Quantitation and Characterization of Phenolic Compounds in Butanol Fraction of *Ficus Lyrata* Leaves and *In Vivo* Assessment of Hepatoprotective and Hypocholesterolemic Activities in Rats

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Quantitation and Characterization of Phenolic Compounds in Butanol Fraction of *Ficus Lyrata* Leaves and *In Vivo* Assessment of Hepatoprotective and Hypocholesterolemic Activities in Rats

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**ABSTRACT**

*Ficus lyrata* leaf extracts in 95% ethanol was fractionated by solvents of increasing polarity and each fraction was screened for its antioxidant activity. The butanol fraction was the most bioactive with 82.40% inhibition of DPPH free radicals. Flavonoids and phenolics of the butanol fraction were identified and quantified by high performance liquid chromatography (HPLC). *In vivo* assessment against liver fibrosis and hypercholesterolemia in rats showed that the butanol fraction was higher in antioxidant, hepatoprotective, and hypocholesterolemic activities than the standard drugs (silymarin and lipanthyl). These results were confirmed by the observed amelioration in the histopathological features of rat liver.

**ARTICLE HISTORY**

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**KEYWORDS**

Moraceae; flavonoids; phenols; liver fibrosis; hypercholesterolemia

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**Introduction**

*Ficus* genus (Moraceae), contains about 800 species of woody trees and shrubs (1). Egyptian *Ficus* species is used in folk medicine for skin diseases and respiratory disorders. Elevated levels of plasma cholesterol and triglycerides are causative factors in the development of coronary heart disease and atherosclerosis (2). Drugs being used as hypocholesterolemic agents include statins, fibrates, nicotinic acid and resins. Hepatic fibrosis is a condition in which liver cells are replaced by fibrous tissue. Experimentally, hepatic fibrosis has been shown to be produced by the administration of carbon tetrachloride (CCL₄), paracetamol, and thioacetamide (3). CCL₄, as a xenobiotic, caused oxidative stress and injure hepatic cells. Many studies have established the fact that CCL₄ is metabolized in the liver into a highly reactive substance, trichloromethyl, which initiates free radicals that mediate lipid peroxidation. Free radicals that
induce lipid peroxidation cause cell membrane damage leading to pathological changes in liver and in acute and chronic renal injuries (4).

Little phytochemical studies are reported in the literature for this plant species (5). Therefore, the aim of the present work was to evaluate the role of butanol fraction of *F. lyrata* in improving hypercholesterolemia and liver fibrosis in rats induced by high fat diet and CCl₄, respectively.

**Materials and Methods**

**Plant Material**

Fresh leaves of *F. lyrata* were collected from Orman Garden, Giza, Egypt. Specimens of the plants were identified at the Ministry of Agriculture and director of Orman Botanical Garden, Giza, Egypt, air-dried, powdered, and kept in tightly-closed containers until needed. Voucher specimen of *F. lyrata* leaves; 28-4-2015 II was deposited at Pharmacognosy Dept., Faculty of Pharmacy, Cairo University, Cairo, Egypt as a reference.

**Chemicals**

All chemicals and HPLC standards used were of high analytical grade, product of Sigma-USA, Aldrich Chemie-Germany and Biomedicals-France. Solvents were purchased from El Nasr Pharmaceutical Chemicals Co., Egypt.

**Extraction and Isolation**

The air-dried powdered leaves (3.5 kg) of *F. lyrata* were extracted by percolation with 95% ethanol until complete exhaustion, filtered, evaporated to dryness, and weighed. Moreover, 100 g of the yielded extract was then successively fractionated using petroleum ether (60–80°C), chloroform, ethyl acetate, and n-butanol, and were evaporated to dryness under vacuum at 40°C and kept at −80°C until needed.

**Quantitative Estimation of the Total Flavonoid and Phenolic Contents**

The total flavonoid contents were measured as described (6). Quercetin was used as standard for the calibration curve. Folin Ciocalteu reagent was used for the analysis of total phenolic contents (7). Tannic acid was used as standard for the calibration curve. TPC was expressed as mg tannic acid equivalents per gram of sample (mg g⁻¹).

