Original Article

Chemical constituents and protective effect of *Ficus ingens* (Miq.) Miq. on carbon tetrachloride-induced acute liver damage in male Wistar albino rats

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**KEYWORDS**

Phytochemical studies; Flavonoids; Anthracquinone; Hepatoprotective effect

**Abstract** The aim of the present study was to investigate the chemical constituents and hepatoprotective effect of *Ficus ingens* (Miq.) Miq. (Moraceae) extract against carbon tetrachloride-induced acute liver damage in male Wistar albino rats. The ethanol extract of *F. ingens* was subjected to phytochemical study. In addition, its acute and sub-chronic toxicities were assessed. Eight compounds were isolated from this plant and identified as β-sitosterol, β-sitosterol glucoside, chrysophanol, 7-hydroxy-2,5 dimethyl chromen-4-one, quercetin, Aloe emodin glucoside, rutin and Patuletin-3-O-methyl-3-O-rutinoside. The structure elucidation was based on 1H and 13C NMR, proton–proton correlation spectroscopy (1H–1H Cosy), distortionless enhancement by polarization transfer (DEPT), Heteronuclear Multiple-Quantum Correlation (HMQC), and heteronuclear
1. Introduction

Liver is a vital organ that has a wide range of functions, including detoxification, plasma protein synthesis, and production of biochemistry necessary for digestion. Damage to the liver inflicted by hepatotoxic agents is of grave consequence. Today, liver damage is one of very common ailments in the world resulting in serious debilities ranging from severe metabolic disorders to even mortality (Akilavalli et al., 2011). Various xenobiotics are known to cause hepatotoxicity; one among them is CCl₄ that may cause lipid peroxidation (Kodavanti et al., 1989; Demirdag et al., 2004). Many hepatoprotective herbal preparations have been recommended in alternative medicine for the treatment of liver diseases. Therefore, the search of a new natural hepatoprotective agent is of great interest.

Ficus is a genus belonging to family Moraceae, it comprises about 850 species of woody trees, shrubs, vines, epiphytes, and hemiepiphyte. F. ingens is an evergreen deciduous tree up to 10 m height, occasionally higher, with a rounded or spreading crown and with a spread of up to 30 m wide. The plant grows throughout the year but peaking in summer (Myburgh et al., 1994). Multiple bond correlations spectrum (HMBC). Hepatotoxicity induced with CCl₄ was evidenced by elevation of liver marker enzymes (ALT, AST, ALP and LDH) and TB content in serum. In addition, antioxidant enzymes were drastically inhibited with significant reduction of GSH and increased LPO in liver homogenate of CCl₄-intoxicated rats. Pre-treatment with F. ingens (200 and 400 mg/kg) and silymarin (50 mg/kg) avoided the changes observed in CCl₄-intoxicated rats. In conclusion, the ethanol extract of F. ingens showed protective activity against liver injury, which might be developed into a new hepatoprotective agent.

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2. Materials and methods

2.1. Plant material

The aerial parts of Ficus ingens (Miq.) Miq. were freshly collected from Tabouk area-KSA, during Summer 2010. They were housed in polypropylene cages and fed with standard chow diet and water ad libitum. The animals were exposed to alternate cycle of 12 h of darkness and light. Male rats were used because of their constant metabolism compared to the variation in the female physiology. Animals were exposed to alternate cycle of 12 h of darkness and light. Male rats were used because of their constant metabolism compared to the variation in the female physiology. Animals were exposed to alternate cycle of 12 h of darkness and light.

2.2. Extraction

One kg of the dried powder was extracted by percolation in 70% aqueous ethanol for 72 h. The combined ethanol extracts were concentrated under reduced pressure at a temperature not exceeding 45 °C. The extract was fractionated using silica gel column chromatography (350 g) and gradually eluted with chloroform containing increasing proportions of methanol. Fractions (85, 100 mL each) were collected and monitored by TLC (silica gel, chloroform–methanol). Similar fractions were combined together to obtain 5 groups. Each group was reapplied to silica gel column eluted with chloroform containing gradually increasing proportions of methanol. Further purification was carried out using Sephadex LH-20 columns to afford compounds 1–8.

