Study on hepatoprotective effect of *Moringa oleifera* Lam. leaves to prevent hepatocyte lesion induced by CCl₄ in the HepG2 cell line

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Abstract

*Moringa oleifera* Lam. is rich of strong antioxidant reagents, especially isoquercitrin. The present study was an attempt to prove hepatoprotective ability of isoquercitrin (IQ), ethyl acetate (EM) and 70% alcoholic extracts (AM) from *M. oleifera* leaves against CCl₄ induced hepatotoxicity in HepG2 cells. Cells were treated with 3 different samples, including IQ, EM or AM at concentrations corresponding to that of isoquercitrin at 2.5, 5, 10 µg/ml (C1, C2 and C3, respectively) alone or combined with 2 mM CCl₄ for 24h. Hepatoprotective effect was evaluated through assays including cell proliferation, lipid accumulation, necrosis, oxidative stress and apoptosis activation. Results showed that CCl₄ reduced 23.2% of HepG2 cells viability. Anti-proliferative effect was prevented in presence of IQ, EM, AM at 3 tested concentrations. Lipid accumulation was increased 53.5% due to CCl₄; however, IQ and EM at 3 tested concentrations, AM at C1 attenuated this situation. CCl₄ decreased 35.7% GSH content in cells; 3 samples enhanced GSH levels at different concentrations: IQ (C2, C3); EM (C1, C2, C3) and AM (C2, C3). Besides, CCl₄ increased 21.8% of lactate dehydrogenase, but its necrotic effect was decreased in presence of 3 samples at all concentrations. For apoptosis, CCl₄ increased 68.5% DNA fragmentation. This parameter was decreased by most concentrations of IQ, EM and AM. In sum, *M. oleifera* Lam. leaf extracts were shown to prevent HepG2 cells injury induced by CCl₄ treatment for 24h: hepatoprotective effect of IQ was better than that of EM and AM, especially at the concentration of 5-10 µg/ml.

Keyword: HepG2; in vitro; Hepatoprotective; CCl₄; *Moringa oleifera* Lam. leaves

1. INTRODUCTION

Liver is the second largest organ that plays important roles in the metabolism of nutrients and detoxification of xenobiotics as well as drugs. Due to that central functional, it is especially susceptible to reactive oxygen species (ROS) which can reduce cell defense capacity and establish oxidative stress. Cell damage arising from this process may result in inflammation and fibrosis, even cancer and necrosis ¹,².

Carbon tetrachloride (CCl₄) is widely used for induce oxidative stress and liver injury, being extensively used in the evaluation of therapeutic potential of drugs and dietary antioxidants using HepG2 cells³-⁶. This xenobiotic is undergone biotransformation by the cytochrome P450 system into a trichloromethyl free radical (CCl₃⁻) and transformation into a highly reactive trichloromethylperoxy free radical (CCl₃OO⁻), impairing critical cellular events such as lipid peroxidation and DNA damage⁷,⁸. It further damages liver cell membranes and organelles and causes swelling, necrosis of hepatocytes and the release of cytosolic enzymes, aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH), into serum and eventually kills cells⁹-¹¹.

Nowadays, researches of plant-based antioxidants that retard the process of oxidative damage but with low cytotoxicity have been
increasing. *Moringa oleifera* Lam., a tree of Moringaceae family, is a native of India and now extensively distributed throughout many southeastern Asian countries particularly in Thailand, Philippines and Vietnam etc. Traditionally, almost every part of *M. oleifera* has some nutritional, medicinal and other valuable properties. The leaves are widely utilized as a good alternative source of protein, calcium, vitamin A, C and E, β-carotene, amino acids, various polyphenolics and some natural anti-oxidizing agents. Apart from nutritional benefits, *M. oleifera* has been extensively used for the following applications: anti-tumor, anti-inflammatory, antibacterial, antidyssipidemia and especially hepatoprotective as a strong antioxidant activity \(^1\). Hence, the present study was carried out in an attempt to evaluate the hepatoprotective activities of 70% alcoholic extract, ethyl acetate extract of *M. oleifera* leaves and isoquercitrin in CCl\(_4\)-injured HepG2 cell line.