**HPLC Identification of Flavonoid and Phenolic Compounds**

Flavonoid compounds were determined by HPLC (8), while phenolic compounds were determined as described earlier (9).
**Biological Study**

**In Vitro Antioxidant Assay**

The antioxidant activity of serial concentrations (10 and 50 µg mL\(^{-1}\)) of the fractions of *F. lyrata* was estimated (10), where any substance that can donate a hydrogen atom (antioxidant) to the solution of DPPH\(^-\) can reduce the stable free radical and change the color of solution from violet to pale yellow. Non-reacted radical form of DPPH\(^-\) absorbed in the visible range, and the spectroscopic method was based on the measurement of color intensity at 518 nm against a blank solution. The percentage of inhibition of DPPH\(^-\) was calculated as follows:

\[
\%\text{IP} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100
\]

**In Vivo Study**

**Acute Toxicity Study.** Animals were subdivided into three subgroups (six rats each). All rats received one oral dose of 250, 500, and 1000 mg of *F. lyrata* butanol fraction/kg body weight, respectively. There were no dead animals at all concentrations used from 24 h to 15 d. As this revealed fraction safety, the median dose was selected for the further biological determinations.

**Animals.** Male Wistar albino rats (100–120 g) were selected for this study and obtained from the animal house, National Research Center, Egypt. All animals were kept in a controlled environment of air and temp with access to water and diet *ad libitum*.

**Ethics.** Anesthetic procedures and handling of animals were as per the ethical guidelines of Medical Ethical Committee of the National Research Centre, Egypt and were approved (Approval no: 11037).

**Administration Regimen and Animal Groups.** (i) Hepatoprotective Effect

Administration regimen was twice a week for six consecutive weeks. CCl\(_4\) was diluted in olive oil (1:9 v/v) and intraperitoneally injected at a dose 0.5 mL kg\(^{-1}\) b.w (11). Butanol fraction was orally administered at a dose of 500 mg kg\(^{-1}\) b.w. according to the acute toxicity study. Silymarin, a reference herbal drug, was administrated orally at a dose 100 mg kg\(^{-1}\) b.w. (12). Thirty two albino rats were selected for this study and divided to four groups (eight rats in each group): Group 1: normal healthy control rats. Group 2: CCl\(_4\) intoxicated rats. Group 3: forced at the same time and for the same duration with CCl\(_4\) and butanol fraction. Group 4: forced at the same time and for the same duration with CCl\(_4\) and silymarin drug.
(ii) Hypocholesterolemic Effect

Control groups were fed with standard diet (El-Kahira Co. for Oil and Soap), while hypercholesterolemic groups were fed with standard diet containing lard at 150 g kg\(^{-1}\) diet (13). Administration regimens were five times per week for nine consecutive weeks (14). The dose was selected according the toxicity study, 250 mg kg\(^{-1}\) b.w. Cholesterol was orally given at 30 mg (14). Lipanthyl drug (Mina Pharm., Egypt) was orally given at 50 mg kg\(^{-1}\) b.w (15). The dose of lipanthyl drug was calibrated to exactly contain 50 mg of fenofibrate kg\(^{-1}\) b.w. A total of 40 male rats were divided into five groups (eight rats in each group): Group 1: normal healthy control rats. Group 2: normal healthy rats administered with butanol fraction. Group 3: cholesterol-treated rats. Group 4: rats forced with cholesterol and butanol fraction. Group 5: rats forced with cholesterol and lipanthyl drug.

**Sample Preparations.** Blood was collected from each animal by puncture of the sub-lingual vein, left for 10 min to clot and centrifuged at 3000 rpm for serum separation. The separated serum was stored at −80°C for further determinations of liver function enzymes (AST, ALST, ALP, GGT), lipid profile (TC, HDL-C, LDL-C, TG) and serum total protein content.

