Hepatoprotective Potential of Mesembryanthemum forsskalii Fruits Extract Against Carbon Tetrachloride-Induced Liver Toxicity in Mice

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ABSTRACT

The present study aimed to investigate the ameliorative effect of the methanolic extract of Mesembryanthemum forsskalii fruits extract against the hepatotoxicity of carbon tetrachloride (CCl4) in mice at two different doses (low; 100 mg/kg BW and high; 500 mg/kg BW). The obtained results showed that CCl4 injection significantly reduced the body weights of mice and increased the relative liver weight. Treatment with M. forsskalii extract after CCl4 injection restores the weights of mice to the normal range and reduced the relative weight of the liver. Mice that were injected with CCl4 exhibited elevation in the liver enzymes; aspartate aminotransferase (AST) and alanine aminotransferase (ALT), reduction in the cholesterol content, and altered the architecture of the hepatic cells. Animals treated with M. forsskalii extract at a low dose after CCl4 administration enhanced the liver functions as evidenced by histological examination and liver enzymes analysis. Immunohistochemical investigation showed that the administration of M. forsskalii extract at a low dose downregulated the expression pattern of P53 and upregulated the expression of Bcl-2 in liver tissue. In conclusion, our findings indicated that the methanolic extract of M. forsskalii at a low dose (100 mg/kg BW) attenuated the hepatotoxicity induced by CCl4 through upregulation of Bcl-2 expression and restoring liver enzymes to their normal range.

INTRODUCTION

Toxins, infectious agents, and serum inflammatory mediators are the main reasons leading to the loss of functional liver capacity (Edwards and Wanless, 2013). Experimental animals that expose to CCl4 exhibit different morphological alterations in livers (Li et al., 2019; Owojuyigbe et al., 2020; Begum et al., 2022). Fibrosis, focal inflammation, cell infiltration, hepatic cell degeneration and regeneration, early portal cirrhosis, and alteration of the lobular structure are the most prominent changes that appear after exposure to CCl4 (Izzularab et al., 2021; Ammar et al., 2022). CCl4-induced hepatotoxicity was proved by the excess serum contents of alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), reduction in the cholesterol content, and altered the architecture of the hepatic cells. Animals treated with M. forsskalii extract at a low dose after CCl4 administration enhanced the liver functions as evidenced by histological examination and liver enzymes analysis. Immunohistochemical investigation showed that the administration of M. forsskalii extract at a low dose downregulated the expression pattern of P53 and upregulated the expression of Bcl-2 in liver tissue. In conclusion, our findings indicated that the methanolic extract of M. forsskalii at a low dose (100 mg/kg BW) attenuated the hepatotoxicity induced by CCl4 through upregulation of Bcl-2 expression and restoring liver enzymes to their normal range.

Key words
Mesembryanthemum forsskalii, Hepatotoxicity, CCl4, Liver enzymes, immunohistochemistry

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raised serum levels of tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and diminished IL-10, IL-12 (Elshopakey and Elazab 2021). 1.25 ml/kg BW of CCl₄ induced hepatotoxicity related parameters in rats (Al-Yahya et al., 2013). CCl₄ in 2 ml/kg BW induced oxidative stress and hepatotoxicity in mice, where a marked alteration in histology and marked inflammation was induced in CCl₄ treated mice (Ullah et al., 2020).