2. MATERIALS AND METHODS

2.1 Chemicals and reagents

Eagle’s Minimum Essential Medium (EMEM), fetal calf serum (FCS), trypsin-EDTA were purchased from Gibco, USA; L-glutamine, penicillin-streptomycine, phosphate buffer, trypsin blue, ethanol, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT), O Red oil, Brilliant G250, L-lactate dehydrogenase (LDH), carbon tetrachloride (CCl\(_4\)), glutathione (GSH), Ellman, isoquercitrin (IQ), quercetin (Q) from Sigma-Aldrich, USA; LDH detection kit from Roche, Germany; diphenylamine, from Merck, Germany. Other chemicals met cell culture standards.

2.2 Plant extracts

The 70% alcoholic (AM) and ethyl acetate (EM) extracts of *M. oleifera* leaves (harvested at An Giang, Vietnam) were standardized and provided by Ginseng and Pharmacognosy Center at Ho Chi Minh City. Isoquercitrin content was measured by UV spectrophotometric method at 2.41% and 14.09% for AM and EM respectively; moisturize content by infrared moisture balance at 19.50% and 16.18% for AM and EM respectively.

2.3 Cell culture

Human liver hepatoma cells (HepG2) (ATCC HB8065\(^2\)) were seeded and cultured in EMEM containing 10% FCS, 2 mM L-glutamine, 100 IU/ml penicillin, 100 µg/ml streptomycin in an humidified atmosphere of 5% CO\(_2\) at 37°C to attain confluency. Cells then were trypsinized, harvested and counted using trypsin blue, and seeded in 6 or 96-well-plates at 2.5 x 10\(^4\) cells/cm\(^2\).

2.4 Cell treatment

Cells were divided into the following groups of culture condition:

**Physiological control:** Cells were treated with EMEM containing DMSO (0.25%, v/v) for 24 h.

**Pathological control:** Cells were treated with EMEM containing DMSO (0.25%, v/v) and 2 mM CCl\(_4\) for 24h.

**Sample control:** Cells were treated with EMEM containing DMSO (0.25%, v/v) and AM or EM or IQ at concentrations corresponding to that of isoquercitrin at 2.5, 5, 10 µg/ml (C1, C2 and C3, respectively) for 24 h.

**Quercetin control:** Cells were treated with EMEM containing DMSO (0.25%, v/v) and 10 µM quercetin for 24 h.

**Sample treatment:** Cells were treated with EMEM containing DMSO (0.25%, v/v), 2 mM CCl\(_4\) and AM or EM or IQ at concentrations of C1, C2, C3 for 24 h.

**Quercetin treatment:** Cells were treated with EMEM containing DMSO (0.25%, v/v), 2 mM CCl\(_4\) and 10 µM quercetin for 24 h.

Tested concentrations of *M. oleifera* leaf extracts and isoquercitrin were presented in Table 1.

2.5 Cell viability assay

Cell viability was evaluated as mitochondrial succinate dehydrogenase (SDH) activity, a marker of viable cells using MTT test. Briefly, SDH activity was detected after 3h incubation in culture medium without serum containing MTT (3-4,5-dimethyl-thiazol-2-yl-2,5 diphenyl-tetrazolium bromide), which is
Table 1. Concentrations of M. oleifera leaf extracts and isoquercitrin

<table>
<thead>
<tr>
<th></th>
<th>70% alcoholic extract (AM)</th>
<th>Ethyl acetate extract (EM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isoquercitrin (% w/w)</td>
<td>2.41</td>
<td>14.09</td>
</tr>
<tr>
<td>Moisturize content (%)</td>
<td>19.50</td>
<td>16.18</td>
</tr>
</tbody>
</table>

**Final concentration in culture medium (µg/ml)**

<table>
<thead>
<tr>
<th></th>
<th>Isoquercitrin (IQ)</th>
<th>70% ethanolic extract (AM)</th>
<th>Ethyl acetate extract (EM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1</td>
<td>2.5</td>
<td>128.9</td>
<td>21.2</td>
</tr>
<tr>
<td>C2</td>
<td>5.0</td>
<td>257.7</td>
<td>42.3</td>
</tr>
<tr>
<td>C3</td>
<td>10.0</td>
<td>515.5</td>
<td>84.7</td>
</tr>
</tbody>
</table>

converted into formazan dissolved in acidified isopropanol. The produced purple solution was spectrophotometrically measured at 570 nm on microplate reader.15.

