QUANTITATION AND HPLC IDENTIFICATION OF FLAVONOIDS AND PHENOLICS OF *FICUS RACEMOSA* (L.) BUTANOL FRACTION AND IN VIVO ASSESSMENT OF ITS HYPOLIPIDEMIC AND HEPATOPROTECTIVE ACTIVITIES IN RATS

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**ABSTRACT**

*Ficus racemosa* Linn. belongs to family Moraceae is used as herbal medicine from ancient times. Total phenolic and flavonoid contents of the dried powdered leaves were quantitated and exhibited 15.17% and 18.72%, respectively. Moreover, different extracts; (Distilled water, 70% ethanol and 95% ethanol) of *F. racemosa* (L.) leaves had been In Vitro assessed as antioxidants. The 95% ethanolic extract gave promising result, consequently, it was fractionated by solvents of increasing polarity and each fraction was screened as well. The butanol fraction was the most bioactive fraction (98.40%). High Performance Liquid Chromatography (HPLC) was used to identify and quantify the flavonoids and phenolics present in it. Further In Vivo assessment against Hypercholesterolemia and liver fibrosis in rats had been done. The butanol fraction exhibited higher antioxidant, hepatoprotective and hypocholesterolemic activities than the standard drugs. These observations were confirmed by the histopathological observation.

**Key Words**: *Ficus racemosa* (L.); Moraceae; Flavonoids; Phenols; Butanol fraction; Liver Fibrosis; Hypercholesterolemia.

**INTRODUCTION**

Medicinal plants continue to be an important therapeutic aid for alleviating the ailments of human kind. Today, there is a renewed interest in traditional medicine and an increasing demand for more drugs from plant sources. This revival of interest in plant-derived drugs is mainly due to the current widespread belief that “green medicine” is safe and more dependable than the costly synthetic drugs, many of which have adverse side effects. According to Ventakamaranm, (1972), the taxonomy of the Moraceae family constitutes large taxa of over fifty genera and nearly 1400 species, including some important groups like Artocarpus, Morus and Ficus. Several species belonging to the genera of *Ficus* were reported to contain furanocoumarins which are important plant phototoxins (Swain & Downum 1990). Ventakamaran (1972) also claimed that Moraceae family contains phytochemistry related to flavonoids, flavonoids with isoprenoid substituents and stilbenes. *Ficus racemosa* Linn (Moraceae) is an evergreen, moderate to large sized spreading, lactiferous, deciduous tree, without much prominent aerial roots. In India, all parts of this plant (leaves, fruits, bark, latex, and sap of the root) are medicinally important in the traditional system of medicine. (Mousa *et al.*, 1994) approved and supported the traditional uses of certain Egyptian *Ficus* species in folk medicine for respiratory disorders and certain skin diseases. Other scientifically studies indicate that *F.*
**racemosa** (L.) posses various biological effects such as hepatoprotective, chemopreventive, antidiabetic, anti inflammatory, antipyretic, antitussive and antidiuretic. The bark has also been evaluated for cytotoxic effects using 1BR3, Hep G2, HL-60 cell lines and found to be safe and less toxic than aspirin, a commonly consumed anti-inflammatory drug (Joseph & Raj, 2010).

Elevated levels of plasma cholesterol and triglycerides have been implicated as causative factors in the development of atherosclerosis and coronary heart disease (Ross, 1999). Several modern drugs are being used as hypocholesterolemic agents such as statins, fibrates, nicotinic acid and resins (Satoskar et al., 2003). The usage of plant based formulations as remedial measures against various human and animal ailments is a globally new trend nowadays.

Hepatic fibrosis is a common condition in which major amounts of liver parenchyma cells are replaced by fibrous connective tissue. Experimentally, hepatic fibrosis has been shown to be produced by the administration of CCl4, thioacetamide, paracetamol, etc. (Geesin et al., 1998). Quercetin was used as standard for the calibration curve.

The aim of the present work is to evaluate the role of the flavonoid and phenolic compounds present in the butanol fraction of *F. racemosa* (L.) in improving hypercholesterolemia and liver fibrosis in rats which were induced, intentionally, by high fat diet and CCl4, respectively.

**MATERIALS AND METHODS**

**Plant material**

*F. racemosa* (L.) fresh leaves were collected in March 2011, from Orman Garden, Giza, Egypt. Specimens of the plants were identified by Dr. Trease Labib Consultant of Plant Taxonomy at the Ministry of Agriculture and director of Orman Botanical Garden, Giza, Egypt. The collected leaves were air-dried, powdered and kept in tightly-closed containers until needed. Voucher specimens of *F. racemosa* (L.) leaves; 28-4-2015 I was deposited at Pharmacognosy Dept., Faculty of Pharmacy, Cairo University, Cairo, Egypt as a reference.

**Chemicals**

All chemical 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 (4 kg) of *F.racemosa* (L.) were extracted by percolation with 95% ethanol until complete exhaustion, filtered, evaporated till dryness and weighed. One hundred grams of the yielded extract then successively fractionated using petroleum ether (60-80°C), chloroform, ethyl acetate and butanol. These fractions were evaporated to dryness under vacuum at 40°C and kept at -80°C until needed.

**Quantitative Estimation of The Total Flavonoid Contents**

The total flavonoid contents in the dried powdered leaves of *F. racemosa* (L.) was measured by spectrophotometric assay (Zhishen et al., 1999; Zou et al., 2004). Quercetin was used as standard for the calibration curve.

**Quantitative Estimation of The Total Phenolic Contents**

Folin Ciocalteu reagent was used for analysis of total phenolic contents (TPC) of the dried powdered leaves of *F. racemosa* (L.), according to Chun et al., (2003). TPC was expressed as mg tannic acid equivalents per gram of sample (mg/g).