Many drugs are now available for the treatment of liver failure disease; however, the majority showed some serious side effects besides their cost-effectiveness. Therefore, several studies have been focused on finding alternative natural sources including plants to replace conventional drugs in the last two decades (Katiyar et al., 2012). Many desert medicinal plants showed therapeutic and hepatoprotective effects against CCl₄-induced hepatotoxicity in mice and rats (Wahid et al., 2016; Ammar et al., 2022; Ayoka et al., 2022; Doudach et al., 2022; Nwaechefu et al., 2022; Ouassou et al., 2021). The methanolic extract of Corrigiola telephifolia roots showed hepatoprotective potentiality through improving the blood biochemical parameters as well as enhancement of the hepatic tissues in CCl₄ induced hepatotoxicity in mice (Doudach et al., 2022). Liver enzymes in rats treated with the methanol extract of Cajanus cajan at 100 and 200 mg/kg BW showed lower levels of liver enzymes compared to CCl₄ treated group (Nwaechefu et al., 2022). The extract of C. cajan also reduced the inflammatory cell infiltration, hepatic fibrosis and necrosis and severe hepatocytes apoptosis induced by CCl₄ (Nwaechefu et al., 2022). The aqueous extract of Caralluma europaea stem showed hepatoprotective potentiality in CCl₄ induced hepatotoxicity rats (Ouassou et al., 2021). The ethanolic flower extract of Salix subserata reduced the liver enzymes in CCl₄ treated rats and also ameliorated the negative impact of CCl₄ on liver tissues (Wahid et al., 2016). Naringenin isolated from Citrus sinensis ameliorated the increased levels of liver enzymes induced by CCl₄ as well as attenuated the pathological changes in liver tissues. Naringenin also enhanced the expression of Bcl-2 (Ammar et al., 2022). CCl₄ induced hepatotoxicity in rats through destruction of the liver tissues. The administration with the alkaloid fraction of the Vitex doniana showed protective effect in rats through improving various blood parameters and also restoration of the normal appearance of the destructive liver tissues induced by CCl₄ (Ayoka et al., 2022).

Desert plants that are growing wildly in the kingdom of Saudi Arabia (KSA); have remarkable medicinal and commercial value to local communities (Aati et al., 2019). Among these plants, Mesembryanthemum forsskallii (family Aizoaceae) which has multiseed and is considered a rich source of natural products and bioactive secondary metabolites that have not been well exploited until now (Moawad et al., 2016; El-Amier et al., 2021). It was reported that M. forsskallii has more than 14 unsaturated fatty acids representing about 5.6% dominated by linoleic and oleic acids and also saturated fatty acids dominated by palmitic acid (Bilel et al., 2020). In addition to the fatty acids, significant amounts of carbohydrates, protein, and amino acids were also detected in seeds of M. forsskallii (Al-Jassir et al., 1995). In the northern part of KSA, the seeds of M. forsskallii are used as a traditional food for the public due to its high content of carbohydrates, protein, and fats (Najib et al., 2004; Abdel-Hamid et al., 2021).

Seeds oil extract of M. forsskallii showed antifungal activity against different fungal strains such as Penicillium chrysogenum, P. silicacinus, P. oxalicum, Fusarium oxysporum, and Aspergillus fumigatus, A. niger, A. flavus, A. carneus, Alternaria alternata, Rhizopus oryzae, Cladosporium cladosporioides and Paecilomyces lilacinus (Bilel et al., 2020). The effect of seeds oil extract of M. forsskallii was more prominent on P. chrysogenum, A. fumigatus, A. flavus and A. carneus (Bilel et al., 2020). Sanh seeds diets decreased the lipid peroxidation in streptozotocin (STZ)-induced diabetic of Wistar Albino rats (Al-Faris et al., 2010). The nanoparticle prepared using M. forsskallii seeds showed antimicrobial activity against Gram positive bacteria (Staphylococcus aureus) and Gram-negative bacteria (Pseudomonas aeruginosa and Escherichia coli) as well as antifungal activity against Candida albicans. The nanoparticles also showed anticancer activity against LoVo cancer cell lines with low IC₅₀ (28.3 µg/ml) (Aabed and Mohammed, 2021). The biological activity of one species of Mesembryanthemum (M. nodiflorum) showed an analgesic effect and significant inhibitory activity against colon, cervix, liver and melanocyte carcinoma. Moreover, the ethanolic extract of the aerial parts of M. nodiflorum had a significant hypoglycemic effect, anti-oxidant, anti-inflammatory and hepatoprotective activity (El-Hawary et al., 2020). Some of the secondary metabolites of M. forsskallii such as flavonoids, alkaloids, saponins, and phenolics are proven to act as potent antioxidant agents (Bilel et al., 2020; El-Amier et al., 2021). Hence, it may contribute individually or in combination (synergistic effect) to protective capability against liver toxicity. Many researchers demonstrated that drugs of herbal origin may have potential antioxidant activities which may contribute to the protective action of liver toxicity if added as supplementary medications (Hamzawy et al., 2015; Singh et al., 2016). So far, there is no previous study had evaluated the phytochemical and biological activity of M. forsskallii. Accordingly, the main objective of our study is to evaluate the hepatoprotective potency of the aqueous methanol extract of M. forsskallii.
Hepato-Protective Activity of *Mesembryanthemum forsskalii* Fruits

**MATERIALS AND METHODS**

Plant collection and extract preparation

*M. forsskalii* was collected from Al-Adare region in the Al Jouf district in the Northern part of KSA. Fruits were separated from the rest of the plant, dried at room temperature, and grounded into powder using an electrical grinder. Powdered materials were macerated in aqueous methanol until exhaustion for 24h. The solvent was evaporated under vacuum at 45°C using a rotary evaporator.

