Effect of Portulaca Oleracea Extract and Its Interactions with Propranolol in Cirrhotic Portal Hypertensive Rats

Eman Ahmed Ibrahim Mohamed Abdou, M Sc [1]; Sohair Hanem Samir Elmenshawy, MD[1]; Amin Mahmoud Sekena, MD[1]; Samia Salah Mohamed Hafez, MD [2]; Dalia I Hemdan, MD[3]

[1] Department of clinical pharmacology, Faculty of medicine, Zagazig University, Egypt
[2] Department of pharmacognosy, Faculty of pharmacy, Zagazig University, Egypt
[3] Department of pharmacognosy, Faculty of pharmacy, Menoufia University, Egypt
Corresponding Author: Eman Ahmed Ibrahim Mohamed Abdou
Email: Dr_emepharma@yahoo.com

Abstract:

Background and aim: portal hypertension (PHT) is a common complication of liver cirrhosis and/or fibrosis. Propranolol is a nonselective beta blocker that is commonly used to reduce portal pressure. Portulaca oleracea extract has been shown to have hepatoprotective properties. The aim of this study is to assess the effects and interactions of portulaca oleracea extract and propranolol in cirrhotic PHT in rats.

Materials and methods: Seventy adult male albino rats were divided into 7 groups. Group I: control group, Group II: CCl_4 untreated group, Group III: treated with propranolol at dose of 75mg/kg with CCl_4, Groups IV, V and VI: treated with portulaca oleracea extract at doses of 0.05, 0.1, and 0.15 g/kg with CCl_4, Group VII: treated with portulaca oleracea 0.15 g/kg plus propranolol 75 mg/kg with CCl_4. After 12 weeks, the portal pressure was measured in all groups then rats were sacrificed and blood samples were collected for estimation of liver enzymes (ALP, AST & ALP) levels then hepatic tissues were obtained for estimation of oxidative stress markers (MDA & SOD) and inflammatory markers (TNF-α, IL-6 & IL-10) as well as histopathological examination.

Results: Portulaca oleracea extract at doses of 0.1& 0.15 gm/kg, propranolol and portulaca oleracea 0.15 g/kg-propranolol combination significantly decreased the elevated portal pressure when compared to CCl_4 group. Portulaca oleracea alone, and in combination with propranolol significantly improved ALT, AST, ALP, MDA, SOD, TNF-α, IL-6, IL-10 levels as well as hepatic histopathological score of both fibrosis (staging) and necrosis (grading) when compared to CCl_4 group. Propranolol alone produced non-significant improvement in hepatic histopathological score of fibrosis (staging), liver enzymes, hepatic oxidative stress markers levels as well as hepatic level of IL-10 when compared to CCl_4 group.

CONCLUSION: Portulaca oleracea may be effective in the treatment of CCl_4 induced PHT. This is probably mediated through its anti-inflammatory and antioxidant properties.

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1. INTRODUCTION

Recently, cirrhosis has been ranked as the 11th most common cause of death accounting for 2.4% of all deaths worldwide [**1**]. **[2]** Reported that portal hypertension (PHT), defined as portal

pressure more than 12 mmHg, is the most common non-neoplastic complication of liver cirrhosis. Clinically, PHT is the major determinant of degree of decompensation in patients with liver cirrhosis **[3]**

Experimental PHT can be induced by several methods. Among these, carbon tetrachloride (CCl_4) induced hepatotoxicity is the most commonly used **[4].** Main advantages of the CCl_4 model are the considerable mimicry to clinical chronic liver disease and its reproducibility. Moreover, no special equipment or surgical intervention are needed. Also, it can be induced by various application routes **[5-6].**

Portulaca oleracea L. (p. oleracea) exhibits a wide range of pharmacological effects, including antibacterial **[7]**, anti-ulcerogenic **[8]**, anti-inflammatory **[9]** and antioxidant properties **[10]**. **[11]** found that p. oleracea extracts significantly reduce lipopolysaccharide-induced synthesis of nitric oxide, the production of tumor necrosis factor- α (TNF-α), interleukin-6 (IL-6), and the expression levels of various transcription factors as P65, p-P65, p-MEK and p-IκB-α which were inhibited dose-dependently. They reported that luteolin, kaempferol, and quercitrin components identified in the extracts were postulated to account for these anti-inflammatory effects.

To the best of our knowledge, the effect of P. oleracea in PHT has not been previously studied. In the current study, we aimed to evaluate the effect of P. oleracea alone and in combination with propranolol on CCl_4 induced PHT in adult male albino rats.

2. MATERIALS AND METHODS

2.1. Drugs and chemicals:

Propranolol hydrochloride, powder, Sigma, St. Louis, MO., USA; **Ethyl carbamate,** crystals, Prolabo, Paris; **Carbon tetrachloride (CCl_4)** Merck, Darmstadt, Germany; **Kits for estimation of liver enzymes [alanine transferase (ALT), aspartate transferase (AST)** and **alkaline phosphatase (ALP)]** Biodiagnostic CO., USA ; **Kits for estimation of inflammatory markers [tumor necrosis factor alpha (TNF-α), Interleukin 6 (IL-6)** and **Interleukin 10 (IL-10)],** Quantikine® ELISA, USA & Canada | R&D Systems, Inc. ; **Kits for estimation of oxidative stress parameters [malondialdahyde (MDA)** and **superoxide dismutase (SOD)],** Biodiagnostic CO., USA.

2.2. Plant material:

P. oleracea was collected from local fields in Ash Skarqia governorate, Egypt. The plant was kindly identified at plant taxonomy department, faculty of Sciences, Zagazig University, Egypt.

2.3. Animals:

We obtained 70 adult male albino rats weighing 200-250 gm from Faculty of Veterinary Medicine, Zagazig University, Egypt. All experimental protocols were approved by the ethics committee of Zagazig University. The animals were kept in cages with mesh bottoms (to prevent coprophagy) at air temperature ranging between 20- 26°C with 12 hours light/dark cycle. Standard food was allowed ad labitum and tap water was freely accessed.

2.4. Experimental design:

Rats were divided into 7 equal groups; each consisting of 10 rats.

- Group I (control group) received distilled water (oral route; daily) and olive oil (0.5 ml/kg; every 3 days, i.p.) for a period of 12 wks.
- Group II (CCl_4 group) received distilled water (oral route; daily) and CCl_4 (0.5 ml/kg, 50% CCl_4 in olive oil; every 3 days, i.p.) for a period of 12 wks.
- Group III (group treated with propranolol) received propranolol dissolved in distilled water at dose of 75 mg/kg (oral route; daily) and CCl_4 (0.5 ml/kg, 50% CCl_4 in olive oil; every 3 days, i.p.) for a period of 12 wks.
- Groups IV, V and VI (groups treated with p. oleracea extract) received p. oleracea extract dissolved in distilled water at mild, medium, and high doses of 0.05, 0.1, and 0.15 g/kg, (oral route; daily); respectively with CCl_4 (0.5 ml/kg, 50% CCl_4 in olive oil; every 3 days, i.p.) for a period of 12 wks.
- Group VII (group treated with p. oleracea & propranolol combination) received p. oleracea extract 0.15 g/kg plus propranolol 75 mg/kg dissolved in distilled water (oral route; daily) with CCl_4 (0.5 ml/kg, 50% CCl_4 in olive oil; every 3 days, i.p.) for a period of 12 wks.

In the current study, the selected doses of p. oleracea extract and CCl4 were based on previous published works **[6, 12].**

2.5. Preparation of plant extract:

According to the method described by [**12]** the aerial parts were shade dried and ground to moderately fine powder. The powder was extracted with 80% ethanol (3×2L) until complete exhaustion. The combined extract was evaporated under reduced pressure at 50 °C using rotary evaporator to give a dark brown viscous residue. About 5gm of the residue was dissolved in methanol/water mixture (2:8) and extracted with light petroleum 60-80°C and the solvent was distilled off under reduced pressure to afford about 0.5gm residue (Light petroleum extract). The remaining of the alcoholic extract was kept in refrigerator for pharmacological study, determination of total flavonoid and phenolic contents as well as UPLC- ESI-MS-MS analysis.