**HPLC Identification of Flavonoidal Compounds**

Butanol fraction of *F. racemosa* (L.) was subjected to (HPLC) technique alongside with available flavonoids standards at wavelength 320 nm to investigate the presence of some flavonoidal compounds and determine their concentrations according to the method described by Mattila et al., (2000).

**HPLC Identification of Phenolic Compounds**

Butanol fraction of *F. racemosa* (L.) was subjected to high performance liquid chromatography (HPLC) technique alongside with available phenolic standards at wavelength 280 nm to investigate the presence of some phenolic compounds and determine their concentrations according to the method described by Goupay et al., (1999).

**BIOLOGICAL STUDY**

**In Vitro Antioxidant Assay**

The antioxidant activity of two concentrations (10 and 100µg) of the extracts and fractions of *F. racemosa* (L.) was estimated by the method of (Chen et al., 2007), 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- was expressed as mg tannic acid equivalents per gram of sample (mg/g).

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

**In Vivo Study**

**Acute toxicity study**

Animals were subdivided into 3 subgroups (6 rats each). All rats received one oral dose of 250, 500 and 1000mg of *F. racemosa* (L.) butanol fraction /kg body weight, respectively. After 24 hours and along for 15 days there were no dead animals, revealed fraction safety.
**Animals**

Male Wistar albino rats (100:120g) were selected and obtained from the animal house, National Research Center, Egypt. All animals were kept in a control environment of air and temp with access of water and diet adlibitum.

**Ethics**

Anesthetic procedures and handling with animals were complied with the ethical guidelines of Medical Ethical Committee of the National Research Centre in Egypt and performed to ensure that the animals do not suffer at any stage of the experiment (Approval no:11037).

**Administration regimen and animal groups**

**Hepatoprotective effect**

Administration regimen was twice a week for six consecutive weeks. CCl4 was diluted in olive oil (1:9 v/v) and intraperitoneally injected at a dose 0.5 ml/kg body weight (Marsillach *et al.*, 2009). Butanol fraction was orally administered at a dose of 500mg/kg body weight according to the acute toxicity study. Silymarin; a reference herbal drug was administrated orally at a dose 100mg/kg body weight (Yuvaraj and Subramoniam, 2009). Thirty two albino rats were selected for this study and divided to four groups (eight rats each) as follows: Group 1: normal healthy control rats. Group 2: CCl4 intoxicated rats. Group 3: forced at the same time and for the same duration with CCl4 and butanol fraction. Group 4: forced at the same time and for the same duration with CCl4 and silymarin drug.

**Hypholesterolemic effect**

Control groups were fed with standard diet (El-Kahira Co. for Oil and Soap), while hypercholesterolemic groups were fed with standard diet containing 150g lard/kg diet (Auger *et al.*, 2002). The modified diet was taken along with oral administration of cholesterol to get a condition of high fat and cholesterol level (Kim *et al.*, 2008) and to ensure triglycerides elevation (Gershkovich and Hoffman, 2007). Administration regimens were five times per week for nine consecutive weeks (Adaramoye *et al.*, 2008). The dose was selected according the toxicity study, 250 mg/kg body weight. Cholesterol was orally given at a dose 30 mg/animal (Adaramoye *et al.*, 2008). Lipanthyl drug (Mina Pharm., Egypt) was orally given at a dose 50 mg/kg body weight (Petit *et al.*, 1988). The dose of lipanthyl drug was calibrated to exactly contain 50mg of fenofibrate/kg body weight. A total of 40 male rats were divided into five groups (eight rats each) as follows: 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 PREPARATION**

Blood was collected from each animal by puncture of the sub-tongual 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 was assayed according to the method of Buege and Aust, (1978), glutathione (GSH) by (Moron *et al.*, 1979), total superoxide dismutase by Nishikimi *et al.*, (1972), aspartate and alanine aminotransferases by the method of Reitman and Frankel, (1957), alkaline phosphatase by the method of (Belfield and Goldberg, 1971). GGT was estimated by the method of (Szasz, 1969), total protein was assayed by the method of (Bradford, 1976), cholesterol was determined by the method of (Meiattini *et al.*, 1978), HDL-C by (Bustein *et al.*, 1980), LDL-C assay method by (Assmann, and Cagen, 1984), triglycerides by (Fossati and Prencipe, 1982).

**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 4 mm thickness were stained by haematoxylin and eosin (H&E) (Hirsch *et al.*, 1997).

**STATISTICAL ANALYSIS**

All data were expressed as mean ± SD of animal numbers in each group. Statistical analysis was carried out by one-way analysis of variance (ANOVA), Costat Software Computer Program accompanied with least significance difference between groups (LSD) at *P*< 0.05.

**RESULTS**

**Total flavonoids and phenols**

The total flavonoids of the dried powdered leaves of *F. racemosa* (L.) was measured. It recorded 18.720 mg/1g. While, the total phenols recorded 15.173 mg/1g, both are calculated against standards. Table (1) illustrates different concentrations and different absorbance for the standards used. Additionally, Fig (1) and Fig (2) are representing the standard calibration curves.

Concerning with the antioxidant activity of *F. racemosa* (L.) leaves extracts; the results revealed that the
95% ethanol extract recorded the highest percentage of inhibition of the DPPH free radicals than the 70% ethanol and water extracts. The 95% ethanolic showed inhibition by 82.35, 92.11 and 98.50 % at concentrations 10, 50 and 100µg of the extract, respectively. Vitamin C as a standard recorded inhibition of DPPH free radicals by 43.47, 80.85 and 95.63% at concentrations of 10, 50 and 100µg/ml.