Carbon tetrachloride (*CCl*_4) preparation

*CCl*_4 of 100% concentration and olive oil were obtained from Algomhoria Company, Egypt. *CCl*_4 dissolved in olive oil was given by intraperitoneal (i.p) injection in a dose of 0.8 ml/Kg body weight twice a week for three weeks.

**Experimental design**

Based on the LD50 value, low and high doses of *M. forsskalii* extract were used in the study as a 100 and 500 mg/kg BW, respectively. Six experimental groups of mice (6 mice/group) were divided as follows: Group 1 (Gp1): Mice were administered with 200 µl of olive oil and served as vehicle control; Gp2: mice were injected intraperitoneal (i.p) with *CCl*_4 as 0.8 ml/kg BW twice a week for three weeks (3 days intervals); Gp3: mice had administered orally with *M. forsskalii* extract as 100 mg/kg BW, 7 times/15 day (every other day); Gp4: mice had injected with *CCl*_4 as in Gp2, then followed by administration with *M. forsskalii* extract as in Gp3 (100 mg/kg BW); Gp5: mice had administered with *M. forsskalii* extract as 500 mg/kg BW (7 times/15 day, day after day) and Gp6: mice had injected i.p with *CCl*_4 as in Gp2, then followed by administration with *M. forsskalii* extract as in Gp5.

**Determination of the total body weights and liver relative weight**

The body weight of mice was assessed at the beginning of the experiment and at the end of the experiment after treatment with *CCl*_4 and/or *M. forsskalii* extract. The significant difference between the body weight before and after treatment was assessed. After decapitation, livers were removed, dried by blotting paper, and weighed on a digital balance. To calculate the relative liver weight, the liver weight was divided by the total body weight x 100.

**Biochemical analysis**

Twenty-four hours after the last treatment, mice were sacrificed. The blood was collected from the orbital plexus in heparinized glass tubes from each mouse, allowed to stand for 30 min at room temperature, and centrifuged at 3000 rpm for 15 min to separate the blood serum samples. The separated serum was used for the estimation of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) using the protocol described by Ahmadi et al. (2011) and Habibi et al. (2015). Total serum protein was determined by the method of Biuret (Perez Gutierrez et al., 2011). Albumin was determined using the method of Webster (Webster, 1977). The contents of cholesterol and triglycerides were also determined using commercial kits. All the above estimations were performed using standard autopak kits using a spectrophotometer.

**Histological examinations**

Liver tissues were sliced into small pieces and then immersed in neutral formalin buffered (10%) for 24h. The fixed tissues were processed routinely, embedded in paraffin (to get paraffin sections 4-5 µm), sectioned, deparaffinized, and dehydrated using the standard techniques (Suvarna et al., 2018). Sections were then stained with hematoxylin-eosin and studied for histopathological changes. During the microscopic examination representative photos were captured (Nikon TE 2000-U microscope (NIKON, Tokyo, Japan)).

**Immunohistochemical studies**

Representative sections of the formalin-fixed, paraffin-embed liver tissue from different groups were used for P53 and Bcl-2 expression investigation. Tissues were deparaffinized in xylene and rehydrated in descending ethanol series. Antigen retrieval was accomplished through microwave irradiation of the sections in 10 mM sodium citrate buffer. After microwave antigen repair, the sections
were incubated at 4°C overnight with rabbit anti-P53 and anti-Bcl-2 antibody (1:100, dilution), followed by incubation at 37°C in PV6001 for 30 min. The bound immune complexes were developed by the addition of 3, 3'-diaminobenzidine tetrahydrochloride (DAB) substrate and the nuclei were stained with hematoxylin (Sigma-Aldrich). The sections were incubated with a normal goat serum as a negative control. Samples were viewed by using Nikon TE 2000-U microscope (NIKON, Tokyo, Japan). Immunohistochemical positivity was evaluated by proportion and staining intensity for each protein.