2.3. Acid hydrolysis

Proton (1H) and carbon 13 (13C NMR) spectra were recorded on Bruker VXS500 NMR spectrometer operating at 500 and 125 MHz respectively. 1H–13C correlations were established by using HMOC and HMBC pulse sequences respectively. 1H–1H correlations were determined by double quantum filtered COSY.

2.5. Experimental animals

Male Wistar albino rats (160–180 g) and albino mice of both sexes (27–30 g) were maintained in the Laboratory Animal Unit of the College of Pharmacy, Salman bin AbdulAziz University. They were housed in polypropylene cages and fed with standard chow diet and water ad libitum. The animals were exposed to alternate cycle of 12 h of darkness and light. Male rats were used because of their constant metabolism compared to the variation in the female physiology. Animals were exposed to alternate cycle of 12 h of darkness and light.
2.6. Preparation of the extract for biological studies

The concentrated ethanol extract of *Ficus ingens* was suspended in 3% v/v Tween 80 in distilled water (vehicle).

2.7. Acute toxicity experiment

Albino mice were divided into control and test groups (6 animals each). Control group received the vehicle (3% Tween 80) while the test groups got graded doses (1000–4000 mg/kg) of *F. ingens* ethanol extract orally and were observed for mortality till 48 h and the LD$_{50}$ was calculated (Ghosh, 1994).

2.8. Doses

The dose selection for the ethanol extract of *F. ingens* was based on the acute toxicity study, which did not show any adverse effect following oral administration of doses up to 4000 mg/kg. Accordingly, experimental oral doses of 100, 200 and 400 mg/kg that equal to one-fortieth, one-twentieth and one-tenth of the maximum possible dose of the extract that did not cause mortalities in mice were selected.

2.9. Sub-chronic toxicity

Twenty-four male Wistar albino rats were randomly divided into 4 groups of 6 animals. The 1st group was kept as control (5 mL/kg of 3% Tween 80), while 2nd, 3rd and 4th groups were administered the ethanol extract of *F. ingens* in doses of 100, 200 and 400 mg/kg, respectively. All medications were administered orally with the aid of an orogastric cannula for 35 consecutive days. Rats were maintained under identical conditions with food and water ad libitum for the entire period with close observation. At the end of the experimental period, blood samples (2 mL) were drawn by puncturing retro-orbital venous sinus of each rat (under ether anesthesia) and centrifuged at 10,000 rpm for 5 min. Sera were separated to be used for the biochemical estimations.

2.10. Measurement of liver and kidney function markers

Liver functions were evaluated by measuring the serum activity of ALT and AST following the method of Reitman and Frankel (1957) while the activities of ALP and LDH were estimated by the methods of Babson et al. (1966) and King (1965), respectively. The serum concentrations of TB (Walter and Gerarde, 1995), total protein (TP) and albumin (Alb) were determined in serum. glutathione (GSH) and lipid peroxidation (LPO). Hematoxylin and eosin (H&E) staining assays deployed to evaluate liver damage were estimated in serum of rats.

2.11. Experimental induction of hepatic damage

CCl$_4$ was dissolved in corn oil in the ratio 1:1 v/v. Liver damage was induced in rats following subcutaneous (SC) injection of CCl$_4$ in the lower abdomen at a dose of 3 mL/kg (Theophile et al., 2006).

2.12. Hepatoprotective activity

Thirty-six adult male Wistar albino rats were randomly divided into six groups of six animals, each. Rats of the 1st (normal control) and 2nd (CCl$_4$-intoxicated control) groups received the vehicle in a dose of 5 mL/kg. Animals of the 3rd group (reference) received silymarin at a dose of 50 mg/kg. The 4th, 5th and 6th groups were treated with the ethanol extract of *F. ingens* in doses of 100, 200 and 400 mg/kg, respectively. All medications were administered orally by gastric intubation for 7 consecutive days. Two h after the last dose, normal control rats were given a single dose of corn oil (3 mL/kg, SC), while animals of the 2nd to 6th groups received a single dose of CCl$_4$ (3 mL/kg, SC).