The In Vitro antioxidant activity of 95% ethanol extract fractions of _F. racemosa_ (L.) showed that the butanol fraction recorded the highest antioxidant effect at concentration of 50µg/ml (Table 2). Data are inhibition percentages (IP) of DPPH free radicals at different concentrations.

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

Therefore, we selected the butanol fractions to be further in vivo evaluated as antioxidant, hepatoprotective, and hypolipidemic agents. Besides, secondly, investigation of its chemical composition responsible for these activities.

**HPLC Identification of Flavonoids of _F. racemosa_ (L.) butanol fraction**

The results of the identification and quantitation of flavonoids in _F. racemosa_ (L.) butanol fraction are summarized in Table 3. From Table (3) it could be concluded that HPLC identified ten flavonoids in the butanol fraction of _F. racemosa_ (L.) Hesperidin is the most abundant flavonoid followed by quercetin, naringin and rutin. They represented the following concentrations; 860, 526, 220.21 and 121.42 mg/100 gm, respectively. On the contrary, apigenin has the least concentration; 1.50 mg/100 gm in the butanol fraction.

**HPLC Identification of phenolic compounds of _F. racemosa_ (L.) butanol fraction**

The results of the identification and quantitation of phenolics in _F. racemosa_ (L.) butanol fraction are summarized in Table (4). Table (4) revealed that twenty three phenols in the butanol fraction of _F. racemosa_ (L.) are identified. Pyrogallol is the major phenol followed by chlorogenic, benzoic, catechol and vanillic. at concentrations of 2054.99, 1424.67, 1088.14, 488.23 and 268.26 mg/100 gm, respectively. On the contrary, cinnamic concentration is the least; 8.65 mg/100 gm in the butanol fraction.

**Hepatoprotective Activity of _F. racemosa_ (L.) Butanol Fraction**

Regarding to the liver function indices in CCl4 injured rats; the results revealed significant increase in AST, ALT, ALP and GGT levels by 70.76, 68.60, 42.54 and 33.35%, respectively as compared with the control group Table (5). Treatment of CCl4 injured rats with _F. racemosa_ (L.) butanol fraction recorded significant decrease in AST and GGT by 18.49 and 10.14%, respectively as compared with CCl4 group. Contradictory, ALT, ALP and serum total protein contents recorded insignificant changes after treatment Table (5).

**In Vivo antioxidant Activity of _F. racemosa_ (L.) Butanol Fraction in Hypatoprotective Experiment**

Regarding to oxidative stress markers in CCl4 injured rats; the results revealed significant increase in MDA and SOD levels by 105.00 and 29.93%, respectively as compared with the control group, while GSH recorded significant decrease by 46.44% Table (6).

Treatment of CCl4 injured rats with _F. racemosa_ (L.) butanol fraction recorded significant decrease in MDA and SOD by 41.46 and 9.07%, respectively as compared with CCl4 group. Contradictory, the GSH level recorded significant increase by 50.00% after treatment. CCl4 group treated with silymarin recorded significant decrease in MDA and SOD levels by 36.58 and 22.21%, respectively comparing with the CCl4 injured group, while GSH recorded significant increase by 50.68%.

**The Histopathological Examination of Rat Livers of Hepatoprotective Experiment**

Regarding to the histopathological analysis of rat livers, the normal liver showed intact lobular hepatic architecture and normal appearance of hepatocytes with insignificant pathological changes (Fig. 3 & a).

CCl4 injured rat livers showed distorted and loss lobular hepatic architecture and formation of micro and macro regenerating nodules, mild to moderate ballooning of hepatocytes, moderate infiltration by lymphocytes to portal tract (Fig.3, c & d). Treatment of CCl4 intoxicated rats with _F. racemosa_ (L.) extract showed partial distortion or loss lobular hepatic architecture, moderate steatotic changes, mild fibrous bands and congested blood vessels (yellow arrow) (Fig.3, e & f). CCl4 intoxicated rats treated with silymarin showed intact (preserved) lobular hepatic architecture and mild steatotic changes with mild blood vessel congestion (Fig. 3, g & h).

**Hypolipidemic Activity of _F. racemosa_ (L.) Butanol Fraction**

With respect to the lipid profile in hypercholesterolemic rats, the results revealed significant elevation in total cholesterol (15.15%), high density lipoprotein-cholesterol (54.45%), low density lipoprotein cholesterol (56.46%) and triglycerids (77.77%) levels as compared with the normal control group (Table 7). Treatment of hypercholesterolemic rats with _F. racemosa_ (L.) butanol fraction recorded significant decrease in TC, HDL-C levels by 34.20, 25.04%, respectively as compared with the hypercholesterolemic rats (Table 7). Lipanthyl drug attenuated the TC, HDL-C, LDL-C and TG level by...
b7.88, 18.71, 6.38 and 8.74%, respectively as compared with the hyperlipidemic rats.

Regarding to the liver function indices in hypercholesterolemic rats, the results revealed significant increase in AST, ALT, ALP and GGT levels by 36.93, 110.78, 88.02 and 86.48%, respectively as compared with the control group Table (8). Treatment of hypercholesterolemic rats with F. racemosa (L) attenuated the level of ALP and GGT by 21.02 and 28.53%, respectively as compared with the hyperlipidemic rats Table(8). Lipanthyl treatment attenuated the level of AST, ALP and GGT by 6.57, 52.17 and 44.03%, respectively as compared with the hypercholesterolemic group Table(8). The level of total serum protein in hypercholesterolemic rats was insignificantly decreased as compared with the control group. It was also not significantly affected by treatment of either butanol fraction or the drug.