**Statistical analysis**

Analysis of variance (ANOVA) using Minitab (ver. 12.21) was used to evaluate the significant difference in blood parameters in control and treated groups of mice. ANOVA was also used to assess the difference between the relative body weight before and after treatment and also the relative liver weight in the control and treated groups.

**RESULTS**

*LD* 50 of M. forsskalii fruit extract

The estimation of the median lethal dose (*LD* 50) in the experimental animals was carried out according to Finney, 1985. The result showed that the *LD* 50 of *M. forsskalii* extract was 3.625 g/kg BW (Table I).

<p>| Table I. Determination of <em>LD</em> 50 of Mesembryanthemum forsskalii on albino Swiss mice. |</p>
<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Dose</th>
<th>No. of dead mice</th>
<th>Z_d</th>
<th>Z^d</th>
<th>% of mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6</td>
<td>5 g /kg wt.</td>
<td>6</td>
<td>100 %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>6</td>
<td>4.5 g /kg wt.</td>
<td>4</td>
<td>5</td>
<td>0.5</td>
<td>2.5</td>
</tr>
<tr>
<td>3</td>
<td>6</td>
<td>4 g /kg wt.</td>
<td>4</td>
<td>0.5</td>
<td>2</td>
<td>66.7 %</td>
</tr>
<tr>
<td>4</td>
<td>6</td>
<td>3.5 g /kg wt.</td>
<td>4</td>
<td>0.5</td>
<td>2</td>
<td>66.7 %</td>
</tr>
<tr>
<td>5</td>
<td>6</td>
<td>3 g /kg wt.</td>
<td>3</td>
<td>3.5</td>
<td>0.5</td>
<td>1.75</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>8.25</td>
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</table>

**Effect of *M. forsskalii* extract on the body weight and relative liver weight**

The total body weight of mice was reduced significantly after CCl 4 treatment. The weight was not significantly changed after treatment with either *M. forsskalii* alone or after CCl 4 followed by 100 mg/kg BW from *M. forsskalii* extract (Gp5) and increased significantly after CCl 4 followed by 500 mg/kg BW from *M. forsskalii* extract (Gp6) (Table II).

The relative liver weight of mice was increased significantly after CCl 4 treatment (Gp2) compared to vehicle control (Gp1). The reduction of relative liver weight was observed after treatment with CCl 4 followed by *M. forsskalii* extract (either 100 or 500 mg/kg BW) (Gp5 and Gp6). Low or high doses of *M. forsskalii* after CCl 4 treatment (Gp5 and Gp6) reduced liver weight observed in treated mice (Gp2). There was no significant change between the relative weights of vehicle control (Gp1) and that of CCl 4 followed by 100 or 500 mg/kg BW (Gp5 and Gp6) (Table II).

**Biochemical changes**

Treatment of the animals with CCl 4 (Gp2) resulted in a significant increase in ALT compared to the vehicle control (Gp1). Both low dose (100 mg/kg BW) (Gp5) and high dose (500 mg/kg BW) (Gp6) significantly reduced the levels of ALT in the treated mice compared to the CCl 4 treated mice (Gp2) (p<0.05). The reduction of the ALT content was more prominent after treatment with the low dose (100 mg/kg BW). No significant difference was observed in the animal group which received 100 mg/kg BW alone (Gp3) and the vehicle control group (Gp1). But the treatment of mice with 500 mg/kg BW alone (Gp4) significantly increased the content of ALT compared to the vehicle group (Gp1) (Table III).

CCl 4 administration to the mice increased AST levels (Gp2) when compared to the vehicle group (Gp1). Treatment of mice with a high dose (500 mg/kg BW) after CCl 4 treatment (Gp6), enhanced the AST content, whereas treatment with a low dose (100 mg/kg BW) (Gp5) reduced the AST content in the serum comparing to the CCl 4 treated group (Gp2) to a level almost normal as indicated by that of the vehicle control group (Gp1) indicating that low dose of *M. forsskalii* has improved the liver enzymes.