2.6. Measurement of portal pressure: It was done at the end of experiment just before scarification**.** Rats were anesthetized with Ethyl carbamate (1.3gm/kg i.p. as 25% freshly prepared aqueous solution) **[13]**. A midline abdominal incision was made and the portal pressure was measured by inserting a normal saline filled 20-gauge needle into the portal vein. The needle was joined to a PE-50 tube which was fixed to a metered recording scale fixed to make the zero reading in mid-axilla. The pressure reading (in cm water) was considered satisfactory when a stable recording was produced **[14].**

2.7. Measurement of Serum biochemical parameters: It was done at the end of experiment just before scarification. Blood samples were collected by direct cardiac puncture and centrifuged at 2200 rpm for 15 min to separate serum. Then serum was transferred into sterile polypropylene tubes and kept at -20°C for assay. ALP was measured by colorimetric method **[15].** Also, ALT and AST were measured by colorimetric method **[16].**

2.8. Assessment of hepatic oxidative stress markers and hepatic inflammatory markers: All animals were sacrificed through decapitation to remove their livers shortly after blood samples were taken. Liver was dissected and freed of fat then its weight was measured. A portion of the left lobe of the liver was spared for pathologic analysis in 10% neutral formalin solution. The rest of the

liver was immediately frozen in liquid nitrogen and stored at −80°C which was then homogenized for biochemical parameters assay. MDA was measured by colorimetric method **[17, 18].** SOD was measured by colorimetric method **[19]**. TNF-α, IL-6 and IL-10 were measured by the quantitative sandwich enzyme immunoassay technique.

2.9. Histopathological examination: Liver tissues were fixed in buffered formalin (10%). Paraffin embedded sections (4 μm thick) were taken after fixation and slides were stained using H & E by the method of **[20]**. The slides were examined by light microscope to set out a numerical score amenable for statistical analysis. Grading of liver inflammation and staging for liver fibrosis were done as shown in **tables (1) and (2).**

2.10. GLC analysis of the fatty acids constituents and GLC/MS analysis of the unsaponifiable matter of p. oleracea:

- ***Saponification of light petroleum soluble fraction of the plant [21,22]:***

About 0.5g of light petroleum soluble fraction was refluxed with10 ml alcoholic potassium hydroxide (10%) for 6 hours. The alcohol was distilled off and the obtained residue was diluted with water (10 ml) and extracted with n-hexane (3x50 ml). The combined n-hexane extract was washed several times with water (to remove any alkalinity), dried over anhydrous sodium sulphate and evaporated to give 100 mg of the unsaponifiable matter (USM). The alkaline aqueous layer remained after extraction of the unsaponifiable matter was acidified with concentrated hydrochloric acid and extracted with Successive portions of n-Hexane (3x50 ml).The combined n-hexane extract was washed several times with water, dried over anhydrous sodium sulphate and distilled off to afford 70 mg of fatty acid contents (FA).

- ***Preparation of fatty acid methyl esters [22,23]***

About 50 mg of fatty acids residue was dissolved in 10 ml of dry methanol/ sulphuric acid (5% v/v) and left at room temperature (25 C) overnight, then refluxed for four hours. Methanol was distilled off under reduced pressure and 10 ml of brine solution was added to the residue. The solution was extracted with successive portions of mixture of (1: 1) light petroleum: ether (3 x20 ml). The combined ethereal extract was washed several times with distilled water, dried over anhydrous sodium sulphate, and evaporated to afford about 60 mg of fatty acids methyl esters (FAME). The obtained fatty acid methyl esters were subjected to GLC/MS analysis. The GC/MS analysis was performed using a Thermo Scientific, Trace GC Ultra / ISQ Single Quadrupole MS, TG-5MS fused silica capillary column (30m, 0.251mm, 0.1mm film thickness).For GC/MS detection, an electron ionization system with ionization energy of 70 eV was used, Helium gas was used as the carrier gas at a constant flow rate of lmL/min. The injector and MS transfer line temperature was set at 280 °C. The oven temperature was programmed at an initial temprature 50 °C (hold 2 min) to150 °C at an increasing rate of 7 °C /min .then to 270 at an increasing rate 5 °C /min (hold 2min) then to 310 as a final temprature at an increasing rate of 3.5 °C /min (hold 10 min). The quantification of all the identified components was investigated using a percent relative peak area. A tentative identification of the compounds was performed based on the comparison of their relative retention time and mass spectra with those of the NIST, WILLY library data of the GC/MS system.

2.11. Determination of total flavonoid and phenolic contents:

- ***Total flavonoid content:***

Total flavonoid content was evaluated using aluminum chloride-potassium acetate method **[24]**, where 0.5 ml of each extract was mixed with 1.5 ml 95% ethanol (v/v), 0.1 ml 10% aluminum chloride (w/v), 0.1 ml of 1M potassium acetate and 2.8 ml distilled water. The volume of 10% (w/v) aluminum chloride is substituted by the same volume of distilled water in blank. The samples under test were incubated at room temperature for 30 min and the absorbance of the reaction mixture was measured at λmax 415 nm. Three replicates were carried out. Flavonoids concentration was expressed as rutin and quercetin equivalents. The calibration curves of rutin and quercetin were constructed by dissolving standard rutin and quercetin in 95% ethanol at a final concentration of 1200 µg/ml. This stock solution was serially diluted with 80% ethanol to obtain the required concentrations (6.25-200 µg/ml) and (3.125-200 µg/ml), for rutin and quercetin respectively and treated similarly.

- ***Total phenolic content:***

Total phenolic content was determined using Folin-Ciocalteau colorimetric method **[25]**. 0.5 ml of each sample was mixed with 0.5 ml of distilled water, 5 ml of 0.2 N Folin-Ciocalteau reagent and 4 ml of saturated sodium carbonate solution (75 g/l) and incubated in darkness at room temperature for 2 hours .The absorbance was measured at λmax 765 nm. Three replicates were carried out.The same volume of distilled water was used as blank instead of the sample. The concentration of phenolic content was expressed as gallic acid equivalent. The calibration curve of gallic acid was constructed by dissolving 30 mg of gallic acid in 100 ml distilled water at a final concentration of 300 µg/ml. This stock solution was serially diluted with 80% ethanol (v/v) to obtain the required concentration (equivalent to 40- 300 µg/ml).

2.12. Methods for UPLC-ESI-MS-MS analysis:

- The sample solutions of alcoholic extract of *p. oleracea* L. was prepared at a concentration of 100 µg /mL using high performance liquid chromatography analytical grade methanol. The samples were filtered using a membrane disc filter (0.2 µm) then subjected to LC-ESI-MS analysis. Sample solutions were stored in a refrigerator at 4◦C until analysis. UPLC-ESI-MS-MS analysis was carried out on a XEVO TQD triple quadruple instrument using ESI-MS negative ion acquisition modes. The instrument was coupled to Waters Corporation Milford, MA01757 U.S.A, mass spectrometer. The chromatographic separation was performed on ACQUITY UPLC - BEH reversed phase C18 column (50 × 2.1 mm, 1.7 µm). The injection volume was set at 10 µL. Mobile phase elution was made with the flow rate of 0.2 mL/min using gradient mobile phase comprising two eluents: eluent A (H2O acidified with 0.1% formic acid) and eluent B (methanol acidified with 0.1% formic acid). The following gradient elution program was applied: 10% B (0-2 min), 10-30% B (2-5 min), 30-70% B (5-15 min), 70-90% B (15-22 min), 90% B (22-225 min), 90-100% B (25-26 min), 100% B (26- 29 min, and 10% B (29-32 min). The parameters for analysis were carried out using negative ion mode as follows: source temperature 150 °C, cone voltage 30 eV, capillary voltage 3 kV, solvation temperature 440 °C, cone gas flow 50 L/h, and solvation gas flow 900 L/h. Mass spectra were detected between m/z 100–1000. The peaks and spectra were processed

using the Maslynx 4.1 software and tentatively identified by comparing its retention time (R_t) and mass spectrum with reported data **[26].**

2.13. Statistical analysis:

The collected data was statistically analyzed using SPSS program (Statistical Package for Social Sciences) software version 26.0. Descriptive statistics were done for numerical parametric data as mean ± SE (standard error). Comparison between different groups were performed using one way analysis of variances (one-way ANOVA) followed by Post-Hoc (least significant difference) tests as described by **[27]**. The differences were considered to be significant when $p < 0.05$.