Liver tissue was homogenized in normal physiological saline solution (0.9% NaCl) (1:9 w/v). The homogenate was centrifuged at 4°C for 5 min at 3000 rpm and the supernatant was stored at −80°C for further estimation of hepatic oxidative stress markers: glutathione (GSH), malondialdehyde (MDA), and superoxide dismutase (SOD).

**Biochemical Determinations.** Malondialdehyde (22), GSH (16), total superoxide dismutase (17), aspartate and alanine aminotransferases (18), and alkaline phosphatase (19) were assayed. GGT was estimated as described (20), and total protein (21), cholesterol (23), HDL-C (24), LDL-C (25), and triglycerides (26) were determined.

**Histopathological Study.** Representative slices of liver tissues were taken from the eviscerated animals and fixed in buffer formalin (10%). After fixation, the paraffin-embedded sections in 4mm thickness were stained by hematoxylin and eosin (H&E) (27).

**Statistical Analysis.** All data were expressed as mean ± SD and analyzed by one-way analysis of variance (ANOVA), and means separated by least significance difference (LSD, \(p \leq 0.05\)) by Costat Software Computer Program.
Results

Total Flavonoid and Phenolic Contents

The total flavonoid and phenolic contents of the dried powdered leaves of *F. lyrata* were 9.56 mg g\(^{-1}\) and 21.08 mg g\(^{-1}\), respectively, as calculated against standard calibration curves.

The *in vitro* antioxidant activity of 95% ethanol extract fractions of *F. lyrata* showed that the butanol fraction had the highest inhibition percentages of DPPH free radicals at concentrations of 10 and 50 µg mL\(^{-1}\) compared to other fractions (Table 1). Therefore, the butanol fraction was selected to be further examined *in vivo* as an antioxidant, hepatoprotective and hypolipidemic agent. The characterization of its chemical composition was also be done.

HPLC Identification of Flavonoids of *F. lyrata* Butanol Fraction

Ten flavonoids were identified in the butanol fraction of *F. lyrata* (Table 2). Naringin was the most abundant, followed by rutin, hispertin, and hesperdin.

<table>
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<tr>
<th>Fractions</th>
<th>Inhibition Percentages</th>
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<td>10 µg mL(^{-1})</td>
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<tr>
<td>Petroleum Ether</td>
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<tr>
<td>Chloroform</td>
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</tr>
<tr>
<td>Ethyl Acetate</td>
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</tr>
<tr>
<td>Butanol</td>
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</tr>
<tr>
<td>Aqueous</td>
<td>45.05</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>43.47</td>
</tr>
</tbody>
</table>

Data are inhibition percentages (IP) of DPPH\(^-\) at different concentrations.

\[
IP = \frac{\text{mean of control (3 reading) } - \text{mean of sample (3 reading)}}{\text{Mean of control}} \times 100
\]

<table>
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<tr>
<th>No.</th>
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<th>Conc. (mg100 g(^{-1}))</th>
<th>Retention time</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Naringin</td>
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<td>12.79</td>
</tr>
<tr>
<td>2</td>
<td>Rutin</td>
<td>71.94</td>
<td>12.94</td>
</tr>
<tr>
<td>3</td>
<td>Hisperdin</td>
<td>63.39</td>
<td>12.96</td>
</tr>
<tr>
<td>4</td>
<td>Quercetin</td>
<td>23.14</td>
<td>13.95</td>
</tr>
<tr>
<td>5</td>
<td>Quercetin</td>
<td>6.29</td>
<td>15.55</td>
</tr>
<tr>
<td>6</td>
<td>Narengenin</td>
<td>13.98</td>
<td>15.90</td>
</tr>
<tr>
<td>7</td>
<td>Kampferol</td>
<td>4.70</td>
<td>16.09</td>
</tr>
<tr>
<td>8</td>
<td>Hispertin</td>
<td>65.79</td>
<td>16.18</td>
</tr>
<tr>
<td>9</td>
<td>Apegenin</td>
<td>2.00</td>
<td>17.20</td>
</tr>
<tr>
<td>10</td>
<td>7 – OH flavones</td>
<td>1.75</td>
<td>18.15</td>
</tr>
</tbody>
</table>
representing concentrations equal to 279.29, 71.94, 65.79, and 63.39 mg 100 g\(^{-1}\), respectively; 7 hydroxy flavones was the lowest at 1.75 mg 100 g\(^{-1}\).