Although *M. forsskalii* extract at any dose has not significantly changed the albumin and the total protein levels, protein content was elevated after treatment with low or high doses (Gp5 and Gp6) compared to that of CCl 4 treated group (Gp2). Albumin content also was elevated after the administration of 500 mg/kg BW from *M. forsskalii* (Gp6) but the elevation of the content was not significant (Table III).

Even the CCl 4 treated mice (Gp2) showed lower cholesterol content than vehicle control (Gp1), the content of cholesterol in blood serum showed a significant increase after treatment with *M. forsskalii* either with low or high doses (Gp5 and Gp6), respectively comparing to CCl 4 treated group (Gp2) (Table III).

A high dose of *M. forsskalii* (Gp6) had significantly increased the content of triglycerides compared to CCl 4 treated group (Gp2), whereas a low dose (Gp5) elevated the content of triglycerides compared to the CCl 4 treated group (Gp2) but the increase was not significant (Table III).
Table II. Effect of *M. forsskalii* fruits extract on the total body weight and relative liver weight of mice.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Gp-1</th>
<th>Gp-2</th>
<th>Gp-3</th>
<th>Gp-4</th>
<th>Gp-5</th>
<th>Gp-6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total body weight (g)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before treatment</td>
<td>33.4 ± 0.09</td>
<td>34.9 ± 2.3</td>
<td>30.7 ± 1.0</td>
<td>31.8 ± 1.0</td>
<td>34.5 ± 6.7</td>
<td>30.9 ± 4.9</td>
</tr>
<tr>
<td>After treatment</td>
<td>34.3 ± 1.7</td>
<td>33.7 ± 1.4</td>
<td>29.0 ± 1.6</td>
<td>33.6 ± 0.9</td>
<td>35.8 ± 7.1</td>
<td>32.0 ± 5.2*</td>
</tr>
<tr>
<td>Relative weight (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>4.8 ± 0.7</td>
<td>6.4 ± 1.1*</td>
<td>6.0 ± 0.2</td>
<td>5.6 ± 0.4</td>
<td>6.0 ± 0.3</td>
<td>5.1 ± 2.5</td>
</tr>
</tbody>
</table>

Data are expressed as means ± SD. Gp-1, control mice (negative control); Gp-2, mice that were injected with CCl₄ (positive control); Gp-3, mice were injected with 100 mg/kg BW of *O. forsskalii*; Gp-4, mice were injected with 500 mg/kg BW of *O. forsskalii*; Gp-5, mice were injected with 100 mg/kg BW after CCl₄ administration; and Gp-6, mice were injected with 500 mg/kg BW of *M. forsskalii* after CCl₄ administration. * Denote significant difference at p < 0.05. Difference was assessed between Gp1 vs. Gp2, Gp2 vs. Gp5, Gp2 vs. Gp6, Gp1 vs. Gp3 and Gp1 vs. Gp4.

Table III. Effect *M. forsskalii* fruits extract on biochemical parameters and liver function in CCl₄ induced hepatotoxicity in mice.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>ALT U/L</th>
<th>AST U/L</th>
<th>Protein</th>
<th>Albumin</th>
<th>Cholest.</th>
<th>Triglyc.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gp-1</td>
<td>Vehicle  (negative control)</td>
<td>32.5 ± 3.5</td>
<td>24.0 ± 5.5</td>
<td>4.12 ± 0.4</td>
<td>3.14 ± 0.26</td>
<td>61.2 ± 6.5</td>
<td>106 ± 20</td>
</tr>
<tr>
<td>Gp-2</td>
<td>CCl₄ (positive control)</td>
<td>60.0 ± 2.8*</td>
<td>39.5 ± 17.6</td>
<td>4.15 ± 0.06</td>
<td>3.14 ± 0.21</td>
<td>47.9 ± 6.4*</td>
<td>111 ± 24</td>
</tr>
<tr>
<td>Gp-3</td>
<td>Extract (100 mg/kg)</td>
<td>37.0 ± 4.2</td>
<td>33.5 ± 8.5</td>
<td>4.18 ± 0.19</td>
<td>3.09 ± 0.04</td>
<td>60.3 ± 7.7</td>
<td>123 ± 32</td>
</tr>
<tr>
<td>Gp-4</td>
<td>Extract (500 mg/kg)</td>
<td>47.0 ± 5.2*</td>
<td>46.0 ± 0.0’</td>
<td>4.76 ± 0.09</td>
<td>3.46 ± 0.09’</td>
<td>62.7 ± 14.4</td>
<td>171 ± 13’</td>
</tr>
<tr>
<td>Gp-5</td>
<td>CCl₄ + Extract (100 mg/kg)</td>
<td>40.0 ± 5.5’</td>
<td>26.5 ± 5.5</td>
<td>4.18 ± 0.16</td>
<td>3.05 ± 0.08</td>
<td>56.9 ± 5.0’</td>
<td>139 ± 24</td>
</tr>
<tr>
<td>Gp-6</td>
<td>CCl₄ + Extract (500 mg/kg)</td>
<td>41.5 ± 3.5’</td>
<td>40.5 ± 5.3</td>
<td>4.67 ± 0.41</td>
<td>3.41 ± 0.18</td>
<td>84.6 ± 9.1*</td>
<td>179 ± 21*</td>
</tr>
</tbody>
</table>