After 24 h of corn oil and CCl$_4$ injections, blood sample from each rat (2 mL) was withdrawn by puncturing their retro-orbital plexus of veins and collected in previously labeled centrifuging tubes and allowed to clot for 30 min at room temperature. Serum was separated by centrifugation at 10,000 rpm for 5 min. Livers were dissected out and divided into two parts. One part was kept in liquid nitrogen for determination of antioxidant status and the other part was immediately fixed in buffered formalin 10% and was used for histopathological examination.

2.13. Assessment of liver biochemical markers

The levels of alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), total bilirubin (TB), total protein (TP) and albumin (Alb) were determined in serum. glutathione (GSH) and lipid peroxidation (LPO). Hematoxylin and eosin (H&E) staining assays deployed to evaluate liver damage were estimated in serum of rats.

2.14. Assessment of CCl$_4$ mediated oxidative stress

Liver tissue was homogenized in 10 volume of 100 mM KH$_2$PO$_4$ buffer containing 1 mM EDTA (pH 7.4) and centrifuged at 12,000 rpm for 30 min at 4°C. The activities of the antioxidant enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT), were assayed in the hepatic tissue homogenate of the control and experimental rats according to the methods of Sun and Zigman (1978), Mohandas et al. (1984) and Chance and Maehley (1955), respectively. Moreover, the extent of lipid peroxidation (LPO) was estimated in liver homogenate of all rats as the concentration of thiobarbituric acid reactive product (malondialdehyde – MDA) according to (Ohkawa et al., 1979), while GSH tissue content was measured according to the method described by Moron et al. (1979).

2.15. Histopathological study

Liver from each rat was removed after dissection and preserved in 10% formalin. Then representative blocks of liver tissues from each lobe were taken and possessed for paraffin embedding using the standard microtechnique (Galighor and Kozloff, 1971). Sections (5 μm) of livers stained with hematoxylin and eosin were observed microscopically for the evaluation of histopathological changes.

4. Statistical analysis

The values are expressed as mean ± standard error of six observations in each group. All groups were subjected to one-way analysis of variance (ANOVA), which was followed
Table 1 Effect of prolonged oral administration of ethanol extract of F. ingens for 35 days on the serum activity of liver marker enzymes in rats, (n = 6).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dose (mg/kg)</th>
<th>ALT (U/L)</th>
<th>AST (U/L)</th>
<th>ALP (U/L)</th>
<th>LDH (U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>00</td>
<td>61.96 ± 3.22</td>
<td>134.91 ± 5.77</td>
<td>73.52 ± 4.22</td>
<td>55.74 ± 2.45</td>
</tr>
<tr>
<td>Ficus ingens</td>
<td>100</td>
<td>63.08 ± 2.74</td>
<td>135.87 ± 5.36</td>
<td>71.45 ± 4.47</td>
<td>60.15 ± 2.11</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>66.36 ± 3.16</td>
<td>137.65 ± 6.15</td>
<td>77.10 ± 4.50</td>
<td>58.22 ± 2.68</td>
</tr>
</tbody>
</table>