**HPLC Identification of Phenolic Compounds of F. lyrata Butanol Fraction**

Twenty-two phenolic compounds were identified in the butanol fraction of *F. lyrata* (Table 3). Pyrogallol was the highest followed by chlorogenic, rosmarinic, protocatchuic, and ferulic, representing 2340.31, 1583.30, 802.87, 657.13, and 588.63 mg 100 g\(^{-1}\), respectively; cinnamic was the lowest at 14.00 mg100 g\(^{-1}\).

**Hepatoprotective Activity of F. lyrata of Butanol Fraction**

**Liver Function Indices**

The results revealed an increase in AST, ALT, ALP, and GGT levels by 70.76%, 68.60%, 42.54%, and 33.35%, respectively compared to the control group (Fig. 1). Treatment with *F. lyrata* butanol fraction decreased AST, ALT, ALP, GGT, and total protein levels by 30.82%, 13.82%, 10.80%, 18.53%, and 13.19%, respectively, compared to the CCl\(_4\) group. CCl\(_4\) group treated with silymarin as standard drug showed a decrease in AST and GGT levels by 34.41% and 26.92%, respectively, compared to the CCl\(_4\) injured group. Treatment of CCl\(_4\) injured rats with *F. lyrata* improved AST, ALT, ALP, GGT, and total protein levels by 52.63%, 23.31%, 15.40%, 66.67%, and

<table>
<thead>
<tr>
<th>NO.</th>
<th>Phenolic compounds</th>
<th>Conc. (mg 100 g(^{-1}))</th>
<th>Retention time</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Gallic</td>
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<tr>
<td>2</td>
<td>Pyrogallol</td>
<td>2340.31</td>
<td>7.15</td>
</tr>
<tr>
<td>3</td>
<td>4- Amino-benzoic</td>
<td>94.76</td>
<td>8.32</td>
</tr>
<tr>
<td>4</td>
<td>Protocatchuic</td>
<td>657.13</td>
<td>8.58</td>
</tr>
<tr>
<td>5</td>
<td>Chlorogenic</td>
<td>1583.30</td>
<td>9.40</td>
</tr>
<tr>
<td>6</td>
<td>Catechol</td>
<td>541.28</td>
<td>9.49</td>
</tr>
<tr>
<td>7</td>
<td>P-OH-benzoic</td>
<td>441.82</td>
<td>10.17</td>
</tr>
<tr>
<td>8</td>
<td>Caffeic</td>
<td>469.24</td>
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</tr>
<tr>
<td>19</td>
<td>Vanillic</td>
<td>143.31</td>
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</tr>
<tr>
<td>10</td>
<td>Ferulic</td>
<td>588.63</td>
<td>12.33</td>
</tr>
<tr>
<td>11</td>
<td>Iso-ferulic</td>
<td>118.25</td>
<td>12.74</td>
</tr>
<tr>
<td>12</td>
<td>E-vanillic</td>
<td>378.23</td>
<td>13.26</td>
</tr>
<tr>
<td>13</td>
<td>Reversetrol</td>
<td>67.34</td>
<td>13.28</td>
</tr>
<tr>
<td>14</td>
<td>Rosmarinic</td>
<td>802.87</td>
<td>13.37</td>
</tr>
<tr>
<td>15</td>
<td>Ellagic</td>
<td>46.50</td>
<td>13.68</td>
</tr>
<tr>
<td>16</td>
<td>Alpha-coumaric</td>
<td>150.26</td>
<td>13.86</td>
</tr>
<tr>
<td>17</td>
<td>Benzoic</td>
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<td>13.95</td>
</tr>
<tr>
<td>18</td>
<td>Salycilic</td>
<td>214.61</td>
<td>14.51</td>
</tr>
<tr>
<td>19</td>
<td>3,4,5-methoxy-cinnamic</td>
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<td>14.66</td>
</tr>
<tr>
<td>20</td>
<td>Coumarin</td>
<td>60.60</td>
<td>14.73</td>
</tr>
<tr>
<td>21</td>
<td>P-coumaric</td>
<td>36.42</td>
<td>15.46</td>
</tr>
<tr>
<td>22</td>
<td>Cinnamic</td>
<td>14.00</td>
<td>15.94</td>
</tr>
</tbody>
</table>
14.33%, respectively, while the silymarin treatment improved AST, ALT, ALP, GGT, and serum protein by 58.77%, 5.38%, 0.24%, 35.90%, and 5.54%, respectively.