**In Vivo antioxidant Activity of F. racemosa (L.) Butanol Fraction in Hypocholesterolemic Experiment**

Regarding to oxidative stress markers in hypercholesterolemic rats; the results revealed significant increase in MDA and SOD levels by 72.72 and 32.51%, respectively as compared with the control group, while GSH recorded significant decrease by 44.03% Table (9). Treatment of hypercholesterolemic rats with F. racemosa (L.) butanol fraction recorded significant decrease in MDA and SOD by 36.84 and 7.26%, respectively as compared with the hypercholesterolemic group. Contrary, the GSH level recorded significant increase by 42.94% after. Hypercholesterolemic rats treated with lipanthyl recorded significant decrease in MDA and SOD levels by 34.21 and 18.78%, respectively comparing with the hyperlipidemic group, while GSH recorded significant increase by 37.51%.

**The Histopathological Examination of Rat Livers of Hypolipidemic Experiment**

With respect to the histopathological finding of normal rat livers, the results showed preserved (intact) lobular hepatic architecture and insignificant pathological changes (Fig. 6 a & b).

Hypercholesterolemic rats liver showed 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. 6, c & d).

Treatment of hypercholesterolemic rats with F. racemosa (L.) fraction showed intact (preserved) lobular hepatic architecture, normal hepatocytes with mild sinusoidal congestion and dilatation (red arrow) and mild interlobular inflammation (black arrow) (Fig. 6, e & f).

Hypercholesterolemic rats treated with lipanthy drug showed intact lobular hepatic architecture and normal hepatocytes with fibrotic improvement. Mild sinusoidal and blood vessels congestion and dilatation were also seen (Fig. 6, g & h).

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**Table 1. The absorbance measured for different concentrations of the quercetin and tannic acid standard solutions**

<table>
<thead>
<tr>
<th>Standard conc. (mg/ml)</th>
<th>Absorbance of quercetin</th>
<th>Absorbance of tannic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.2</td>
<td>0.144</td>
<td>0.245</td>
</tr>
<tr>
<td>0.4</td>
<td>0.277</td>
<td>0.51</td>
</tr>
<tr>
<td>0.6</td>
<td>0.435</td>
<td>0.701</td>
</tr>
<tr>
<td>0.8</td>
<td>0.59</td>
<td>0.997</td>
</tr>
<tr>
<td>1</td>
<td>0.709</td>
<td>1.207</td>
</tr>
</tbody>
</table>

**Table 2. Percentages of In vitro antioxidant activities of 95% ethanol extract fractions of F. racemosa (L.)**

<table>
<thead>
<tr>
<th>Fractions</th>
<th>F. racemosa (L.) (10µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Petroleum Ether</td>
<td>62.22</td>
</tr>
<tr>
<td>Chloroform</td>
<td>29.62</td>
</tr>
<tr>
<td>Ethyl Acetate</td>
<td>24.07</td>
</tr>
<tr>
<td>Butanol</td>
<td>62.33</td>
</tr>
<tr>
<td>Aqueous</td>
<td>55.56</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>43.47</td>
</tr>
</tbody>
</table>

**Table 3. Identification and quantitation of flavonoids in F. racemosa (L.) butanol fraction**

<table>
<thead>
<tr>
<th>No</th>
<th>Flavonoids</th>
<th>Concentration results of Flavonoids (mg /100g )</th>
<th>Retention time</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Naringin</td>
<td>220.21</td>
<td>12.73</td>
</tr>
<tr>
<td>2</td>
<td>Rutin</td>
<td>121.42</td>
<td>12.88</td>
</tr>
<tr>
<td>3</td>
<td>Hisperdin</td>
<td>860.27</td>
<td>12.99</td>
</tr>
<tr>
<td>4</td>
<td>Quercetrin</td>
<td>526.27</td>
<td>14.02</td>
</tr>
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</table>
Table 4. Identification and quantitation of phenolics in *F. racemosa* (L.) butanol fraction

<table>
<thead>
<tr>
<th>NO</th>
<th>Phenolic compounds</th>
<th>Concentration results of phenolic Compounds (mg/100g)</th>
<th>Retention time</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Gallic</td>
<td>15.16</td>
<td>7.08</td>
</tr>
<tr>
<td>2</td>
<td>Pyrogallol</td>
<td>2054.99</td>
<td>7.15</td>
</tr>
<tr>
<td>3</td>
<td>4- Amino-benzoic</td>
<td>15.73</td>
<td>8.29</td>
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<tr>
<td>4</td>
<td>Protocatechuic</td>
<td>77.34</td>
<td>8.53</td>
</tr>
<tr>
<td>5</td>
<td>Chlorogenic</td>
<td>1424.67</td>
<td>9.39</td>
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<tr>
<td>6</td>
<td>Catechol</td>
<td>488.23</td>
<td>9.48</td>
</tr>
<tr>
<td>7</td>
<td>Catecheine</td>
<td>217.93</td>
<td>10.00</td>
</tr>
<tr>
<td>8</td>
<td>P-OH-benzoic</td>
<td>143.22</td>
<td>10.19</td>
</tr>
<tr>
<td>9</td>
<td>Caffeic</td>
<td>58.87</td>
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<tr>
<td>10</td>
<td>Vanillic</td>
<td>268.26</td>
<td>10.59</td>
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<td>11</td>
<td>Ferulic</td>
<td>235.27</td>
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<td>Iso-ferulic</td>
<td>51.36</td>
<td>12.70</td>
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<tr>
<td>13</td>
<td>e-vanillic</td>
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<td>14</td>
<td>Reversetrol</td>
<td>34.69</td>
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<td>15</td>
<td>Rosmarinic</td>
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<td>16</td>
<td>Ellagic</td>
<td>44.58</td>
<td>13.74</td>
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<tr>
<td>17</td>
<td>Alpha-coumaric</td>
<td>31.03</td>
<td>13.86</td>
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<tr>
<td>18</td>
<td>Benzoic</td>
<td>1088.14</td>
<td>14.00</td>
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<tr>
<td>19</td>
<td>Salycilic</td>
<td>117.61</td>
<td>14.49</td>
</tr>
<tr>
<td>20</td>
<td>3,4,5-methoxy-cinnamic</td>
<td>19.63</td>
<td>14.67</td>
</tr>
<tr>
<td>21</td>
<td>Coumarin</td>
<td>18.47</td>
<td>14.69</td>
</tr>
<tr>
<td>22</td>
<td>P-coumaric</td>
<td>86.31</td>
<td>15.46</td>
</tr>
<tr>
<td>23</td>
<td>Cinnamic</td>
<td>8.65</td>
<td>15.94</td>
</tr>
</tbody>
</table>