SC injection of CCl₄ to rats showed significant elevation of liver marker enzymes (ALT, AST, ALP and LDH) in their serum after 24 h of intoxication. The level of TB in the serum of CCl₄-intoxicated control was also significantly increased when compared to the normal control group. Administration of silymarin (50 mg/kg) and the ethanol extract of F. ingens (200 and 400 mg/kg) once daily for 7 days prior to CCl₄ exhibited a significant hepatoprotective activity, resulting in reduc-
tion in the elevated serum activities of liver marker enzymes (Table 3) and level of TB (Table 4) when compared to CCl4-intoxicated rats. Furthermore, pretreatment with the ethanol extract of *F. ingens* in a dose of 100 mg/kg, did not elicit any significant effect. The oxidative stress caused by CCl4 in the liver was assessed by measuring the activity of hepatic antioxidant defense enzymes (SOD, GPx, and CAT), GSH and the level of lipid peroxidation product (MDA). Results presented in Table 5 showed that SC injection of CCl4 induced significant reduction in the activities of SOD, GPx and CAT enzymes with a decreased level of GSH content as compared to the normal control group. On the other hand, it increased the MDA level in liver tissues. Pre-administration of silymarin (50 mg/kg) and *F. ingens* (200 and 400 mg/kg) reduced the severity of CCl4 toxicity, as evident from the non-significant differences observed in the oxidative stress indicators and antioxidant enzyme levels in these groups.

Histopathological examination of the liver sections from normal rats showed hepatocytes with normal parenchymal architecture (Fig. 2A). Liver sections of CCl4-intoxicated animals exhibited diffuse central and peripheral necrosis and destruction of the lobular architecture (Fig. 2B). Liver sections of rats treated with the ethanol extract of *F. ingens* in a dose of 400 mg/kg showed normal hepatic cords (Fig. 2C) and absence of severe congestion and pyknosis indicating pronounced protection of hepatocytes against CCl4-induced hepatic damage.

### 6. Discussion

Preliminary phytochemical study indicates the presence of flavonoids, coumarins, steroids and anthraquinone in the extract of *F. ingens*.

#### 6.1. Isolated compounds

Eight compounds were isolated from *Ficus ingens*. Compounds were identified examining their 1H NMR, 13C NMR and as well comparison with the published data (Anand et al., 2010). Acid hydrolysis and TLC of the sugar part (ethyl acetate–methanol–acetic acid–water (65:15:10:10) revealed that compounds 2 and 6 contain glucose, compounds 7 and 8 contain glucose and rhamnose. Compounds 1 and 2 were identified as β-sitosterol and β-sitosterol glucoside by comparing their data with previous published data (Maridass & Ramesh, 2010).

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Table 2  Effect of prolonged oral administration of ethanol extract of *F. ingens* for 35 days on the serum levels of TB, TP, Alb, urea and creatinine in rats, (n = 6).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dose (mg/kg)</th>
<th>TB (mg/dL)</th>
<th>TP (g/dL)</th>
<th>Alb (g/dL)</th>
<th>Urea (mg/dL)</th>
<th>Creatinine (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>00</td>
<td>1.42 ± 0.08</td>
<td>8.12 ± 0.23</td>
<td>3.70 ± 0.16</td>
<td>37.34 ± 1.63</td>
<td>0.34 ± 0.03</td>
</tr>
<tr>
<td><em>Ficus ingens</em></td>
<td>100</td>
<td>1.45 ± 0.09</td>
<td>8.15 ± 0.27</td>
<td>3.72 ± 0.14</td>
<td>38.58 ± 1.17</td>
<td>0.36 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>1.45 ± 0.07</td>
<td>8.22 ± 0.20</td>
<td>3.79 ± 0.15</td>
<td>38.70 ± 1.20</td>
<td>0.38 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>1.48 ± 0.06</td>
<td>8.20 ± 0.26</td>
<td>3.80 ± 0.10</td>
<td>40.58 ± 1.43</td>
<td>0.38 ± 0.02</td>
</tr>
</tbody>
</table>

The results are expressed as mean ± S.E.M., n = 6 rats/group. * indicate significance compared to CCl4 group (p < 0.05).

Table 3  Effect of the ethanol extract of *F. ingens* on the serum activity of liver marker enzymes in rats with CCl4 induced – hepatotoxicity.