3. RESULTS:

3.1. Effect on portal pressure: The portal pressure was significantly increased in CCl_4 group compared with control group. Administration of propranolol, p. oleracea extract at medium and high doses (0.1, and 0.15 g/kg) and p. oleracea extract-propranolol combination significantly decreased the portal pressure in comparison to CCl_4 group. There were no significant differences in portal pressure among propranolol, p. oleracea extract and the combination groups regarding portal pressure (**Table 3**).

3.2. Effect on Liver enzymes: Regarding ALP, ALT and AST, their levels were significantly increased in CCl_4 group compared with control group. Administration of p. oleracea extract and p. oleracea extract-propranolol combination significantly decreased their levels in comparison to CCl_4 group. Compared to CCl_4 group, administration of propranolol alone produced non-significant difference in ALP, ALT and AST levels (**Table 3).**

3.3 Effect on hepatic oxidative stress markers: Compared to control group, SOD activity decreased significantly while MDA activity increased significantly in CCl_4 group. Treatment with p. oleracea extract and its combination with propranolol, hepatic level of SOD significantly increased while hepatic level of MDA significantly decreased in a dose-dependent manner compared to CCl_4 group. Administration of propranolol alone produced non-significant difference in SOD and MDA levels in comparison to CCl_4 group (**Table 4**).

3.4 Effect on hepatic inflammatory markers: Hepatic TNF-α and IL-6 levels were significantly increased in CCl_4 group compared to control group. Administration of propranolol, p. oleracea extract and p. oleracea extract-propranolol combination significantly decreased the TNF-α and IL-6 levels in comparison to CCl_4 group. On the contrary, administration of portulaca oleracea extract and p. oleracea extract-propranolol combination, but not propranolol alone, significantly increased IL-10 level in comparison to CCl_4 group (**Table 4**).

3.5 Effect on hepatic tissue: Microscopic examination of hepatic tissues in control group showed preserved lobular architecture with no fibrosis, lobular inflammation or necrosis. In contrast, CCl_4 group showed cirrhosis with extensive bridging fibrosis in the presence of regenerating nodules where normal portal areas and normal central veins couldn't be seen. Also, areas of hepatocellular damage were seen. Compared to CCl_4 group, livers from propranolol-treated rats showed significantly milder grades of hepatic inflammation. In the same group, stages of hepatic fibrosis were milder albeit being non-significant. P. oleracea extract, at high dose, alone and together with propranolol significantly mitigated grades of hepatic inflammation as well as stages of hepatic fibrosis **(Table 5, Figure 1**).

3.6 Analysis of fatty acid methyl esters: Analysis of fatty acid methyl esters was performed using GLC/MS. Identification of the different peaks was done by comparing the mass fragments of the isolated peaks with these of library references **(WILEY)**. The quantitative estimation was carried out by the peak area measurements **(Figure 2)** and the results were summarized in **table (6)**.

3.7 Quantitative estimation of the total flavonoid and phenolic contents:

Total flavonoid content was estimated by aluminum chloride potassium acetate spectrophotometric method, and expressed as quercetin and quercetin equivalents by reference to calibration curves where y = 0.001 x - 0.012 and r2 = 0.985 in case of rutin as standard while y = 0.0008 x + 0.0097 and r2 = 0.986 in case of quercetin as standard, **Table 7.** Total phenolic content was estimated by Folin-Ciocalteu method, are expressed as gallic acid equivalents (GAE) by reference to standard calibration curve y = 0.006x - 0.200, r^2 = 0.988.

3.8 UPLC-ESI-MS-MS analysis: Identification of phytoconstituents in alcoholic extract of *P. oleracea* L. achieved by using advanced analytical technique of Ultra Performance Liquid Chromatography coupled to Electrospray Ionization Mass/Mass Spectrometry (UPLC-ESI-MS-MS**)** in negative ionization mode. The tentative identification was based on comparison of the retention time, molecular ions, MS/MS fragmentation patterns with the previous literatures and library search such as Chemspider (http: // www.Chemspider.com), Massbank (http: //www.massbank.eu), MetLin (htt:// metlin.scripps.eud) and FooDB (http:// www.Foodb.ca).

Ninety three compounds were detected and tentatively identified; the results are illustrated in **Table 8**.

3.8.1 Organic acids and their derivatives: Twenty seven organic acids and their derivatives were detected and identified, from which eighteen free organic acids as *p*-coumaric acid (1), syringic acid (2), 4-hydroxy benzoic acid (5), vanillic acid (11), ferulic acid (17), malic acid (20), neochlorogenic acid (22), caffeic acid (27), hydroxyglutaric acid (37), *m*-coumaric acid (38), methoxycinnamic acid (41), chlorogenic acid (42), gallic acid (44), oxalic acid (45), 5-O-p-coumaroyl quinic acid (46), dihydroferulic acid (54), feruloyl malic acid (57), gentisic acid; 2,5-dihydroxy benzoic acid (82). Nine phenolic acid derivatives as protocatechuic acid hexoside (8), coumaric acid derivative (29), dihydroxybenzoic acid hexoside (31), di-O-caffeoylquinic acid derivatives (34), ferulic acid derivative (59), quinic acid derivative (60), methoxydihydroxy benzoic acid (73), ferulic acid methyl ester (79), *p*-coumaric acid hexoside (86) were identified as previously published.

Compound **(5)** is identified as 4-hydroxy benzoic acid as it showed molecular ion peak at *m/z* 137 [M-H] and base peak at *m/z* 93 $[M\text{-}H\text{-}COO]^-$ **[28].** Compound (17) exhibited $[M\text{-}H]^-$ at *m/z* 193 was suggested to be ferulic acid with fragment at *m/z* 178 $[M\text{-}H\text{-}CH_3]^-$ and fragment ion at *m/z* 161 $[M\text{-}H\text{-}OCH_3]^-$ **[28-29]**. Total current ion chromatogram of total alcoholic extract revealed the presence of two peaks at different retention times with the same molecular ion $[M\text{-}H]^-$ at *m/z* 353. Compounds (22) and (42) at R_t 8.65 and 11.53 min. were suggested to be neo chlorogenic and chlorogenic acids, respectively; based on the presence of fragment at *m/z* 191.0 (100%) due to quinic acid moiety **[28]**. Also the two compounds (17) and (59) were detected as ferulic acid and its derivatives **[26]**. Compound (46) is tentatively identified as 5-O-*p*-coumaroyl quinic acid

which showed [M-1] $^-$at m/z 337 and MS^2 fragment at m/z 191 [337-146]$^-$ indicating quinic acid residue in the structure after neutral loss of coumaroyl moiety **[30].**

With respect of phenolic acid derivatives, they are mostly glycosidated. The first step in their fragmentation is the cleavage of the glycosidic linkage to give fragment corresponding to the free phenolic acid and neutral mass loss of sugar moieties as hexose (162 m.u.) and pentose (132 m.u.) then neutral losses of CO_2 (44 m.u.), H_2O (18 m.u.) and and 15 m.u.for CH_3 group. Compound (8) was suggested to be protocatechuic acid hexoside gave precursor ions 315 at m/z $[M-H]^-$. The MS^2 of the compound showed the fragment ion at m/z 153 which corresponds to protocatechuic acid after lossing a hexose moiety **[31]**

3.8.2 Flavonoids and their glycosides: Four flavonoid aglycones; luteolin-7,3',4'-trimethyl ether (48), eriodictyol (56), apigenin (70) and myricetin (93) were identified as reported data. Compound (48) showed molecular ion peak at m/z 327 $[M-1]^-$ and MS^2 at m/z 313, 299 and 285 due to sequential losses of methyl groups, so it was suggested to be luteolin-7,3',4'-trimethyl ether **[32]**. Also, four flavonoid glycosides were tentatively identified; apigenin-O-hexoside (9), quercetin-O-pentoside (12), quercetin-3-O-glucouronide (55), and luteolin-7-O-rutinoside (85) as the mass fragmentation of this class of compounds is characterized by loss of the sugar moieties **[33].**

Apigenin-O-hexoside (9), showed molecular ion peak at m/z 431, in MS^2 spectrum the fragment ion at m/z 269 $[M-H-162]^-$ appeared after loss of hexose moiety **[34].** While, compounds (12) and (55) produced base peak at m/z 301 (for quercetin as aglycone) in the MS^2 analysis after loss of 132 m.u. and 176 m.u. as sugar moieties for compound (12) and compound (55) respectively so, identified as quercetin-O-pentoside and quercetin-3-O-glucouronide, respectively. Also, compound (85) showed deprotonated ion at m/z 593 and base peak at m/z 285$[M-H-146-162]^-$ due to loss of rutinoside moiety **[33]** and identified as luteolin-7-O-rutinoside. Five isoflavones are identified; genistin (10), lonchocarpic acid (13), portulacanones C (14), portulacanone D (51) and portulacanone A (61).