**Figure 1.** Effect of treatment with *Ficus lyrata* butanol fraction on liver function indices and oxidative stress markers in CCl₄ injured rats. AST, ALT, ALP and GGT are expressed as U/L, serum protein as mg mL⁻¹, GSH and MDA as µg mg⁻¹ protein, SOD as µmol mg⁻¹ protein. Data are mean ± SD of eight rats in each group. Mean separation by Least significance level (LSD, p ≤ 0.05). Means with the same grouping letter are not different.
Antioxidant Parameters
The results revealed an increase in MDA and SOD levels by 105.00% and 129.93%, respectively, compared to the control group, while GSH decreased by 46.44% (Fig. 1). Treatment of CCl₄ injured rats with F. lyrata butanol fraction decreased MDA and SOD levels by 46.34% and 21.49%, respectively, compared to the CCl₄ group, while GSH level increased by 65.55%. CCl₄ group treated with silymarin treatment showed a decrease in MDA and SOD levels by 36.58% and 22.21%, respectively, compared to the CCl₄ injured group, while GSH increased by 50.68%. Treatment of CCl₄ injured rats with F. lyrata improved GSH, MDA, and SOD by 35.10%, 95.00%, and 28.94%, respectively, while the Silymarin treatment improved GSH, MDA, and SOD by 27.14%, 75.00%, and 28.86%, respectively.

Histopathological Examination in Hepatoprotective Rats
The histopathological profile of rat livers showed that the normal liver had intact lobular hepatic architecture and normal appearance of hepatocytes with insignificant pathological changes (Fig. 2). CCl₄ injured rat livers showed distorted and loss of lobular hepatic architecture and formation of micro and macro regenerating nodules (yellow arrows), mild to moderate ballooning of hepatocytes (red arrows), and moderate infiltration by lymphocytes to portal tract (black arrows) (Fig. 2c, d). Treatment of CCl₄ intoxicated rats with F. lyrata butanol fraction showed intact (preserved) lobular hepatic architecture and almost normal hepatocytes, with mild interlobular inflammation (black arrow) (Fig. 2e, f); whereas, CCl₄ intoxicated rats treated with Silymarin drug showed intact (preserved) lobular hepatic architecture and normal appearance of hepatocytes.
architecture and mild steatotic changes (black arrows) with mild blood vessel congestion (red arrows) (Fig. 2g,h).

**Hypolipidemic Activity of F. lyrata Butanol Fraction**

**Lipid Profile**

The results revealed an elevation in total cholesterol (15.15%), high-density lipoprotein-cholesterol (54.45%), low-density lipoprotein cholesterol (56.46%), and triglycerides (77.77%) levels in the CCl4 injured rates compared to the normal control group (Fig. 3). Treatment of hypercholesterolemic rats with *F. lyrata* butanol fraction reduced TC, HDL-C, and LDL-C by 34.47%, 35.97%, and 29.30%, respectively, compared to the hypercholesterolemic group. Lipanthyl drug attenuated the TC, HDL-C, LDL-C, and TG level by 7.88%, 18.71%, 6.38%, and 8.74%, respectively, compared to the hyperlipidemic rats. Treatment with *F. lyrata* improved TC, HDL-C, LDL-C and TG levels by 39.69%, 55.56%, 45.84%, and 20.00%, while lipanthyl drug improved these parameters by 9.08%, 28.89%, 9.98%, and 15.55%, respectively.