<table>
<thead>
<tr>
<th>Groups</th>
<th>ALT (U/L)</th>
<th>AST (U/L)</th>
<th>ALP (U/L)</th>
<th>LDH (U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>68.0 ± 2.11 †</td>
<td>140.5 ± 4.18 †</td>
<td>72.6 ± 2.65 †</td>
<td>52.5 ± 2.44 †</td>
</tr>
<tr>
<td>CCl4-intoxicated control</td>
<td>378.6 ± 12.95 †</td>
<td>436.3 ± 14.91 †</td>
<td>131.8 ± 5.43 †</td>
<td>142.3 ± 5.78 †</td>
</tr>
<tr>
<td>Silymarin (50 mg/kg) + CCl4</td>
<td>154.0 ± 5.07 †</td>
<td>264.5 ± 9.40 †</td>
<td>79.0 ± 3.45 †</td>
<td>68.0 ± 3.27 †</td>
</tr>
<tr>
<td><em>F. ingens</em> (100 mg/kg) + CCl4</td>
<td>355.6 ± 16.19 †</td>
<td>418.3 ± 11.61 †</td>
<td>123.1 ± 5.48 †</td>
<td>130.6 ± 5.22 †</td>
</tr>
<tr>
<td><em>F. ingens</em> (200 mg/kg) + CCl4</td>
<td>284.1 ± 10.43 †</td>
<td>363.8 ± 10.57 †</td>
<td>95.1 ± 5.88 †</td>
<td>94.1 ± 4.79 †</td>
</tr>
<tr>
<td><em>F. ingens</em> (400 mg/kg) + CCl4</td>
<td>200.1 ± 7.50 †</td>
<td>318.0 ± 12.88 †</td>
<td>84.5 ± 5.01 †</td>
<td>76.5 ± 4.95 †</td>
</tr>
</tbody>
</table>

The results are expressed as mean ± S.E.M., n = 6 rats/group. * indicate significance compared to CCl4 group (p < 0.05). † indicate significance compared to silymarin group (p < 0.05).

Table 4  Effect of the ethanol extract of *F. ingens* on the serum levels of TB, TP and Alb in rats with CCl4 induced – hepatotoxicity.

<table>
<thead>
<tr>
<th>Groups</th>
<th>TB (mg/dL)</th>
<th>TP (g/dL)</th>
<th>Alb (g/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>1.24 ± 0.06 †</td>
<td>8.47 ± 0.39 †</td>
<td>3.65 ± 0.18 †</td>
</tr>
<tr>
<td>CCl4-intoxicated control</td>
<td>3.44 ± 0.13 †</td>
<td>5.16 ± 0.25 †</td>
<td>2.21 ± 0.16 †</td>
</tr>
<tr>
<td>Silymarin (50 mg/kg) + CCl4</td>
<td>1.79 ± 0.12 †</td>
<td>7.71 ± 0.21 †</td>
<td>3.48 ± 0.15 †</td>
</tr>
<tr>
<td><em>F. ingens</em> (100 mg/kg) + CCl4</td>
<td>3.22 ± 0.17 †</td>
<td>5.80 ± 0.23 †</td>
<td>2.41 ± 0.15 †</td>
</tr>
<tr>
<td><em>F. ingens</em> (200 mg/kg) + CCl4</td>
<td>2.48 ± 0.13 †</td>
<td>6.70 ± 0.42 †</td>
<td>2.95 ± 0.11 †</td>
</tr>
<tr>
<td><em>F. ingens</em> (400 mg/kg) + CCl4</td>
<td>2.13 ± 0.13 †</td>
<td>7.56 ± 0.34 †</td>
<td>3.21 ± 0.17 †</td>
</tr>
</tbody>
</table>

The results are expressed as mean ± S.E.M., n = 6 rats/group. * indicate significance compared to CCl4 group (p < 0.05). † indicate significance compared to silymarin group (p < 0.05).
Compound 3 from the given data of $^1$H NMR, $^{13}$C NMR, COSY, HMQC and HMBC (Fig. 1) this compound is identified as 7-hydroxy-2,5-dimethyl-chromen-4-one, also the spectral data were in agreement with the literature reported (Kimura et al., 1992; Gu, 2009 & Königs et al., 2010).