Table 5. Effect of treatment with *F. racemosa* (L.) on liver function indices in CCl₄ injured rats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>CCl₄</th>
<th>Ficus racemosa</th>
<th>Silymarin</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST</td>
<td>3.42±0.07 (U/L)</td>
<td>5.84±0.13 (U/L)</td>
<td>4.76±0.36 (U/L)</td>
<td>3.83±0.13 (U/L)</td>
</tr>
<tr>
<td>ALT</td>
<td>2.23±0.07 (U/L)</td>
<td>3.76±0.21 (U/L)</td>
<td>3.53±0.21 (U/L)</td>
<td>3.64±0.11 (U/L)</td>
</tr>
<tr>
<td>ALP</td>
<td>170.41±5.80 (U/L)</td>
<td>224.91±5.15 (U/L)</td>
<td>236.24±7.27 (U/L)</td>
<td>243.33±1.55 (U/L)</td>
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<tr>
<td>GGT</td>
<td>37.63±26.95 (U/L)</td>
<td>50.18±7.22 (U/L)</td>
<td>45.09±9.84 (U/L)</td>
<td>36.67±11.89 (U/L)</td>
</tr>
<tr>
<td>Serum protein</td>
<td>8.65±0.53 (mg/dL)</td>
<td>9.40±0.79 (mg/dL)</td>
<td>8.56±0.79 (mg/dL)</td>
<td>8.92±0.075 (mg/dL)</td>
</tr>
</tbody>
</table>

Data are mean ± SD of eight rats in each group. Values are expressed as U/L and serum protein as mg/dL. Statistical analysis are done using one way analysis of variance (ANOVA) using Co Stat Computer program accompanied with least significance level (LSD) between groups at p<0.0001. Unshared superscript letters are significant values between groups at p<0.05. Values between brackets are % changes over control group = [(mean treated)- mean control]/mean control x 100. Values between parenthesis are % changes over CCl₄ group = [(mean control - mean treated)/mean of CCl₄] x 100.

Table 6. Effect of treatment with *F. racemosa* (L.) on oxidative stress markers in CCl₄ rats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>CCl₄</th>
<th>Ficus racemosa</th>
<th>Silymarin</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSH (μg/mg protein)</td>
<td>18.97±1.54 (μg/mg)</td>
<td>10.16±0.72 (μg/mg)</td>
<td>15.24±1.59 (μg/mg)</td>
<td>15.31±2.34 (μg/mg)</td>
</tr>
<tr>
<td>MDA (μg/mg protein)</td>
<td>0.20±0.01 (μg/mg)</td>
<td>0.41±0.03 (μg/mg)</td>
<td>0.24b±0.04 (μg/mg)</td>
<td>0.26±0.03 (μg/mg)</td>
</tr>
<tr>
<td>SOD (μmol/mg protein)</td>
<td>99.17±9.10 (μmol/mg)</td>
<td>128.86±5.93 (μmol/mg)</td>
<td>118.17±0.94 (μmol/mg)</td>
<td>100.23±5.72 (μmol/mg)</td>
</tr>
</tbody>
</table>

Data are mean ± SD of eight rats in each group. Values are expressed as mg/dL. Statistical analysis are done using one way analysis of variance (ANOVA) using Co Stat Computer program accompanied with least significance level (LSD) between groups at p<0.05. Unshared superscript letters are significant values between groups at p<0.0001. Values between brackets are % changes over control group = [(mean control - mean treated)/mean of control] x 100. Values between parenthesis are % changes over hypercholesterolemic group = [(mean of hypercholesterolemia - mean treated)/mean of hypercholesterolemia] x 100.
Table 7. Effect of treatment with F. racemosa (L.) on lipid profile in hypercholesterolemic rats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Cholesterol</th>
<th>Ficus racemosa</th>
<th>Lipanthyl</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC</td>
<td>59.45±4.41 ---</td>
<td>68.46±11.10 (+15.15)</td>
<td>45.04±6.74 [-34.20]</td>
<td>63.06±15.50 [-7.88]</td>
</tr>
<tr>
<td>HDL-C</td>
<td>53.22±1.44 ---</td>
<td>82.20±3.64 (+54.45)</td>
<td>61.61±6.31 [-25.04]</td>
<td>66.82±4.65 [-18.71]</td>
</tr>
<tr>
<td>LDL-C</td>
<td>12.52±1.55 ---</td>
<td>19.59±3.64 (+56.46)</td>
<td>17.55±2.11 [-10.41]</td>
<td>18.34±2.23 [-6.38]</td>
</tr>
<tr>
<td>TG</td>
<td>93.75±5.10 ---</td>
<td>166.66±7.79 (+77.77)</td>
<td>152.08±36.91 [-8.74]</td>
<td>152.08±5.89 [-8.74]</td>
</tr>
</tbody>
</table>

Data are mean ± SD of eight rats in each group. Values are expressed as mg/dl. Statistical analysis are done using one way analysis of variance (ANOVA) using Co Stat Computer program accompanied with least significance level (LSD) between groups at p<0.05. Unshared superscript letters are significant values between groups at p<0.0001. Values between parenthesis are % changes over control group = [(mean of variance (ANOVA) using Co Stat Computer program accompanied with least significance level (LSD)] between groups at p<0.0001. Values between brackets are % changes over control group = [(mean control - mean treated)/mean of control] x 100. Values between parenthesis are % changes over hypercholesterolemic group= [(mean of hypercholesterolemia - mean treated)/mean of hypercholesterolemia] x 100.