3.8.3 Coumarins and coumarin derivatives: Four compounds are tentatively identified as scopoletin (35), bergapten (43), propoxy-methoxy coumarin (49) and meranzin hydrate (78). Compounds (35) identified as scopaletin showed precursor ion at m/z 191 and a fragment ions at m/z 177 due to loss of methyl group, while meranzin hydrate (78) gave fragment ion at m/z 259 after loss of 18 m.u. for water molecule.

3.8.4 Alkaloids and other nitrogen containing compounds: Fifteen alkaloids and nitrogen containing compounds were identified as uridine (4), 3-formylindole (15), benzamide (18), β-carboline-carboxylic acid (19), indole-3-carboxilic acid (24), N- trans-feruloyloctopamine (25), N-cis-feruloyloctpamine (26), N-trans-feruloyltyramine (30), guanosine (33), N-trans-feruloyl-3-methoxytyramine (39), 1-(5-bicyclo [2.2.1] heptyl) ethylamine (47), β-carboline-1-carboxylic acid (50), oleracein A (64), 1-carbomethoxy-β-carboline (76), and oleracimine A (88). Two isomers for β- carboline-carboxylic acid (19) and (50) showed a deprotonated molecular ion at m/z 211 with MS^2 fragment at m/z 167 $[M-H-CO_2]^-$ revealing the presence of β- carboline nucleus known as norharmane **[35],** while N-trans-feruloyltyramine (30) produced $[M-H]^-$ ion at m/z 312 fragmented to give MS^2 ions at m/z 178 $[M-H-CH_3-C_8H_7O]^-$ **[36]**

3.8.5 Amino acids and their derivatives: Seven amino acids are identified as previousely reported as lysine (6), valine (16) phenylalanine (21), methionine (36), tyrosine (40), thymine (53), and ethyl-N-acetylphenylalaninate (69). Fragmentation pattern of amino acids is characterized by sequential losses of CO and H_2O **[37].**

3.8.6 Tannins: Catechol (7), (epi)catechin-(epi)catechin (81), digalloyl hexose (91) and (epi) gallocatechin (92) were tentatively identified. Compound (81) showed $[M-H]^-$ at *m/z* 577 with MS^2 base peak at *m/z* 425 $[M-H-152]^-$ produced by retro-Diels-Alder fission for ring C or F followed by removal of water to give *m/z* at 407 $[M-H-152-18]^-$. The fragment at *m/z* 451 [M-H-126] is due to heterocyclic ring fission of ring C which then fragmented by retro-Diels-Alder fission for ring F to give *m/z* 299 $[451-152]^-$ with other fragments at *m/z* 289 and 287 due to ring C and D fission indicating the presence of two monomers of (epi) catechin type **[38- 40]**.

Compound with the precursor ion at m/z 483 has been assigned to be digalloyl-hexoside (91), MS and MS/MS spectra that showed product ions at m/z 331 $[M-H-152]^-$ and 169 $[M-H-152-162]^-$ corresponding to the neutral losses of galloyl and hexose moieties respectively **[41]**

3.8.7 Fatty acids and their derivatives: Ten fatty acids were tentatively identified as dihydroxy-octadecatetraenoic acid (52), docosapentaenoic acid (62), dihydroxy-octadecenoic acid (63), dihydroxy-octadecadienoic acid (71), monohydroxy-octadecatrienoic acid (74), monohydroxy-octadecadienoic acid (77), docosahexaenoic acid (83), α-linolenic acid (84), linoleic acid (87) and palmitic acid (89) which are characterized by losses of carboxyl and / or water as reported data. The LC-MS data revealed the presence of three methyl esters of polyunsaturated fatty acids hexanedioic acid mono (2-ethylhexyl) ester (23), 9,12-octadecadienoic acid (Z, Z)-2,3 bis [(trimethylsilyl) oxy] propyl ester (66) and tetradecenoic acid, 12-methyl-, methyl ester (90) were identified as reported literature.

3.8.8 Miscellaneous: Vanillin **(**3) is an aldehyde while syringaresinol hexoside (28) is a lignan, the precursor ion was detected at *m/z* 579 $[M-H]^-$ and its characteristic MS^2 $[M-H-hexose]^-$ fragment ion at *m/z* 417 **[42].** Compound (32) is tentatively identified as dihydroxybenzaldehyde **[43]**, it showed precursor ion at *m/z* 137 $[M-H]^-$ and MS^2 fragment at *m/z* 93 $[M-H-CO-H_2O]$, the betalain; portulacaxanthin III (58) and the fatty alcohol trans-2-dodecen-1-ol (67) were also identified. β-carotene (65) has molecular formula $C_{40}H_{56}$, belongs to the group of carotenoids consisting of eight isoprene units and showed a molecular ion peak at *m/z* 535 $[M-H]^-$ and other fragment at *m/z* 268 $[M-H- C_{21}H_{30}]^-$. Daucosterol (68) is a natural phytosterol compound (glucoside of β-sitosterol), exhibited a deprotonated molecular ion peak at *m/z* 575 $[M-H]^-$, which fragmented in MS^2 to produce a base peak fragment ion at *m/z* 413 $[M-H-162]^-$, revealing the neutral loss of glucose moiety, as well as a fragment ion at *m/z* 359, 331 and 308 **[44].**

β-Sitosterol **(**72) is an unsaturated sterol characterized by lossing water molecule upon fragmentation. It showed parent ion peak $[M - H]^-$ at m/z 413 and fragments at m/z 255 [M-side chain $-H_2O]^-$. Copper (75) and tri-butyl phosphate (80) commonly known as TBP is an organophosphorus compound with the chemical formula $(CH_3CH_2CH_2CH_2O)_3$ PO; showed a precursor ion at *m/z* 265 $[M-H]^-$ and MS^2 fragment at *m/z* 209 $[M-H-C_4H_7]^-$were also identified as reported data **[45].**

Table (1): Grades of hepatic inflammation [46]

Grade	Description	Piecemeal necrosis	Lobular inflammation and necrosis
0	No activity	None	None
1	Minimal	Minimal, patchy	Minimal; occasional spotty necrosis
2	Mild	Mild; involving some or all portal tracts	Mild; little hepatocellular damage
3	Moderate	Moderate; involving all portal tracts	Moderate; with noticeable hepatocellular damage
4	Severe	Severe; may have bridging fibrosis*	Severe; with prominent diffuse hepatocelluar damage

* **Bridging fibrosis** refers to the presence of fibrosis that reaches from a portal area to another portal area.

Table (2): Stages of hepatic fibrosis [46]

Stage	Description	Criteria
0	No fibrosis	Normal connective tissue
1	Portal fibrosis	Fibrous portal expansion
2	Periportal fibrosis	Periportal or rare portal-portal septa
3	Septal fibrosis	Fibrous septa with architectural distortion; no obvious cirrhosis
4	Cirrhosis	Cirrhosis*

* **Cirrhosis** consists of extensive bridging fibrosis in the presence of regeneration such that normal portal areas and normal central veins cannot be identified.