*Figure 3.* Effect of treatment with *F. lyrata* butanol fraction on lipid profile in hypercholesterolemic rats. Values are expressed as mg dL$^{-1}$. Data are mean ± SD of eight rats in each group. Mean separation by Least significance level (LSD, $p \leq 0.05$). Means with the same grouping letter are not different.
Liver Function Parameters

The liver function indices AST, ALT, ALP, and GGT in hypercholesterolemic rats increased by 36.93%, 110.78%, 88.02%, and 86.48%, respectively, compared to control group (Fig. 4). Treatment of hypercholesterolemic rats with the butanol fraction reduced AST, ALT, ALP, and GGT levels by 12.82%, 6.51%, 23.04%, and 31.00%, respectively while the Lipanthyl treatment attenuated the level of AST, ALP, and GGT by 6.57%, 52.17%, and 44.03%, respectively, compared to the hypercholesterolemic group.

The level of total serum protein in hypercholesterolemic rats decreased and was not affected by treatment of either the butanol fraction of F. lyrate or the drug. Treatment with F. lyrate improved the AST, ALT, ALP, GGT, and total protein levels by 17.56%, 13.72%, 43.33%, 57.81%, and 5.31%, respectively, while Lipanthyl drug improved the liver function indices by 9.00%, 1.96%, 98.10%, 82.12%, and 4.27%, respectively.

Oxidative Stress Markers

The results revealed an increase in MDA and SOD levels by 72.72% and 32.51%, respectively, in hypercholesterolemic rats compared with the control group, while GSH decreased by 44.03% (Fig. 4). Treatment with F. lyrata butanol fraction decreased MDA and SOD levels by 39.47% and 20.80%, respectively, compared to the hypercholesterolemic rats, while GSH level increased by 46.36% after treatment. Hypercholesterolemic rats treated with lipanthyl showed a decrease in MDA and SOD levels by 34.21% and 18.78%, respectively, comparing to the hyperlipidemic group, while GSH increased by 37.51%. Treatment of hypercholesterolemic rats with F. lyrata improved GSH, MDA, and SOD levels by 25.94%, 68.18%, and 27.57%, respectively, while Lipanthyl treatment improved GSH, MDA, and SOD by 20.99, 59.09, and 24.89%, respectively.

Histopathological Examination of Rat Livers in Hypercholesterolemic Rats

The histopathological finding of normal rat livers showed preserved (intact) lobular hepatic architecture and insignificant pathological changes (Fig. 5a,b). Hypercholesterolemic rats liver had distorted (lost) lobular hepatic architecture with moderate hydropic degeneration (black arrows), sever micro and macrosteatotic changes (red arrow), sever interlobular inflammation (yellow arrow), and congested blood vessels (green arrow) (Fig. 5a,b).

Treatment of hypercholesterolemic rats with F. lyrata fraction showed intact (preserved) lobular hepatic architecture and normal hepatocytes with mild sinusoidal congestion and dilatation (black arrows) (Fig. 5a,b).

Hypercholesterolemic rats treated with lipanthyl drug showed intact lobular hepatic architecture and normal hepatocytes with fibrotic improvement. Mild sinusoidal and blood vessels congestion and dilatation were also seen (black arrow) (Fig. 5a,b).
Liver fibrosis is a process of liver cirrhosis, which is associated with most chronic liver diseases. The use of CCl₄ to induce liver fibrosis is well known (28) and affected liver function indices. The results in the present study regarding the

<table>
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<tr>
<th></th>
<th>Control</th>
<th>Cholesterol</th>
<th>F. lyrata</th>
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<td><img src="chart31.png" alt="" /></td>
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**Figure 4.** Effect of treatment with the plant fraction on liver function indices and oxidative stress markers in hypercholesterolemic rats. AST, ALT, ALP, and GGT are expressed as U/L, serum protein as mg mL⁻¹, GSH and MDA as µg mg⁻¹ protein, SOD as µmol mg⁻¹ protein. Data are mean ± SD of eight rats in each group. Mean separation by Least significance level (LSD, p ≤ 0.05). Means with the same grouping letter are not different.