Table 8. Effect of treatment with F. racemosa (L.) on liver function indices in hypercholesterolemic rats.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Cholesterol</th>
<th>Ficus racemosa</th>
<th>Lipanthyl</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST</td>
<td>2.22±0.08 ---</td>
<td>3.04±0.38 (+36.93)</td>
<td>2.95±0.36 [-2.96]</td>
<td>2.84±0.23 [-6.57]</td>
</tr>
<tr>
<td>ALT</td>
<td>1.02±0.03 ---</td>
<td>2.15±0.02 (+110.78)</td>
<td>2.10±0.06 [-2.32]</td>
<td>2.13±0.06 [-0.93]</td>
</tr>
<tr>
<td>ALP</td>
<td>114.68±21.27 ---</td>
<td>215.63±14.73 (+88.02)</td>
<td>170.31±5.80 [-21.02]</td>
<td>103.12±6.65 [-52.17]</td>
</tr>
<tr>
<td>GGT</td>
<td>36.93±2.23 ---</td>
<td>68.87±3.42 (+86.48)</td>
<td>49.22±3.34 [-28.53]</td>
<td>38.54±5.97 [-44.03]</td>
</tr>
<tr>
<td>Serum protein</td>
<td>8.65±0.53 ---</td>
<td>8.33±0.76 (-3.69)</td>
<td>8.59±0.27 [+3.12]</td>
<td>8.70±0.57 [+4.44]</td>
</tr>
</tbody>
</table>

Data are mean ± SD of eight rats in each group. Values are expressed as U/L and serum protein as mg/ml. Statistical analysis are done using one way analysis of variance (ANOVA) using Co Stat Computer program accompanied with least significance level (LSD) between groups at p<0.0001. Unshared superscript letters are significant values between groups at p<0.05. Values between brackets are % changes over control group = [(mean control - mean treated)/mean of control] x 100. Values between parenthesis are % changes over hypercholesterolemic group= [(mean of hypercholesterolemia - mean treated)/mean of hypercholesterolemia] x 100.

Table 9. Effect of treatment with F.racemosa (L.) on oxidative stress markers in hypercholesterolemic rats.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>CCl4</th>
<th>Ficus racemosa</th>
<th>Silymarin</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSH (µg/mg protein)</td>
<td>21.39±2.80 ---</td>
<td>11.97±5.94 [-44.03]</td>
<td>17.11±2.05 [+42.94]</td>
<td>16.46±2.33 [+37.51]</td>
</tr>
<tr>
<td>MDA (µg/mg protein)</td>
<td>0.22±0.03 ---</td>
<td>0.38±0.03 (+72.72)</td>
<td>0.24±0.04 [-36.84]</td>
<td>0.25±0.05 [-34.21]</td>
</tr>
<tr>
<td>SOD (µmol/mg protein)</td>
<td>95.92±4.88 ---</td>
<td>127.11±4.68 (+32.51)</td>
<td>117.42±6.23 [-7.62]</td>
<td>103.23±6.91 [-18.78]</td>
</tr>
</tbody>
</table>

Data are mean ± SD of eight rats in each group. Values are expressed as U/L and serum protein as mg/ml. Statistical analysis are done using one way analysis of variance (ANOVA) using Co Stat Computer program accompanied with least significance level (LSD) between groups at p<0.05. Unshared superscript letters are significant values between groups at p<0.0001. Values between brackets are % changes over control group = [(mean control - mean treated)/mean of control] x 100. Values between parenthesis are % changes over CCl4 group= [(mean of CCl4 - mean treated)/mean of CCl4]
DISCUSSION AND CONCLUSION

Oxidant free radicals play a relevant role in the etiology and pathogenesis of a variety of diseases such as diabetes mellitus, cancer, hypertension, and cardiovascular diseases and are considered to be the principle causative agents of aging (Jeon et al., 2002).

The results obtained in the present study regarding the effect of CCl₄ intoxication on the concentrations of liver function indices; ALT, AST, ALP and GGT are in agreement with those reported by (Romero et al., 1998; Opoku et al., 2007; Karakus et al., 2011). These authors attributed the increase of these markers in rats’ serum to the process of protein synthesis and the decreased levels of DNA. In addition, the increased serum levels of hepatic markers have been attributed to the liver injury, because these enzymes are place in cytoplasmic area of the cell and are released into circulation in case of cellular damage (Brent and Rumack, 1993). There are many authors’ reports that these enzymes activities were significantly elevated after CCl₄ treatment. The reports about the hepatotoxic effects by CCl₄ are of lipid peroxidation origin due to the presence of the active metabolite of CCl₄; CCl₃ that abstract hydrogen from fatty acids, initiating lipid peroxidation, led to cell injury, and finally liver damage (Mehmet et al., 2008; Arici et al., 2011).

However, the reduced concentrations of these markers as a result of F. racemosa (L.) butanol fraction administration might probably due to the presence of antioxidant compounds in the fraction that contribute to the protection against CCl₄ induced hepatotoxicity in rats. These compounds may be responsible for its hepatoprotective action by scavenge reactive oxygen species such as the superoxide anion (O₂), the hydrogen peroxide (H₂O₂) and the hydroxyl radical (OH) (Jahn et al., 1985).