ALT, alanine aminotransferase; AST, aspartate aminotransferase; Cholest., cholesterol; Triglyc., triglycerides. Protein and albumin were expressed as g/dl, cholesterol and triglycerides were expressed as mg/100 ml. For other abbreviations, details of groups and statistical details see Table II.

From the previous results, it is clear that treating mice with high doses of *M. forsskalii* (500 mg/kg BW) had a negative effect on liver enzyme function either in control mice or the mice treated with CCl₄. Treated mice with low doses either alone or post CCl₄ treatment had significantly improved the liver enzyme’s function and other blood parameters.

**Histopathological changes**

Liver tissue sections were either stained with H and E (Fig. 1) or immune stained for the detection of the expression levels of the cell cycle regulator and tumor suppressor protein, P53 (Fig. 2), and the expression levels of the anti-apoptotic Bcl-2 (Fig. 3). The histological observations of liver tissues support the results obtained from serum enzyme assays. Negative control tissue sections exhibited no apparent pathological alterations (Fig. 1A). No cavitation, necrosis, or fibrosis was found in control sections. In contrast, sections from CCl₄-only treated mice displayed apparent cavitation in broad areas, necrosis with inflammation, and loss of cellular boundaries (Fig. 1B). On the other hand, liver sections treated with low and high dose of *M. forsskalii* extract (100mg and 500mg/kg BW, respectively) separately showed no marked variation in the histological architecture of the liver tissue (Fig. 1C, D). Interestingly, the broad cavitation in the liver was attenuated in mice treated with *M. forsskalii* extract during the experimental periods (Fig. 1E, F). The administration of *M. forsskalii* extract resulted in less cavitation in the liver. Importantly, the remaining cavitation level and the range of the necrotic cells in the 100 mg/kg BW-treated group (Fig. 1E) were lower than that in the 500 mg/kg BW-treated group (Fig. 1F).

Liver sections prepared from *M. forsskalii* -treated groups displayed less fibrosis as compared to the CCl₄-only control group. This group’s liver sections showed regeneration of hepatocytes, almost toward near-normal liver architecture and possessing higher hepatoprotective action. The improvement in the 100 mg/kg group (Fig. 1E) was more obvious in comparison to that of the 500 mg/kg group (Fig. 1F). We also examined the distribution of fibrosis in different liver regions (from the central vein region to hepatic portal veins). Both doses of the extract reduced apparent liver injury caused by CCl₄ compared to the group of mice that were treated with CCl₄ only.

The immunostaining study revealed that CCl₄ enhanced the expression of the cell cycle regulator and necro-apoptotic driver, P53 in the liver tissues (Fig. 2B). However, this expression was relatively reduced after treatment with the extract from *M. forsskalii* at both doses.
Fig. 1. Histopathology of liver tissues. (A) Liver section of normal negative control mice (vehicle) shows central vein surrounded by hepatic cord of cells (normal architecture), (B) liver section of CCl₄-treated mice showing massive fatty changes, focal central vein congestion, a variety of cavitations and necrosis in hepatocytes with inflammation, and loss of cellular boundaries (indicated by arrow), (C) liver section of mice treated with 100mg/kg showing normal liver architecture (magnification 5X), (D) liver section of mice treated with the 500mg/kg showing normal liver architecture, (E) liver section of mice treated with CCl₄ and 100mg/kg of *M. forsskalii* showing absence of cavitations, necrosis, and inflammatory cells, and regeneration of hepatocytes around central vein toward near normal liver architecture but slight congestion in central vein (indicated by arrow), and (F) liver section of mice treated with CCl₄ and 500 mg/kg of *M. forsskalii* showing mild central vein congestion (indicated by arrow), ballooning, and necrosis with sinusoidal dilatation H and E x 50.