**Discussion**

Liver fibrosis is a process of liver cirrhosis, which is associated with most chronic liver diseases. The use of CCl₄ to induce liver fibrosis is well known (28) and affected liver function indices. The results in the present study regarding the
The effect of CCl₄ intoxication on liver function markers ALT, AST, ALP, and GGT are in agreement with past reports (29). The increase of these markers in rats’ serum was attributed to protein synthesis and the decreased levels of DNA (29). Additionally, the increased serum levels of hepatic markers may be due to liver injury that leads to their release into circulation. The hepatotoxic effects by CCl₄ are due to the presence of the reactive metabolite; CCl₃ that abstract hydrogen from fatty acids, initiating lipid peroxidation, led to cell injury, and finally liver damage (31).

However, the reduced concentrations of these markers as a result of F. lyrata butanol fraction administrations might probably be due to the presence of antioxidant compounds in the extracts that contribute to the protection against CCl₄ induced hepatotoxicity in rats. The butanol fraction of 95% ethanolic extract of F. lyrata recorded remarkable improvement percentages in AST, ALT, ALP, GGT, and serum protein levels. It attenuated the increased level of liver function enzymes and caused a subsequent recovery toward normalization. This gives additional support that the butanol fraction of F. lyrata extract is able to condition the hepatocytes, accelerate regeneration of parenchyma cells, protect against membrane fragility, and decrease leakage of the enzymes into circulation.

Oxidative stress is one of the most important stimuli to activate hepatic stellate cells (2), thus playing a key role in the development of liver fibrosis. Reactive oxygen species can damage the polyunsaturated fatty acids (PUFA) in cellular membranes and the substantial number of unprotected protein sulphydryl groups and DNA bases, causing instabilities to cellular homeostasis and even leading to cell death (32). In this study, CCl₄ treatment

Figure 5. Photomicrography of hematoxylin and eosin (H&E) and Masson’s trichrome stained section (100×) of normal rat liver (a, b), hypercholesterolemic (c, d), hypercholesterolemic treated with butanol fraction of F. lyrata (e,f), and hypercholesterolemic treated with lipanthyl drug (g, h).
damaged the defense systems of liver causing serious lipid peroxidation as shown by increased MDA production. In addition, treatment with CCl₄ increased the activity and/or content of SOD, which scavenging free radical scavenger, and simultaneously reduced production of lipid per-oxides, which mildly alleviated the oxidative damage caused by CCl₄. Decrease in GSH activity may be to decreased availability of GSH resulted during the enhanced lipid peroxidation processes (28).

The butanol fraction of 95% ethanolic extract of F. lyrata improved the oxidative stress markers more than that of Silymarin, which has the ability to attenuate free radicals elevation, chelate metal ions, inhibit lipid peroxidation and prevent liver glutathione depletion (33). Therefore, the F. lyrata butanol fraction may have the same mode of action as silymarin.

CCl₄ intoxication induced changes in the process of protein synthesis (29). Hence, increase in total protein content can be deemed as a useful index of the severity of cellular dysfunction in liver diseases as clearly shown in this study.

The histological changes in the liver injury induced by CCl₄ are known as apoptosis, necrosis, steatosis, and mononuclear cell infiltration in both lobular area and portal septa (28). Similar to the previous reports, the findings on the study also revealed high level of inflammation, steatosis, and necrosis within the lobular areas in CCl₄ group. In F. lyrata butanol extract and silymarin treated groups, hepatocytes degeneration, necrosis, and infiltration of inflammatory cells were all ameliorated. Collagen deposition was also markedly reduced. The potential effect of F. lyrata butanol fraction as a potent antifibrotic agent was comparable to the standard hepatoprotective drug Silymarin.

High fat diet (HFD) is also the major cause of liver disease and impairment of liver function (2). The present study showed an increase in total cholesterol, LDL, HDL, and triglycerides in rats administered with cholesterol, which was consistent with other reports (2) that attributed the increase of triglycerides level to the effect of dietary cholesterol that reduced fatty acid oxidation, which, in turn, increased the levels of plasma and hepatic triglyceride.