We noticed that the butanol fraction of 95% ethanolic extract of Ficus racemosa (L.) recorded remarkable improvement percentages. Therefore, the butanol fraction attenuated the increased level of liver function enzymes and caused a subsequent recovery towards normalization. This give an additional support that the fraction is able to condition the hepatocytes, accelerate regeneration of parenchyma cells, protect against
membrane fragility and decrease leakage of the enzymes into circulation. This was also the same action of silymarin on liver function indices as it recorded improvement levels upon treatment of CCl4 intoxicated rats.

Oxidative stress is one of the most important stimuli to activate hepatic stellate cells (Ghatak et al., 2011), 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 sulfhydryl groups and DNA bases, causing instabilities to cellular homeostasis and even leading to cell death (Jaeschke and Ramachandran, 2011). In the present study, CCl4 treatment damaged the defense systems of liver causing serious lipid peroxidation as shown by increased MDA production. In addition, treatment with CCl4 significantly 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 (Yu et al., 2012). Decrease in GSH activity might be also due to decrease availability of GSH resulted during the enhanced lipid peroxidation processes (Motawi et al., 2011; El-Gengaithi et al., 2012 and 2013).

Moreover, Romero et al., (1998) showed that CCl4 intoxication induced changes in the process of protein synthesis. 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 our studies.

Silymarin as an antioxidant flavonoid complex derived from the herb milk thistle (Silybum marianum), has the ability to attenuate free radicals elevation, chelates metal ions, inhibits lipid peroxidation and prevents liver glutathione depletion (Mansour et al., 2006). Therefore, plant fraction acted by the same mode of action of silymarin (Gowri-Shankar et al., 2008).

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 (Karakus et al., 2011; Motawi et al., 2011; El-Gengaithi et al., 2012 and 2013). Similar with the above reports, our findings were revealed high level of inflammation, steatosis and necrosis within the lobular areas in CCl4 group.

In plant and silymarin treated groups, hepatocytes degeneration, necrosis and infiltration of inflammatory cells were all apparently ameliorated. Collagen deposition was also markedly reduced. Silymarin, as a standard herbal hepatoprotective drug confirmed the potential effect of F. racemosa (L.) butanol fraction as a potent hepatoprotective agent.

High fat diet (HFD) is also the major cause of liver disease and impairment of liver function (Slim et al., 1996; Rizvi et al., 2003). Several studies have indicated that diet treatment or drug therapy to regulate cholesterol can reduce subsequent cardio vascular disease (CVD)-associated mortality and morbidity (Yokozawa et al., 2003). On the basis of this, great efforts have been made to reduce the risk of CVD through the regulation of cholesterol, thus the therapeutic benefits of plant foods have been the focus of many extensive dietary studies (Zheng et al., 2008).

Concerning lipid profile, the present study revealed significant increase in total cholesterol, LDL, HDL and triglycerides in rats administered with cholesterol as compared to the control group. These results were in accordance with the results of (Awad et al., 2011, 2012; Hamed, 2011) who founded significant increase in lipid profile after different experimental animals were administrated with cholesterol or fed a high fat diet. (Fungwe et al., 1993) attributed the increase of triglycerides level to the effect of dietary cholesterol that has been shown to reduce fatty acid oxidation, which, in turn, increases the levels of hepatic and plasma triglycerol.

Suggesting that the alteration induced by the HFD is partly due to oxidative damage, therefore the antioxidants may have had a significant protective effect on the pathways leading to transcription of these genes by scavenging ROSs (Yang et al., 2008). Treatment with the plant under investigation recorded improvement in total cholesterol, HDL-C, LDL-C and triglycerides with variable degrees. (Adaramoye et al., 2008) attributed the attenuated of cholesterol and triglyceride levels to the effect of treatment that may be reduced the hepatic triglyceride biosynthesis and favor the redistribution of cholesterol among the lipoprotein molecules. The reduction of total cholesterol by the butanol fraction of F. racemosa (L.) was associated with a decrease of its LDL fraction, which is the target of several hypolipidaemic drugs. This result suggests that cholesterol-lowering activity of the herb extract can be result from 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.

In the present study, treatment with F. racemosa (L.) butanol fraction led to improve the level of serum HDL-cholesterol, indicating its promising protective role against CVD. (Yokozawa et al., 2006) explained the phenomenon of CVD according to the role of HDL that exerts part of its anti-atherogenic effect by counteracting LDL oxidation or HDL that may promotes the reverse cholesterol transport pathway, by inducing an efflux of excess accumulated cellular cholesterol and prevents the generation of an oxidatively modified LDL. On the basis of this explanation, the fraction under investigation may probably plays as 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. This agrees with
the mechanism of action of fibrates (Staels et al., 1998) which their LDL-cholesterol lowering activity is not strongly marked, but the triglycerides decreasing effect of them is very spectacular especially by both stimulation of the gene expression of lipoprotein lipase leading to enhanced catabolism of VLDL, synthesis of fatty acids and reduced VLDL secretion.

The relationship between oxidative stress and cholesterol level was confirmed in the present 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. Therefore, Ficus extract may elicit some health benefits through the modulation of physiologic functions including the atherogenic lipid profile.

In the present study, treated rats with F. racemosa (L.) recorded amelioration of glutathione, malondialdehyde and superoxide dismutase levels. (Shukla et al., 2004) attributed the amelioration of the antioxidant levels after treatment to the constituents present in the fraction which may decrease the levels of lipid peroxidation products by scavenging free radicals like superoxide anion, hydroxyl and peroxy-free radicals. In addition, its hypolipidaemic and hypcholesterolaemic effects led to decrease lipid peroxidation, by decreasing the availability of lipid substrates.