2.6 Intracellular lipid droplet accumulation - O red oil staining

To measure intracellular lipid droplet accumulation, HepG2 cells were stained by the O red oil method. Cells were rinsed with PBS and fixed with formalin 10%, rinsed with water followed by 60% isopropanol, stained with O red oil solution. Excess stain was removed by washing with water. The stained cells were observed under microscopy. Later, isopropanol 100% were added to dissolve stained oil droplets. The dye-triglyceride complex was spectrophotometrically measured at 500 nm.14.

2.7 Determination of glutathione content

Glutathione (GSH) is an antioxidant tripeptide in hepatocyte which can be decreased in the presence of ROS. Briefly, cell lysates in cold KCl 1.15% were homogenized with Tris-HCl 25 mM pH 7.4 (2:1, v/v), then incubated in 60 min at 37 °C. Later, mixture was added TCA 10% (1:1, v/v) then was centrifuged. 100 µl the supernatant was mixed with 180 µl EDTA-phosphate buffer and 20 µl 5,5'-dithio-bis 2-nitrobenzoic acid 5 mM (Ellman reagent). GSH levels were spectrophotometrically measured at 412 nm and deduced from a calibration curve and corrected from protein level of cell lysates. The protein content was also determined using BSA as standard.15 Results were expressed as µM GSH/mg protein.

2.8 Assay of cell necrosis

Extracellular lactate dehydrogenase (LDH) was considered as an index of cell necrosis. LDH activity was measured in culture medium, using “Cytotoxicity detection LDH” kit. LDH participates in a coupled reaction which converts a yellow tetrazolium salt into a red, formazan-class dye which was measured by absorbance at 492 nm. A LDH standard curve (0 - 1000 mU/mL) was used for quantification of enzyme activity.16

2.9 Acridine orange/ethidium bromide (AO/EB) fluorescence staining

Changes in cell morphology were evaluated using AO/EB fluorescence staining. Briefly, cells were stained with dye mixture containing 100 µg/ml acridine orange and 100 µg/ml ethidium bromide in PBS, and then washed with PBS. After staining, cells were visualized immediately under a fluorescence microscope.17

2.10 Determination of percentage (%) of DNA fragmentation

Briefly, DNA from the cells was released into the lysis buffer [0.2% Triton X-100, 10 mM Tris-HCl, and 10 mM EDTA (pH 8.0)] by rupturing the nucleus. The supernatant received
after centrifugation at 13000 rpm for 10 min at 4°C, contained fragmented DNA while the intact DNA was in the pellet. The amount of DNA in both the supernatant and pellet was estimated by diphenylamine (DPA) assay. The percentage of DNA fragmentation was calculated as the ratio of DNA in supernatant and DNA in pellet.

2.11 Detection of DNA fragmentation by agarose gel electrophoresis

Fragmented DNA was released into the lysis buffer (0.2% Triton X-100, 10 mM Tris-HCl, and 1 mM EDTA, pH 8.0) by rupturing the nucleus. DNA in the supernatant was extracted using a 25:24:1 (v/v/v) equal volume of phenol: chloroform: isoamyl alcohol. DNA was precipitated overnight with absolute ethanol and 300 mM NaCl, dissolved in TE buffer [10 mM Tris-HCl pH 8.0 and 1 mM EDTA containing RNase A (0.1 mg/ml)]. The samples were analyzed electrophoretically on 2% agarose gel containing 0.01% SYBR Green.

2.12 Evaluation of hepatoprotective effect

Hepatoprotective effect was evaluated via prevention percentage that means attenuation of hepatocyte injury induced by 2 mM CCl<sub>4</sub> and calculated as the following function.

\[
\text{Prevention percentage} = \frac{[(\text{CCl}_4 \text{ treatment} - \text{sample treatment})/\text{CCl}_4 \text{ treatment} - \text{physiological control}]}{\times 100%}
\]

2.13 Data analysis

Each assay was done twice or three times. The results were expressed as Mean + SEM of six culture of one representative experiment and statistically analyzed by Mann-Whitney test with SPSS 22.0 for Windows software. Threshold of significance was set at \( p < 0.05 \).

