attenuation of the degree of liver damage and a reduction of hepatocellular necrosis, inflammatory cell infiltration. Few activated kupffer cell and dilated blood sinusoid were also noticed (Fig 4D). Low dose of *Nannorrhops ritchiana* and galactosamine showing moderately improved and dilated blood sinusoids (S) with activated kupffer cells and pyknotic nuclei (Fig 5A). On the other hand, in some cases of low dose of *Nannorrhops ritchiana* and galactosamine showed moderately improved (Fig 5B). C) In case of a high dose of *Nannorrhops ritchiana* and galactosamine, results showed resortation of most hepatocytes architecture, with minimal dilated blood sinusoids and activated kupffer cells (Fig 5C).

From these results we concluded that the plant was effectively attenuating DGa1N induced–liver injury and its hepatoprotective action may be related to the fact that they contain a combination of different phytochemicals and bioactive constituents such as phenolics, flavonoids, sterols and terpenoids that were synergistic in their action, such diversity of these compounds magnified their biological properties, so that it can be recommended as a plant of pharmaceutical importance.

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