Due to the ameliorations observed in the oxidative stress markers, the fraction also improved the liver function indices. Similarly, lipanthyl drug recorded an amelioration level of liver enzyme activities due to the attenuation of the hypercholesterolemic state and the elaborated free radicals, which in turn ameliorate the liver function. These results were in accordance with the results of (Awad et al. 2011; Hamed, 2011; Awad et al., 2012) who confirmed the role of lipanthyl drug in treatment of hypercholesterolemia and attenuation of free radicals elevation secondary to hypercholesterolemia.

In the present study, the light microscopic examination of the liver sections of hypercholesterolemic rat livers revealed a large accumulation of macrovesicular and microvesicular fat in the livers as well as dark inflammatory infiltrates in the high fat/cholesterol fed liver. This was in agreement with (Zheng et al., 2008; Awad et al. 2011; Hamed, 2011; Awad et al., 2012) who observed the same structure of liver after high fat diet. This was attributed to the presences of fatty liver as a result of 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. racemosa (L.) 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 plant.

In conclusion, F. racemosa(L.) butanol fraction approved to be antioxidant, hepatoprotective, and hypolipidemic agent.

To correlate between the biological effects obtained by the butanol fraction of F. racemosa (L.) and its polyphenolic ingredients; the HPLC results revealed the presence of twenty three phenols and ten flavonoids. Regarding to phenolic compounds, their ability to penetrate into lipid bilayers is undoubtedly crucial to the protection against oxidation. For polyphenols that partition in the non-polar region of the bilayer, they can inhibit the propagation of lipid oxidation by two mechanisms: (i) by intercepting intramembrane radicals and/or (ii) by increasing membrane fluidity, which disorganizes lipid chains and hinders radicals’ propagation (Fadel et al., 2011).

The approval of the antioxidant properties of chlorogenic acid on liver inflammation and Fibrosis induced by carbon tetrachloride is done by (Mikulski et al., 2014). The authors have demonstrated that the hepatic mRNA expression and serum levels of tumour necrosis factor α, interleukin- 6 and interleukin-1β were significantly increased in CCl4- treated rats and attenuated by cotreatment with chlorogenic acid. Also in that study the authors have proved that the chlorogenic acid may efficiently inhibit CCl4-induced liver fibrosis in rats and the protective effect may be due to the inhibition of TLR4/MyD88/NF- _B signaling pathway. Moreover, the antioxidant potency of chlorogenic acid has been investigated in vitro and in vivo by (Sato et al., 2011) who also investigated the activity of caffeic acid, which can be obtained by hydrolys of chlorogenic acid in the intestines. The results obtained show that caffeic acid has stronger antioxidant activity than chlorogenic acid. This explains that chlorogenic which is the second major phenolic in F. racemosa (L.) butanol fraction (1424.67 mg/100gm) and caffeic acid (58.87 mg/100 gm) are both, synergistically, have great responsibility for the investigated biological activities. Additionally, (Biskup et al., 2013) stated that the antioxidant character of chlorogenic acid is due to the presence of a carbonyl group like ester, besides, they confirmed the antioxidant characters of pyrogallol, and protocatechuic. (Lamaison et al., 1991) approved that of Rosmarinic acid.

The evidence of the antioxidant activities of ferulic, p – coumaric acid and vanillic acid is done by (Goupy et al., 1999). They explained that the antioxidant activity of phenolic acids is dependent on the number of hydroxyl groups.

According to the flavonoids, they have attracted the interest of researchers because they show promise of being powerful antioxidants that can protect the human body from free radicals and against oxidative stress (Bors
et al., 1996). Chemically, there are three features that confer on flavonoids their remarkable antioxidant properties (Rice-Evans et al., 1997): the hydrogen donating substituents (hydroxyl groups) attached to the aromatic ring structures of flavonoids, which enable the flavonoids to undergo a redox reaction that helps them to scavenge free radicals more easily; a stable delocalization system, consisting of aromatic and heterocyclic rings as well as multiple unsaturated bonds, which helps to delocalize the resulting free radicals; and the presence of certain structural groups which are capable of forming transition metal-chelating complexes that can regulate the production of reactive oxygen species such as hydroxyl radicals and oxygen radicals.

Naringin (glycoside) which is present by 220.21 mg/100 gm has metal-chelating, antioxidant and free radical scavenging properties (Chen et al., 1990) and has been reported to offer some protection against lipid peroxidation (Maridonneau- Parini et al., 1986). In addition, our results were in accordance with that mentioned by (Jeon et al., 2001) who confirmed that naringin is potent cholesterol-lowering agent. Moreover, (Wahsha et al., 2010) explained that hesperidin and naringin could decrease the formation of MDA through their ability to scavenge the hydroxyl radicals.

CONCLUSION

After monitoring the polyphenolic compounds in the butanol fraction of F. racemosa (L.) and correlate their chemical nature with its biological activities as antioxidant, hepatoprotective and hypolipidemic, F. racemosa (L.) proved that it has the ability to down regulate free radicals elevation, improves liver functions, reduces fibrosis severity, and normalizes hepatic cells architecture. F. racemosa (L.) recorded potent effect in improving the selected parameters. F. racemosa (L.) may be used as a new safe therapy that may enhance the antifibrotic mechanism, delay disease progression, or reduce complications, which was clearly seen in the histopathological analysis. Further study is needed to identify, specifically, the molecules responsible for pharmacological and clinical uses.

REFERENCES


Fadel O, El Kirat K, and Morandat S. The natural antioxidant rosmarinic acid spontaneously penetrates membranes to inhibit lipid peroxidation in situ. *Biochimica et Biophysica Acta*. 2011; 1808: 2973–2980