Table (3): Effect (mean ± SE) of propranolol (75mg\kg), p. oleracea (0.05 g/kg), p. oleracea (0.1 g/kg), p. oleracea (0.15 g/kg) and combination of propranolol (75mg\kg) and p. oleracea (0.15 g/kg) on portal pressure as well as serum liver enzymes (ALP, ALT and AST) levels in experimentally induced portal hypertension using CCL4 (n=6):

	Portal pressure (CmH_2O) (mean±SE)	**ALP (IU/L) (mean ± SE)**	**ALT (U/mL) (mean ± SE)**	**AST (U/mL) (mean ± SE)**
Control	**11.5 ± 0.9 A**	**100.5 ± 4.1 A**	**23.9 ± 2.4 A**	**32.8 ± 3.9 A**
CCL4	**31.2 ± 0.7 B**	**194.3 ± 6.2 B**	**73.4 ± 12.1 B**	**104.5 ± 8.5 B**
Propranolol	**23.0 ± 1.5 C**	**191.4 ± 5.9 B**	**75.8± 14.2 B**	**106.3 ± 9.1 B**
p. oleracea 0.05g/kg	**29.8 ± 2.1 B**	**170.3 ± 3.9 C**	**58.9 ± 9.0 C**	**83.9 ± 6.7 C**
p. oleracea 0.1g/kg	**24.3 ± 0.7 C**	**151.9± 3.5 D**	**56.0 ± 10.1 C**	**79.2 ± 7.3 C**
p. oleracea 0.15g/kg	**22.9 ± 1.2 C**	**132.3 ± 5.9 E**	**44.8 ± 7.6 D**	**55.9 ± 6.2 D**
Propranolol plus p. oleracea 0.15g/kg	**20.4 ± 2.4 C**	**.1251 ± 5.4 E**	**41.0± 10.7 D**	**51.8± 8.4 D**

Table 4 Effect (mean ± SE) of propranolol (75mg\kg), p. oleracea (0.05 g/kg), p. oleracea (0.1 g/kg), p. oleracea (0.15 g/kg) and combination of propranolol (75mg\kg) and p. oleracea (0.15 g/kg) on hepatic SOD, MDA, TNF-α and IL-6 and IL-10 levels in experimentally induced portal hypertension using CCL4 (n=6):

	MDA (nmol/gm) (mean ± SE)	SOD (U/mg) (mean ± SE)	TNF-α (Pg/mg) (mean ± SE)	IL6 (Pg/mg) (mean±SE)	IL10 (Pg/mg) (mean ± SE)
Control	1.7 ± 0.2 A	22.6 ± 2.8 A	78.3 ± 8.1 A	35.5 ± 5.8 A	52.8 ± 7.5 A
CCL4	4.1 ± 0.3 B	11.1 ± 1.3 B	169.8 ± 11.8 B	103.4 ± 10.2 B	53.2 ± 7.0 A
Propranolol	3.9 ± 0.2 B	11.8 ± 1.5 B	129.3 ± 6.3 C	76.6 ± 5.1 C	55.1 ± 4.8 A
p. oleracea 0.05g/kg	3.3 ± 0.1 C	16.8 ± 0.9 C	138.1 ± 9.9 C	79.1 ± 7.4 C	73.0 ± 10.3 B
p. oleracea 0.1g/kg	3.2 ± 0.2 C	17.2 ± 1.1 C	112.6 ± 5.5 D	77.5 ± 6.2 C	74.6 ± 9.5 B
p. oleracea 0.15g/kg	2.6 ± 0.1 D	21.4 ± 1.6 A	107.4 ± 7.6 D	63.8 ± 2.3 D	77.8 ± 7.9 B
Propranolol plus p. oleracea 0.15g/kg	.24 ± 0.2 D	22.5 ± 1.7 A	85.1 ± 6.4 A	49.7 ± 4.3 E	75.9 ± 10.5 B

Table 5 : Effect (mean ± SE) of propranolol (75mg\kg), portulaca oleracea (0.05 g/kg), portulaca oleracea (0.1 g/kg), portulaca oleracea (0.15 g/kg) and combination of propranolol (75mg\kg) and portulaca oleracea (0.15 g/kg) on histopathological score of hepatic damage in experimentally induced portal hypertension using CCL4(n=6):

	Pathological score (Grading)	Pathological score (Staging)
Control	0.17 ± 0.03 A	0.00 ± 0.00A
CCL4	3.3 ± 0. 23 B	3.5 ± 0.24 B
Propranolol	2.0 ± 0.00 C	3.0 ± 0.00 B
Portulaca oleracea 0.05g/kg	2.0 ± 0.00 C	2.8 ± 0.18 B
Portulaca oleracea 0.1g/kg	1.2 ± 0.18 D	2.8 ± 0.18 B
Portulaca oleracea 0.15g/kg	1.00 ± 0.00 D	1.3 ± 0.23 C
Propranolol plus Portulaca oleracea 0.15g/kg	1.00 ± 0.00 D	1.3 ± 0.23 C

Table (6) GLC/MS analysis of fatty acid methyl esters of p. oleracea

No	Rt	Area %	M. Wt	Molecular formula	Mass fragment	Name
1	5.38	4.06	216	$C_{11}H_{20}O_4$	185, 152, 143, 124, 111, 98, 83, 55	Nonanedioic acid dimethyl ester (Azelaic acid dimethylester)
2	6.09	0.33	214	$C_{14}H_{30}O$	196, 185, 152, 124, 111, 97, 69, 59, 54	3-Tetradecanol
3	7.19	0.58	230	$C_{12}H_{22}O_4$	199, 166, 138, 98, 87, 74	Decanedioic acid dimethylester (Sebacic acid dimethyl ester)
4	8.64	3.57	242	$C_{15}H_{30}O_2$	211, 199, 157, 143, 74	Tetradecanoic acid methyl ester (Methyl myristate)
5	9.26	0.58	244	$C_{13}H_{24}O_4$	213, 185, 152, 139, 121, 111, 98, 87	Undecanedioic acid dimethyl ester (dimethyl undecanedioate)
6	9.96	0.19	256	$C_{16}H_{32}O_2$	213, 199, 155, 143, 111, 109, 97, 87, 55	12 Methyl tetradecanoic acid methyl ester (methyl 12-ethyltetradecanoate)
7	10.45	0.62	256	$C_{16}H_{32}O_2$	256, 222, 195, 178, 161, 150, 123, 110, 111, 95, 87, 74	Pentadecanoic acid methyl ester (methyl pentadecanoate)

8	13.00	7.16	270	$C_{17}H_{34}O_2$	239, 227, 185, 143, 128, 101, 87, 74, 55	Hexadecanoic acid methyl ester (methyl palmitate)
9	14.26	2.58	268	$C_{17}H_{32}O_2$	236, 194, 152, 127, 113, 87	9-Hexadecenoic acid methyl ester (methyl palmitoleate)
10	14.54	0.92	284	$C_{18}H_{36}O_2$	241, 199, 143, 129, 125, 87	Hexadecanoic acid 14-methyl methyl ester (Methyl 14-methylhexadecanoate) (14- methyl palmitic acid methyl ester)
11	14.7	1.08	284	$C_{18}H_{36}O_2$	241, 199, 143, 129, 125, 87, 74	Hexadecanoic acid 15-methyl methyl ester (Methyl isoheptadecanoate)
12	14.97	0.93	240	$C_{15}H_{28}O_2$	208, 153, 125, 110, 98, 81, 69	Methyl Z-11-tetradecenoate
13	15.33	4.09	282	$C_{18}H_{34}O_2$	253, 241, 199, 185, 143, 129, 87, 74	11-Heptadecenoic acid methyl ester
14	15.78	1.27	286	$C_{17}H_{32}O_2$	254, 228, 207, 185, 158, 138, 125, 111, 97, 90, 83, 69, 57	Hexadecanoic acid, 2-hydroxy-, methyl ester
15	16.99	11.94	296	$C_{19}H_{36}O_2$	264, 222, 180, 132, 110, 83, 69, 54	9-Octadecenoic acid methyl ester (Methyl elaidate)
16	17.5	14.28	298	$C_{19}H_{38}O_2$	267, 255, 241, 213, 199, 162, 142, 129, 101, 97, 83, 74, 69, 55	Octadecanoic acid methyl ester (Methyl stearate)
17	18.78	0.31	294	$C_{19}H_{34}O_2$	263, 220, 164, 150, 125, 109, 95, 81, 67, 55	linoleic acid methyl ester (9,12-Octadecadienoic acid)
18	21.61	1.67	326	$C_{21}H_{42}O_2$	295, 283, 227, 199, 143, 129, 111, 101, 97, 87, 74	Eicosanoic acid methyl ester (Methyl arachidate)
19	23.57	0.19	340	$C_{22}H_{44}O_2$	309, 297, 241, 199, 185143, 129, 97, 87, 74, 55	Heneicosanoic acid methyl ester (Methyl heneicosanoate)
20	25.48	1.17	354	$C_{23}H_{46}O_2$	311, 255, 199, 143, 129, 87, 74	Behenic acid methyl ester (Docosanoic acid, methyl ester)
21	27.87	0.29	370	$C_{23}H_{46}O_3$	338, 311, 111, 97, 81	2-hydroxy Docosanoic Acid methyl ester (Docosanoic acid, 2-hydroxy-, methyl ester)
22	29.08	0.37	382	$C_{25}H_{50}O_2$	339, 283, 241, 199, 143, 129, 97, 87, 74	Lignceric acid methyl ester (Tetracosanoic acid methyl ester, Methyl lignocerate)

[a] Compounds are listed in order of their elution from the column , [b] Rt, Retention time , [C] MWt Molecular weight, [d] % Relative percentage.