3. RESULTS

3.1 Hepatoprotective effect on the proliferation of HepG2 cells

After 24 hours, almost concentrations of AM, EM, IQ and 10 μM quercetin increased the percentage of viable cells \( (p < 0.05) \) (Table 2). Cell viability was found to decrease 23.2% by 2 mM CCl<sub>4</sub> treatment for 24h (Table 3). However, antiproliferative effect of CCl<sub>4</sub> significantly attenuated in the presence of all concentrations of AM, EM, IQ and 10 μM quercetin (Table 4).

<table>
<thead>
<tr>
<th>Extract/Concentration</th>
<th>Percent change (+ or -) compared to physiological control (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cell proliferation</td>
</tr>
<tr>
<td>Q</td>
<td>+1.35*</td>
</tr>
<tr>
<td>AM</td>
<td>+15.05*</td>
</tr>
<tr>
<td>C1</td>
<td>+30.15**</td>
</tr>
<tr>
<td>C2</td>
<td>+21.79*</td>
</tr>
<tr>
<td>C3</td>
<td>+32.57**</td>
</tr>
<tr>
<td>EM</td>
<td>+24.87**</td>
</tr>
<tr>
<td>C1</td>
<td>+30.49**</td>
</tr>
<tr>
<td>C2</td>
<td>+19.76*</td>
</tr>
<tr>
<td>C3</td>
<td>+25.72**</td>
</tr>
<tr>
<td>IQ</td>
<td>+26.39**</td>
</tr>
</tbody>
</table>

*: \( p < 0.05 \) and **: \( p < 0.01 \): compared to physiological control
Study on hepatoprotective effect of Moringa oleifera Lam. leaves to prevent hepatocyte lesion induced by CCl4 in the HepG2 cell line

3.2 Hepatoprotective effect against lipid accumulation in HepG2 cells

When HepG2 cell were treated alone with AM and EM, its intracellular lipid levels raised (Table 2). The same results were found in pathological control where 2 mM CCl4 treatment for 24h increased 53.5% intracellular lipid content in comparison with physiological control. However, in sample and quercetin treatments, all three concentrations of IQ and EM; AM at C1; 10 µM quercetin showed a decrease in lipid accumulation. Interestingly, this effect of IQ and EM on CCl4-injured HepG2 cells were insignificantly different from that of 10 µM quercetin. Microscopic images of O red oil-stained lipid droplet in HepG2 cells shown in Figure 1 confirmed the results found in Table 4.

### Table 3. Effect of 2 mM CCl4 on the proliferation, lipid accumulation, oxidative stress, necrosis and DNA fragmentation in HepG2 cells

<table>
<thead>
<tr>
<th>Extract/Concentration</th>
<th>Percent change (+ or -) compared to physiological control (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cell proliferation</td>
</tr>
<tr>
<td>Physiological control</td>
<td>-23.2**</td>
</tr>
<tr>
<td>Pathological control</td>
<td>-53.5**</td>
</tr>
</tbody>
</table>

**P < 0.01:** compared to physiological control

### Table 4. Hepatoprotective effect of 70% alcoholic extract (AM), ethyl acetate extract (EM), isoquercitrin (IQ) and quercetin (Q) against the antiproliferation, lipid accumulation, oxidative stress, cell necrosis and DNA fragmentation in HepG2 cells induced by 2 mM CCl4

<table>
<thead>
<tr>
<th>Extract/Concentration</th>
<th>Prevention percentage against CCl4-induced hepatotoxicity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Anti-proliferation</td>
</tr>
<tr>
<td>Q</td>
<td>91.3</td>
</tr>
<tr>
<td>AM</td>
<td></td>
</tr>
<tr>
<td>C1</td>
<td>251.9</td>
</tr>
<tr>
<td>C2</td>
<td>223.3</td>
</tr>
<tr>
<td>C3</td>
<td>111.4</td>
</tr>
<tr>
<td>EM</td>
<td></td>
</tr>
<tr>
<td>C1</td>
<td>126.0</td>
</tr>
<tr>
<td>C2</td>
<td>194.2</td>
</tr>
<tr>
<td>C3</td>
<td>111.2</td>
</tr>
<tr>
<td>IQ</td>
<td></td>
</tr>
<tr>
<td>C1</td>
<td>248.8</td>
</tr>
<tr>
<td>C2</td>
<td>205.6</td>
</tr>
<tr>
<td>C3</td>
<td>220.1</td>
</tr>
</tbody>
</table>