Fig. 2. Immunohistochemistry of P53 in the liver tissues. (A) Section from a negative control mice liver shows the normal pattern of P53 with mild staining. (B) The liver section obtained from CCl₄-intoxicated mice shows extensive staining (indicated by brown color) for P53. (C) and (D) a liver section from normal mice treated at low and high doses, respectively, showing a non-appreciated difference in P53 staining pattern (magnification, x 50 for the main photo and x 100 for the hyper-focused region in the small box at the left bottom of each panel). (E) and (F) Liver tissue sections prepared from the 100 mg/kg and 500mg/kg of *M. forsskalii* after CCl₄ treatment, respectively, show less P53 staining compared to (B), however (E) shows a closer staining pattern to the normal liver (A).
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(Fig. 2E, F) but the effect was more prominent when the extract was administered at a low dose (Fig. 2E). There was no appreciated change in the expression level in P53 under the extract treatment when administered to normal animals as represented in (Fig. 2C, D) if compared to the negative control (Fig. 2A). On the other side, the expression level of the tumor-promoting and cytoprotective molecule, Bcl-2, was significantly enhanced in the liver section of animals challenged by CCI\(_4\) (Fig. 3B). Interestingly, this elevated expression had been reverted after treatment with the plant extract at both doses (Fig. 3E, F). Similarly, the expression of Bcl-2 in the liver from normal animals did not show detectable changes before and after the administration of the extract neither at a low dose nor a higher one (Fig. 3C, D) when compared to the negative control (Fig. 3A).

**DISCUSSION**

The change in dietary habits as well as the chemoprevention show considerable effective strategies against oxidative stress and are the main focus of the area of research these days (Lee and Park, 2003). CCI\(_4\) is a known, reliable, and commonly used chemical to induce liver damage through oxidative degradation in the adipose tissue which resulted in fatty infiltration of the hepatocytes (Pal *et al*., 2014; Wang *et al*., 2019; Begum *et al*., 2022) and increased permeability, acute toxicity and may be hepatic necrosis (Naji *et al*., 2017). Increasing relative liver weight and the level of liver enzymes such as AST and ALT in the serum is the first clue to liver toxicity (Tsai *et al*., 2009; Bahashwan *et al*., 2015). The current investigation was undertaken to evaluate the possible protective effect of *M. forsskalii* extract against carbon tetrachloride-induced hepatotoxicity and oxidative stress in mice. CCI\(_4\) causes acute hepatocyte injuries and altered membrane integrity and as a result enzymes in hepatocytes leak out (Hu *et al*., 2000). However, after treatment with *M. forsskalii*, the pathological increases in AST and ALT were significantly restored at least after treatment with a low dose. These results indicate that *M. forsskalii* can protect against CCI\(_4\)-induced hepatocyte injuries. Extract from *M. forsskalii* fruits showed the protective capability against treatment through the restoration of the liver enzymes level to their normal values and restoration of the normal relative weights. The protective potentiality of *M. forsskalii* against CCI\(_4\)-induced hepatotoxicity may be attributed to the presence of some secondary metabolites well known as an antioxidant and radical scavenging agents such as flavonoids, saponins, and phenolics.

Many plants are used as hepatoprotective natural sources against CCI\(_4\)-induced hepatotoxicity. For instance, the aqueous ethanol extract of the pods of *Acacia senegal*

![Fig. 3. Immunohistochemistry of the cytoprotective molecule, Bcl-2, in the liver tissues.](image-url)
was used efficiently as a hepatoprotective agent against hepatotoxicity induced by CCl4 in rats through the restoration of the blood serum enzymes and bilirubin and also restoration of the architecture of the liver through the presence of normal hepatic cords, absence of necrosis and fatty infiltration through histology of liver sections (Pal et al., 2014; Azubuike et al., 2018). It was reported that leaves extract of Pyrenacantha staudtii has a protective effect against CCl4 induced liver toxicity and damage, as well as CCl4 induced elevations in the liver enzymes were significantly reduced by 750 mg/kg and 1500 mg/kg BW of the plant extract (Anosike et al., 2008). As well as (Ouassou et al., 2021) found that Caralluma europaea stem extract (250 mg/kg BW) had a significant hepatoprotective effect by ameliorating CCl4-induced alterations of some biochemical parameters.