Table (7): The total flavonoid and phenolic contents of the total alcoholic extract

Total alcoholic Extract	**Total flavonoids mg rutin/ g extract**	**Total flavonoids mg quercetin/ g extract**	**Total phenolic mg GAE/ g extract**
Extract	15.43±1.22	19.33±0.5	36.56±1.75

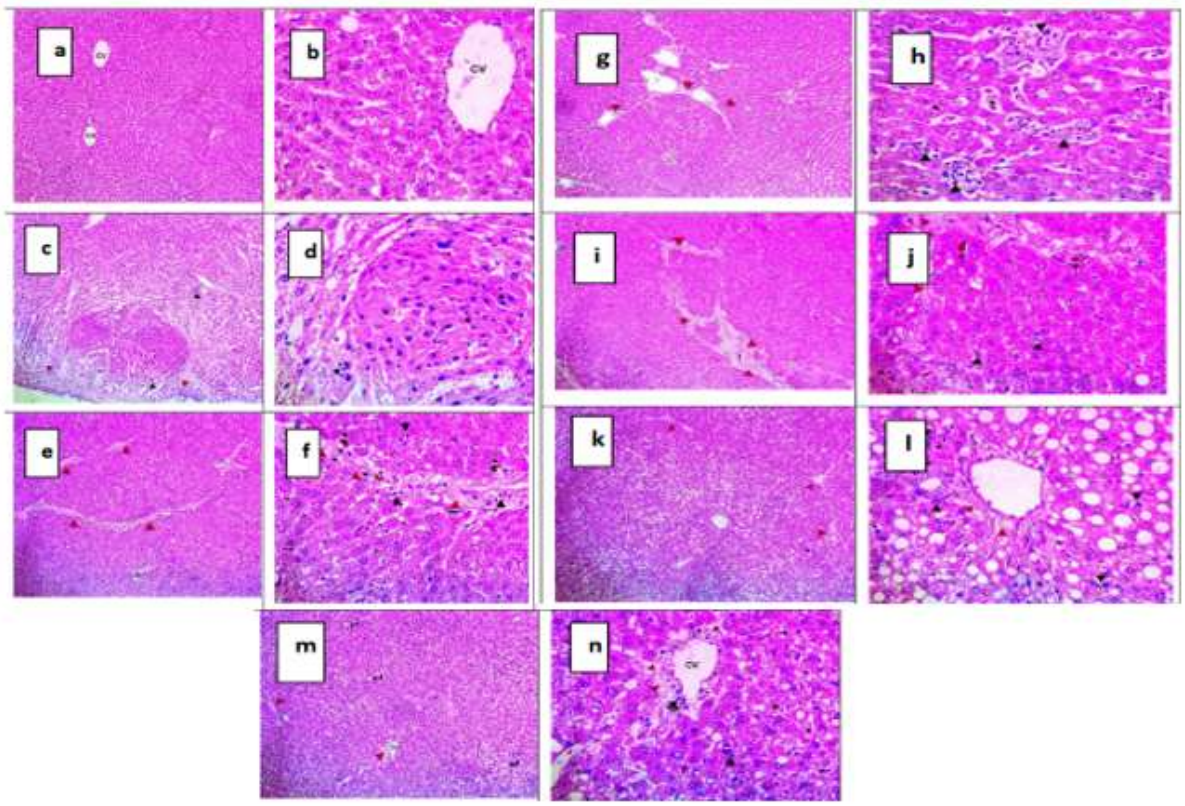
Table (8): Secondary metabolites identified from the total extract of p. *oleracea* L.

No.	**Rt.**	**M-H**	**MS²**	**Tentative identification**	**Ref.**
1	0.81	163.5412	163, 119	*p*-coumaric acid	28
2	0.94	197.8740	182, 153	Syringic acid	32
3	2.75	150.8704	151, 136	Vanillin	28
4	3.07	243.5462	133, 113, 112	Uridine	47
5	3.49	137.3220	137, 93	4-OH benzoic acid	28
6	4.64	145.3187	128, 117, 114, 103,	Lysine	43
7	5.26	108.7023	107, 93, 77	Catechol	44
8	6.53	315.1915	153	Protocatechuic acid hexoside	31
9	7.19	431.3233	269, 252, 214	Apigenin-O-hexoside	34
10	7.30	431.3264	269, 241, 151	Genistin	44

11	7.54	167.0784	152, 123	Vanillic acid	32
12	7.76	433.3552	301, 300, 179	Quercetin-O-pentoside	30
13	8.16	433.3264	419, 285, 257	Lonchocarpic acid	44
14	8.16	329.2137	315, 301	Portulacanones C (5,2'-dihydroxy-6,7-dimethoxy-3-benzyl-chroman-4-one)	44
15	8.21	143.9278	125, 115	3-Formylindole	36
16	8.23	116.0838	88, 85	Valine	43
17	8.29	193.0680	193, 178	Ferulic acid	29
18	8.34	119.9522	171, 155, 103, 77	Benzamide	35
19	8.59	211.9475	167	β-Carboline-3-carboxylic acid	35
20	8.60	133.0377	115, 71	Malic acid	43
21	8.62	164.0836	136, 133	Phenylalanine	43
22	8.65	353.2944	353, 191	Neochlorogenic acid	28
23	8.67	257.3351	145, 101, 57	Hexanedioic acid, mono (2-ethylhexyl) ester	45
24	8.73	160.0748	160, 142, 116	Indole-3-carboxilic acid	44
25	8.96	328.2234	310, 188, 135	N- trans-Feruloyloctopamine	36
26	9.03	328.1948	310, 188, 135	N- cis-Feruloyloctopamine	36
27	9.23	178.8953	179, 135	Caffeic acid	44
28	9.56	579.4005	417, 402, 387	Syringaresinol hexoside	42
29	9.63	377.3070	333, 271, 257, 163, 119	Coumaric acid derivative	48
30	9.66	312.2189	178, 119	N-trans-feruloyltyramine	36
31	9.74	313.8594	153, 109, 93	Dihydroxybenzoic acid hexoside	43
32	9.85	137.0507	137, 93	Dihydroxybenzaldehyde	43
33	10.21	282.1203	167, 139	Guanosine	47
34	10.49	515.2736	353, 179	Di-O-caffeoylquinic acid derivatives	49
35	10.60	191.3832	177, 164, 149, 121	Scopoletin	44
36	10.60	148.1466	120, 117, 101,	Methionine	43
37	10.69	147.2294	129, 115, 87	Hydroxyglutaric acid	50
38	10.75	163.4962	163, 119	*m*-coumaric acid	28
39	11.03	342.2415	312, 119	N-trans-feruloyl-3-methoxytyramine	36
40	11.07	180.5432	152, 149	Tyrosine	43
41	11.44	177.9700	147	Methoxycinnamic acid	51
42	11.53	353.3273	353, 191	Chlorogenic acid	28
43	11.71	215.7094	200	Bergapten	44
44	12.23	169.2735	169, 125	Gallic acid	28
45	13.33	89.1736	45	Oxalic acid	44
46	14.54	337.2885	191, 173, 155	5-O-p-Coumaroyl quinic acid	30
47	15.01	139	44, 43, 41	1-(5-Bicyclo [2.2.1] heptyl) ethylamine	45
48	15.37	327.3223	313, 299, 285, 197, 151,133	Luteolin-7,3',4'-trimethyl ether	32
49	15.99	232.9476	215, 173, 157	Propoxy-methoxy coumarin	
50	16.12	211.1521	167	β-Carboline-1-carboxylic acid	44
51	16.15	329.3350	315	Portulacanones D (5,2'-dihydroxy-7-methoxy-3-benzylidene-chroman-4-one)	44
52	16.46	307.2681	289, 235	Dihydroxy-octadecatetraenoic acid.	52
53	16.47	125.0747	121, 96, 83	Thymine	44
54	16.55	195.6117	177, 151, 136	Dihydroferulic acid	53
55	16.64	477.1714	301, 179, 15	Quercetin-3-O-glucouronide	48
56	16.75	287.3347	151, 135,107	Eriodictyol	44