Compound 4 is identified as chrysophanol as it gave positive test for anthraquinones (Harborne, 1993; Sofowora, 1993 and Trease and Evans, 2002). Its $^1$H NMR spectrum displayed two sharp singlets at $\delta$ 11.97 and 12.04 ppm, assigned for two chelated hydroxyl groups, five signals assigned for aromatic protons at $\delta$ 7.23 (1H, s, H-2), $\delta$ 7.56 (1H, s, H-4), $\delta$ 7.40 (1H, d, J = 8.4 Hz, H-7), $\delta$ 7.80 (1H, d, J = 7.5 Hz, H-5), $\delta$ 7.73 (1H, m, H-6). Besides, the methyl singlet at $\delta$ 2.44 ppm was assigned to the methyl group at C-3. $^{13}$C NMR spectrum and DEPT experiment data are almost identical with the reported data for chrysophanol (Rani et al., 2010; Sosa et al., 2006).

Compound 5 is identified as quercetin through comparison of its $^1$H NMR and $^{13}$C NMR spectral data with those reported for quercetin (Mabry et al., 1970).

Compound 6 based on the characterization, the isolated compound was Aloe emodin glucosidse (Anand et al., 2010).

Compound 7 is identified as rutin. It gave positive test for flavonol glycosides. m.p. 190°C. Its UV, $^1$H NMR and $^{13}$C NMR spectral data were identical with those reported for rutin (Geissman, 1962 and Mabry et al., 1970 & Harborne et al., 1975).

Compound 8 was identified as Patuletin-3’-O-methyl-3-O-rutinoside (5,7,3’ trihydroxy-6,3’-dimethoxy flavone) by comparing data with that published by Lin et al. (2002).

In the current study, oral administration of F. ingens extract in doses up to 4000 mg/kg did not produce any symptom of acute toxicity and none of mice died during 48 h of observation. Accordingly, it suggested that oral LD$_{50}$ of the tested extract was higher than 4000 mg/kg b.wt. Therefore, F. ingens plant can be categorized as highly safe since substances possessing LD$_{50}$ higher than 50 mg/kg are non toxic (Buck et al., 1976).

The non-toxic nature of F. ingens extract in acute toxicity study in mice is well supported by the normal levels of ALT, AST, ALP, LDH, TB, TP and Alb following 35-days treatment period in rats. Urea and creatinine are the most sensitive biochemical markers employed in the diagnosis of renal damage. In kidney damage, there will be retention of urea and creatinine in the blood (Nwanjo et al., 2005), therefore marked increases in serum urea and creatinine are indications of functional damage to the kidney (Panda, 1999). By these indicators, ethanol extract of F. ingens is therefore, not nephrotoxic in rats.

6.2. Hepatoprotective activity

CCl$_4$ is a well-known hepatotoxic agent. It has been used as a tool induced hepatotoxicity in experimental animals (Okuno et al., 1986). Acute exposure to CCl$_4$ produces rapid cellular injury due to its reductive dehalogenation in the endoplasmic reticulum of hepatocytes to generate an unstable highly reactive complex CCl$_3$O, or trichloroperoxyl free radical (CCl$_3$O$_2$). These radicals attack microsomal lipids causing lipid peroxidation. Products of lipid peroxidation may causes damage to the biological membranes leading to serious cellular injury and leakage of liver marker enzymes like ALT, AST, ALP and LDH ( Cotran et al., 1994). The prevention of this phenomenon can be considered as hepatoprotective activity (Mak et al., 1996).