As the alteration induced by the HFD is due to the oxidative damage, the antioxidants may have a protective effect on the pathways leading to transcription of these genes by scavenging ROSs (34). Treatment with the plant extract under investigation improved the total cholesterol, HDL-C, LDL-C, and triglycerides. It was attributed the attenuated of cholesterol and triglyceride levels to the effect of treatment that may reduce the hepatic triglyceride biosynthesis and favor the redistribution of cholesterol among the lipoprotein molecules (14). The reduction of total cholesterol by the butanol fraction of F. lyrata was associated with a decrease of its LDL fraction, which is the target of several hypolipidemic drugs. The results suggest that cholesterol-lowering activity of the extract can be due to the rapid catabolism of
LDL-cholesterol through its hepatic receptors for final elimination in the form of bile acids; therefore LDL-cholesterol level may be used for monitoring the treatment of patients with elevated blood cholesterol levels.

The phenomenon of cardiovascular disease was explained according to the role of HDL that exerts part of its anti-atherogenic effect by counteracting LDL oxidation or HDL that may promote the reverse cholesterol transport pathway, by inducing an efflux of excess accumulated cellular cholesterol and prevent the generation of the oxidatively modified LDL. On the basis of this explanation, *F. lyrata* butanol fraction may play an anti-atherogenic role through the inhibition of lipids oxidation as well as the elevation of HDL cholesterol.

Treatments with lipanthyl drug (fenofibrate), decreased triglycerides and HDL levels (30). This is in agreement with the mechanism of action of fibrates, in which the LDL-cholesterol lowering activity is not strongly marked, but the triglycerides decreasing effect is very clear by stimulation of the gene expression of lipoprotein lipase leading to enhanced catabolism of VLDL, synthesis of fatty acids and reduced VLDL secretion (40).

The relationship between oxidative stress and cholesterol level was confirmed in this study where the hepatic glutathione (GSH), an indicator of antioxidant defense, exhibited a negative correlation with the total cholesterol levels of hypercholesterolemic rats and a positive correlation with the cholesterol levels of the extracts-treated rats.

In this study, the light microscopic examination of the liver sections of hypercholesterolemic rat livers revealed a large accumulation of macrovesicular and micro-vesicular fat in the livers as well as dark inflammatory infiltrates in the high fat/cholesterol fed liver that is in agreement with past reports (2), that attributed the presence of fatty liver to hypercholesterolemia and accumulation of large vacuoles of fat. This microscopic examination also confirmed the present biochemical determinations, where cholesterol and triglycerides were increased. Treatment with *F. lyrata* butanol extract fraction and lipanthyl drug showed only focal area of fatty droplets and less degenerative changes in the liver, which was also confirmed by the reduction observed in the level of cholesterol and triglycerides after treatment with the *F. lyrata* butanol extract fraction.

The biological effects obtained by the butanol fraction of *F. lyrata* may be due to the polyphenolic constituents of 22 phenols and 10 flavonoids that are characterized by the presence of hydrogen donating substituents attached to aromatic rings that enable them to scavenge free radicals. Many phenolics have antioxidant capacities that are much stronger than those of vitamins C and E. Flavones and flavonols are very important flavonoids as they possess antioxidant and free radical scavenging activities in foods (35). In plants, phenolic acids occur mainly in bound forms, as components of complex structures such as lignins and hydrolysable tannins or derivatives.
of sugars and organic acids. Two main classes of phenolic acids are the hydroxycinnamic acids (e.g., coumaric, caffeic, ferulic) and the hydroxybenzoic acids (e.g., gallic, p-hydroxybenzoic, vanillic, and protocatechuic acids). Naringin, present in the butanol fraction of F. lyrata, has been reported as an antioxidant (36) that acts by upregulation of gene expressions in the antioxidant enzymes (CAT, SOD, and GPx) consequently enhancing the scavenging of reactive oxygen species, parallel with lowering of lipid peroxidation (36).

References