57	16.80	309.3211	133	Feruloylmalic acid	43
58	16.81	267.0100	a	Protulacaxanthin III	44
59	16.81	293.2058	275, 235, 193	Ferulic acid derivative	44
60	17.20	533.4659	191	Quinic acid derivative	44
61	17.22	299.1651	285, 271	Portulacanones A (2'-hydroxy- 5,7-dimethoxy-3-benzyl-chroman-4-one)	44
62	17.25	329.3299	273, 245, 233, 205, 181,153	Docosapentaenoic acid	44
63	17.51	313.5873	295, 277, 245, 213, 195, 171, 155	Dihydroxy-octadecenoic acid	54
64	17.61	503.4678	341, 196, 178, 147, 119	Oleracein A (5-hydroxy-1-p-coumaric acyl-2,3-dihydro-1H-indole-2-carboxylic acid-6-O-β-Dglucopyranoside)	28
65	17.94	535.4851	268	β-carotene	50
66	18.31	498.4832	a	9,12-Octadecadienoic acid (Z, Z)-2,3 bis [(trimethylsilyl) oxy] propyl ester	45
67	18.45	183.1410	a	*Trans*-2-dodecen-1-ol	55
68	18.71	575.4490	413, 359, 331, 308	Daucosterol	44
69	18.86	234.6225	205, 163	Ethyl-N-acetylphenylalaninate	WILEY
70	19.45	269.6520	252, 214	Apigenin	44
71	19.50	311.2682	293, 275, 253, 235, 223	Dihydroxy-octadecadienoic acid	52
72	19.54	413.3947	366, 270, 255, 189, 175, 161	β-Sitosterol	44
73	20.06	183.1324	165, 139	Methoxy dihydroxy benzoic acid	44
74	20.44	293.3095	293, 275, 235, 221	Monohydroxy-octadecatrienoic acid	52
75	21.31	63.9171	----------	Copper	44
76	21.45	225.0587	193,167	1-Carbomethoxy-β-carboline	36
77	21.49	295.3376	295, 277, 195, 179	Monohydroxy-octadecadienoic acid	52
78	21.65	277.3567	259, 189	Meranzin hydrate	56
79	21.66	206.9392	193, 178	Ferulic acid methyl ester	57
80	22.21	265.1913	209, 150, 94	Tributyl phoaphate	45
81	22.47	577.4407	559, 451, 425, 407, 299	(epi)Catechin-(epi)Catechin-	40
82	22.33	152.9848	153, 109, 93	Gentisic acid (2,5-dihydroxybenzoic acid)	28
83	22.49	327.5405	a	Docosahexaenoic acid	44
84	22.59	277.1835	235, 220, 206, 192, 164, 150, 135, 109, 95, 81, 67, 55, 41	α-Linolenic acid	44
85	22.71	593.4404	285	Luteolin-7-O-rutinoside	33
86	22.77	325.3003	163	*p*-Coumaric acid hexoside	54
87	22.82	279.0974	237, 222, 208, 194, 166, 152, 137, 111, 97, 83, 69, 57, 43	Linoleic acid	44
88	23.18	299.1123	a	Oleracimine A	29
89	23.56	255.2852	211	Palmitic acid	44
90	23.71	255.2623	241, 227, 183, 169, 155, 141, 113, 85, 57	Tetradecenoic acid, 12-methyl-, methyl ester	45
91	25.16	483.4199	a	Digalloyl hexose	41
92	26.97	304.9768	261, 219, 221, 179, 165, 125	(epi) Gallocatechin	30
93	28.37	317.0052	179, 151	Myricetin	58

[a] Fragmentation was not achieved



a,c,e,g,I,k,m (H&E X100) while b,d,f,h,j,l,n (H&E X400).

Figure 1: Histopathology of various treatment groups;

(a,b) In the normal control group (Group I) animals showed preserved lobular architecture with no fibrosis **(Stage 0)**, Central vein (CV) with no lobular inflammation or necrosis **(grade 0**).

(c,d) Liver sections from the CCl_4 group (Group II) showed cirrhosis with extensive bridging fibrosis (red arrowheads) in the presence of regenerating nodule where normal portal areas and normal central veins cannot be seen **(stage 4)** with mild lobular inflammation (black arrowheads) and noticeable hepatocellular damage (asterisks) **(grade 3)**

(e,f) Animals treated with propranolol 75mg/kg with CCl_4 (Group III) showed septal fibrosis (Bridging fibrosis) (red arrowheads) with architectural distortion but no obvious cirrhosis **(Stage 3)** and mild lobular inflammation (black arrowheads) **(grade 2)** with cholestasis (dashed arrows).

(g,h) Animals treated with p. oleracea extract at dose of 0.05g/kg with CCl_4 (Group IV) showed septal fibrosis (red arrowheads) with architectural distortion but no obvious cirrhosis **(Stage 3)** and mild lobular inflammation (black arrowheads) with little hepatocellular damage (asterisks) **(grade 2)**

(i,j) Animals treated with p. oleracea extract at dose of 0.1g/kg with CCl_4 (Groups V) showed excess fibrosis (red arrowheads) surrounding hepatocytes (red arrowheads) **(stage 3)** with minimal lobular inflammation (black arrowheads) **(grade 1)** and cholestasis (dashed arrows).

(k,l) Animals treated with p. oleracea extract at dose of 0.15 g/kg with CCl_4 (Group VI) showed moderate steatosis (bent arrows) in a slightly distorted lobular architecture with some areas of portal fibrosis (red arrowheads) **(stage 1)** with minimal piecemeal necrosis (asterisks), and minimal lobular inflammation (black arrowheads) **(grade 1)**.

(m,n) Animals treated with p. oleracea extract 0.15 g/kg- propranolol combination with CCl4 (Group VII) showed moderate steatosis (bent arrows) with some areas of fibrosis (red arrowheads) **(Stage 1)** and minimal lobular inflammation (black arrowheads), with little hepatocellular damage (asterisks) **(grade 1)**. Central vein (CV)

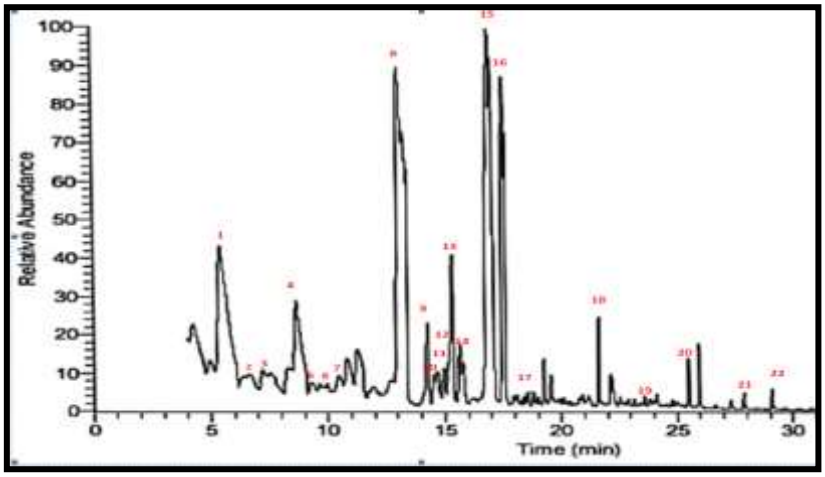


Figure 2: GLC/MS analysis of fatty acid methyl esters of p. oleracea

4. DISCUSSION

The main findings in our study were the hypotensive effect of p. oleracea on portal venous pressure as well as its beneficial additive effect with propranolol on hepatic inflammatory and oxidative stress markers in CCl_4 induced PHT.