Liver marker enzymes are localized in the cytosol of hepatic cells and thus are extruded into the serum when cells are damaged or necrotic. In this study, rats intoxicated with CCl$_4$ developed significant hepatic damage as manifested by a significant increase in the serum activities of ALT, AST, ALP and LDH that are indicators of hepatocyte damage and loss of functional integrity. Pretreatment of rats with the ethanol extract of F. ingens in doses of 200 and 400 mg/kg effectively protected rats against CCl$_4$-induced hepatic damage, resulting in reduction in serum activities of liver marker enzymes when compared to the intoxicated control rats. Decrease in the level of these enzymes with F. ingens is an indication of the stabilization of plasma membrane as well as repair of liver damage caused by CCl$_4$.
Further, the rise in the level of TB in serum following CCl₄ intoxication is also a measure of hepatotoxicity and could be attributed to impaired hepatic clearance due to hepatic parenchymal damage and biliary obstruction (Blanckaert and Schmid, 1982). The ability of the ethanol extract of F. ingens (200 and 400 mg/kg) to reduce the level of TB in the serum of intoxicated rats suggests its potential hepatoprotective effect. The lowered serum levels of TP and Alb due to CCl₄-intoxication are attributed to the initial damage of the endoplasmic reticulum which results in the loss of P-450 leading to fatty liver (Recknagel, 1967). Administration of the ethanol extract of F. ingens in doses of 200 and 400 mg/kg remarkably prevented CCl₄-induced reduction of TP and Alb in serum. This assures the hepatoprotective activity of this extract against damage by CCl₄.

Hepatic antioxidant enzymes (SOD, GPx and CAT) represent one protection against oxidative tissue-injury (Halliwell and Gutteridge, 1990). SOD converted O₂ into H₂O₂ while GPx and CAT metabolize H₂O₂ to non-toxic products. In the present investigation SC injection of CCl₄ to rats was shown to cause oxidative stress in liver and this damage was manifested by reduced activities of the antioxidant enzymes as well as GSH depletion in the liver homogenate. Depletion of GSH leads to cell death. Accordingly, the possible mechanism of the antihepatotoxic effect of F. ingens extract may be, in part, attributed to its antioxidant activity. This effect was evidenced by the ability of F. ingens to return the reduced activities of SOD, GPx and CAT and GSH level in the liver homogenate back to their control levels.

Since CCl₄ induced hepatotoxicity is due mostly to oxidative stress (Weber et al., 2003 and Lin et al., 2008), antioxidant mediated protective role of F. ingens extract has been assessed. Oxidative stress was evidenced by reduced activities of the antioxidant enzymes as well as GSH depletion in the liver homogenate. Depletion of GSH leads to cell death. In this work, the possible mechanism of the antihepatotoxic effect of F. ingens extract may be, in part, attributed to its antioxidant activity. This effect was evidenced by the ability.
of *F. ingens* to return the reduced activities of SOD, GPx and CAT and GSH level in the liver homogenate back to their control levels.

In addition, lipid peroxidation is supposed to be a critical factor in the pathogenesis of CCl4-induced hepatic injuries. Pre-treatment of *F. ingens* extract, on the other hand, prevented the toxic effects of CCl4 by restoring the increased MDA level in the liver homogenate toward the level of control animals, implying that the tested extract may prevent the peroxidation of lipids by CCl4. Increased activities of the antioxidant enzymes with concomitant increase in GSH level and reduced lipid peroxidation product are the indications that *F. ingens* extract offered significant protection. The hepatoprotective effect of *F. ingens* (200 and 400 mg/kg), was also supported by histopathological examination which showed recovery of the damaged liver cells. In accordance with these results, the protective effect of *F. ingens* extract against CCl4 may be attributed to the presence of phytoco-constituents such as flavonoids.

7. Conclusion

In the present study, it has been observed that *F. ingens* offered significant protection against the hepatotoxicant CCl4 in rats, which may be attributed to its phytochemical constituents (which were mainly polyphenolic compounds) with their anti-oxidant and membrane stabilizing properties.

References


by the plants *Ficus ingens* var. *ingens* and *Ficus cordata* subsp. *salicifolia*. J. Vet. Res. 61, 171–176.