In line with the results of the current study, the blood parameters were improved in CCl4 induced animals after administration of different plant extracts such the flowers extract of S. subserrata (Wahid et al., 2016), roots extract of C. telephifolia (Doudach et al., 2022) and the extract of C. cajan (Nwaechefu et al., 2022). In accordance with our results, the inflammatory cell infiltration, hepatic fibrosis, necrosis and severe hepatocytes apoptosis induced by CCl4 were ameliorated by the extract of C. cajan (Nwaechefu et al., 2022). Administration of S. subserrata extract ameliorated the negative impact of CCl4 on liver tissues, where the appearance of the liver tissues was restored to the normal appearance post-administration of S. subserrata extract (Wahid et al., 2016). Naringenin from C. sinensis ameliorated the negative impact of CCl4 on liver tissue appearance. Moreover, it enhanced the expression of Bcl-2 (Ammar et al., 2022). The alkaloids extract of V. doniana restored the normal appearance of the destructive liver tissues induced by CCl4 (Ayoka et al., 2022).

The lower level of cholesterol in CCl4 treated mice than that of normal (vehicle control) may be attributed to loss of an animal’s appetite after CCl4 treatment compared to other groups which could be concluded from the amounts of food remaining in the CCl4 treatment group comparing to other groups. It seems that animals hardly eat after treatment with CCl4. It was reported that feed intake was significantly reduced after CCl4 administration compared with the constant feed intake by the control group (Uemitsu and Nakayoshi, 1984). The effect of decreasing cholesterol and the nonsignificant changes in the content of triglycerides, protein, and albumin in CCl4 treated mice may also be attributed to the decrease in the number of hepatocytes due to liver damage by CCl4 and consequently decrease in the liver capacity to synthesize these metabolites (Bhandarkar and Khan, 2003). The significant increase in cholesterol and triglycerides after treatment with either high or low doses from M. forsskalii indicates the preliminary evidence for the protective role of M. forsskalii. This may refer to the restoring of the liver ability to synthesize these metabolites after its retardation by the CCl4 effect.

It is well established that CCl4 induces liver damage through necrosis. Immunostaining results from our study showed that CCl4 alone induced relative up-regulation of P53 and down-regulation in the expression of Bcl-2 in the liver tissues. Importantly, unlike high dose, administration of the M. forsskalii at a low dose significantly restored this effect by the downregulation of P53 and upregulation of Bcl-2 expression. CCl4 is reported to induce hepatic damage as a result of metabolic conversion of the radicals through lipid peroxidation and disturbance of the activities of the antioxidant enzymes (Adesanoye and Farombi, 2010), induce oxidative stress, and cause liver injury by the formation of free radicals (Manna et al., 2006). On the other hand, carbon tetrachloride causes noticeable toxicity by enhancing liver lipid peroxidation as found by increased concentrations of hepatic malondialdehyde (Poli, 1993; Dalton et al., 2009). Results from the current study have not included any evidences for the antioxidant activity of the M. forsskalii extract, the antioxidant activity of each fraction from the same fruits should be evaluated.

In conclusion, the administration of a low dose of M. forsskalii (100 mg/kg BW) improved the liver function through restoration of liver enzymes, reducing P53 expression, and increasing the Bcl-2 expression to the normal levels after CCl4 treatment. However, 500 mg/kg BW prominently showed no beneficial effect after CCl4 induced toxicity except enhancing Bcl-2 expression; even it exerts some toxic effect on the liver enzymes in mice.

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IRB approval

Jouf University Institutional Animal Ethics Committee approved the protocol under the number: JU-49/2014.

Ethical statement

All procedures were conducted under ethical guidelines of animal care and approved by the animal care and use committee of at Jouf University, Saudi Arabia. We
took all the possible procedures to reduce mice sufferance.

Statement of conflict of interest
The authors declare no competing interests.

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