Cirrhotic portal hypertension induced by CCl_4 has been widely used in the study of the pathophysiology of PHT **[59]**. This model was utilized in the current study to investigate the prophylactic effect of P. oleracea and its interaction with propranolol, a commonly used drug to treat portal hypertension.

4.1. Effect on portal pressure:

Rats in CCl_4 group showed significant elevation in the portal venous pressure which could be explained, according to **[60],** by obstruction of the portal vascular bed resulting from tissue fibrosis and other contractile cells. In addition, **[2]** postulated that in cirrhotic liver, portal hypertension results not only due to hepatic fibrosis but also microcirculatory dysfunction; hepatic sinusoidal vasoconstriction, and endothelial dysfunction **[61-62]**

In our study, administration of propranolol reduced significantly the effect of CCl_4 on portal pressure. These findings are fully compatible with literature data. **[63]** found that low dose of non-selective β-blockers caused unopposed splanchnic vasoconstriction (β2 effect), leading to reduced portal blood flow and, consequently, decreased portal hypertension. With higher doses, portal blood flow is further reduced by the negative effect of β-blockers on cardiac output (β1 effect). **[64]** suggested that propranolol could also decrease portal pressure by its ability to attenuate bacterial translocation.

Regarding the effect of p. oleracea on portal pressure, our study revealed a significant reduction in portal pressure with medium and high doses of p. oleracea extract (0.1, and 0.15 g/kg) when compared to CCl_4 group. Our study is, to the best of our knowledge, the first to report such a finding. We suppose that several mechanisms could explain this reduction. The anti-inflammatory and anti-fibrotic effect of p. oleracea could result in a decrease in intra-hepatic vascular impedance.

Moreover, p. oleracea has been supposed to improve intrahepatic microcirculation and endothelial function. This hemodynamic effect has been recently demonstrated by **[65]** who found that medium-dose and high-dose p. oleracea significantly decreased the expression of cyclooxygenase-2 in hepatocytes, a key rate limiting enzyme for synthesis of prostaglandin from arachidonic acid. Lastly, the potential effect of p. oleracea on gut micobiota **[66]** as well as its anti-angiogenic effect **[67]** could share in the reduction of portal pressure.

In our study, the combination of propranolol with p. oleracea extract did not result in further reduction than either alone. This may stem from the possible β2 agonist effect of p. oleracea extract on smooth muscles **[68]** partially abolishing the splanchnic vasoconstrictor effect of propranolol.

4.2. Effect on biochemical markers

In propranolol treated group, there was significant decrease in hepatic inflammatory cytokines (TNF-α, IL6) in comparison to CCl4 group. In addition, there was a trend toward improvement, albeit statistically insignificant, in hepatic levels of IL-10, oxidative stress markers (MDA and SOD), as well as hepatocyte necrosis markers (ALT, AST and ALP).

[69] reported that non-selective beta-blockers, such as propranolol, reduced inflammation-related transcription factors such as NFκB and STAT3. More specifically, **[70]** found that β1 blockade decreased hepatic expression of proinflammatory cytokines and plasma IL-6. On the other hand, **[71]** found that in RAW 264.7 cells, β2 blockade attenuated lipopolysacharide stimulated TNF-α production; thereby mitigating activation of caspases and apoptosis in hepatocytes.

In the present study, p. oleracea extract administration resulted in a dose dependent significant reduction in hepatic content of TNF-α, IL-6 and MDA when compared to CCl4 group. In addition, hepatocyte necrosis markers (ALT, AST and ALP) were significantly decreased in a dose dependent manner. On the contrary, the hepatic activity of SOD was significantly elevated. Regarding IL-10, our results showed that its hepatic level was significantly increased with all doses of p. oleracea.

Polysaccharides from p. oleracea downregulate the inflammatory cytokines TNF-α, IL-1 and IL-6. Furthermore, they upregulate production of IL-10 **[72]** Polysaccharides isolated from aerial parts of p. oleracea were shown to stimulate $CD4^+/CD25^+$ and $CD8^+/CD25^+$ (human T-cells), $CD14^+$ and $CD64^+$ cells (activated phagocytes) **[73]**. 1-carbomethoxy-carboline disturbed one of the major intracellular inflammatory signaling pathways associated with MAPKs and suppressed the nuclear translocation of NF-B, decreasing proinflammatory mediators such as iNOS, TNF-α, IL-6, and IL-1 **[36, 73].**

In our study, rats treated with propranolol- p. oleracea 0.15g/kg combination showed significant increase in hepatic levels of SOD and IL-10. In the contrary, TNF- α, IL-6, MDA, ALT, AST and ALP were significantly decreased when compared to CCl4 group. This significant reduction was more than reductions associated with either of the treatments alone. Intuitively, this observation can be explained by the additive hepatoprotective, anti-inflammatory and antioxidant effects of propranolol and p. oleracea. Noteworthy, insignificant reduction in MDA and hepatic necrosis markers was noted in the propranolol treated group. However, the combination of propranolol with p. oleracea 0.15g/kg resulted in significant hepatoprotection. In parallel, levels

of SOD and IL-10 were significantly increased with the combination of propranolol with p. oleracea but not with propranolol alone. Taken together, these observations could explain the more favorable outcomes regarding liver histopathology in the group treated with the combination of propranolol with p. oleracea 0.15g/kg.

4.3 Effect on hepatic tissue:

The histological examination of liver of CCl_4 group showed cirrhosis with extensive bridging fibrosis and formation of regenerating nodules. Also, areas of hepatocellular necrosis were seen. **[74]** showed that the mechanism of CCl_4-induced liver injury is involved in impairment of the anti-oxidative defense system during the metabolism of CCl_4**.**

The current study demonstrated that propranolol significantly reduced liver inflammation. However, fibrosis was not improved by propranolol treatment when compared to CCl_4 group. Failure of propranolol to improve hepatic fibrosis in the current study can be explained by the insignificant change in IL-10 which has been proven to be a potent anti-inflammatory and anti-fibrotic cytokine **[75-76]. [5]** reported that IL-10 can repress proinflammatory responses and limit unnecessary tissue disruptions caused by inflammation**.** In their study, **[77]** reported that sympathetic nervous system signaling activates HSC and consequently enhances hepatic fibrogenesis in NAFLD. This activation is both α- and β-adrenoreceptor mediated. Therefore, the authors found that propranolol can only partially mitigate hepatic fibrosis secondary to sympathetic overdrive.

The current study demonstrated that p. oleracea significantly reduced hepatic necrosis and fibrosis. Hesperidin, a bioflavonoid that was identified in p. oleracea extract used in our study, showed significant antifibrotic mechanism associated with its ability to reduce oxidative stress and modulate proinflammatory and profibrotic signals. It was found to modulate the effects of CCl4 toxicity, inhibit NF-κB, decrease the expression of TGFβ1, CTGF and IL-1β and upregulate IL-10 levels **[78].** Moreover, Quercetin (3,3,4,5,7-pentahydroxyfavone), a flavonoid was detected in p. oleracea extract used in our study with a wide safety profile and bioavailability, possesses an anti-inflammatory, antioxidant and antifibrotic activity **[79].** Quercetin was found to attenuate BDL or CCl4-induced hepatic fibrosis by inhibiting HSCs activation. Furthermore, it significantly exerted an antifibrotic activity by suppressing the TGFβ1/Smads signaling pathway and activating the PI3K/Akt signaling pathway.

5. **CONCLUSION**: Portulaca oleracea may be effective in the treatment of CCl_4 induced PHT. This is probably mediated through its anti-inflammatory and antioxidant properties

Abbreviations:

PHT portal hypertension, **CCl_4** carbon tetrachloride, P. oleracea portulaca orelacea, **ALT** alanine transferase, **AST** aspartate transferase, **ALP** alkaline phosphatase, **SOD** superoxide dismutase, **MDA** malondialdahyde, **TNF-α** tumor necrosis factor alpha, **IL-6** Interleukin 6, **IL-10** Interleukin 10

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