*: P < 0.05 and **: P < 0.01: compared to pathological control (CCl4)
@@: P < 0.05 and @@: P < 0.01: compared to quercetin treatment
Figure 1. Images (20X) of HepG2 cells treated with CCl₄, 70% alcoholic extract (AM), ethyl acetate extract (EM), isoquercitrin (IQ), quercetin alone or combined after O red oil staining (O red oil-stained-lipid droplet: arrow)
3.3 Hepatoprotective effect against oxidative stress in HepG2 cells

Glutathione (GSH) plays important role to neutralize oxidative species. After 24 hours of treatment, compared to physiological control, AM at C2, C3; EM at C3 and 10 μM quercetin significantly increased GSH content ($p < 0.05$), whereas AM at C1, IQ at 3 concentrations showed the reverse effect (Table 2). In pathological control, CCl$_4$ decreased 35.7% GSH content ($p < 0.05$) (Table 3). The presence of AM at C2, C3; EM at 3 concentrations; IQ at C2, C3 demonstrated prevention effect from oxidative stress by enhancing GSH content 50-200% in 2 mM CCl$_4$-injured HepG2 cells. Quercetin at 10 μM interestingly boosted the amount of GSH up to 514% in comparison with pathological control (Table 4).

3.4 Hepatoprotective effect against necrosis of HepG2 cells

Plasma membrane leakage from necrotic cells causes the release of intracellular contents into extracellular milieu. Hence, the activity of extracellular LDH reflects cell necrosis status. In sample control, IQ at C2, C3 reduced significantly the amount of LDH comparing to normal control (Table 2). CCl$_4$ evoked necrosis process in HepG2 cells, which released 21.8% LDH comparing to normal control (Table 3). Results in Table 4 showed that all concentrations of AM, EM, IQ prevented necrosis caused by CCl$_4$ in HepG2 ($p < 0.05$), even better than 10 μM quercetin did.

3.5 Hepatoprotective effect against apoptosis activation in HepG2 cells

The execution phase of apoptosis is characterized by DNA fragmentation and marked changes in cell morphology that include contraction and membrane blebbing. Those features of apoptotic cells were found in HepG2 cells treated to 2 mM CCl$_4$ more than in cells not treated (physiological control) or in cells treated to tested samples AM, EM and IQ (Figure 2).

![Physiological control](image1) ![Pathological control (CCl$_4$ 2 mM)](image2) ![CCl$_4$ 2 mM + isoquercitrin 5μg/ml](image3)

**Figure 2.** Hepatoprotective effect of isoquercitrin (5 μg/ml) against apoptosis activation induced by CCl$_4$ in HepG2 cells (DF: DNA fragmentation, MB: membrane blebbing)

The results of DNA fragmentation analysis were presented in Table 5. In sample control, AM and IQ at C1, EM at C2 and C3 as well as quercetin 10 μM increased the DNA fragmentation ($p < 0.05$) (Table 2). An increase of 68.5% DNA fragmentation was found in CCl$_4$ treated cells compared to control ($p < 0.05$) (Table 3). Incubation HepG2 cells with 2 mM CCl$_4$ in the presence of most concentrations of AM, EM and IQ prevented DNA from fragmentation ($p < 0.05$). Especially, the prevention effects of EM and IQ at C2, C3 were better than that of quercetin 10 μM (Table 4). The results were confirmed by agarose gel electrophoresis as shown in Figure 3.
4. DISCUSSION

The present study was an attempt to prove hepatoprotective ability of isoquercitrin (IQ), ethyl acetate (EM) and 70% alcoholic extracts (AM) from *M. oleifera* leaf against CCl$_4$ induced hepatocyte lesion in HepG2 cells. Cells were treated with 3 different samples, including IQ, EM or AM from *M. oleifera* leaves at concentrations corresponding to that of isoquercitrin at 2.5, 5, 10 µg/ml alone or combined with 2 mM CCl$_4$ for 24h. Hepatoprotective effect was evaluated through several assays including cell proliferation, intracellular lipid accumulation, necrosis, oxidative stress and apoptosis activation.

After 24 hours, CCl$_4$ decreased 23.2% cell viability and 35.7% GSH content, on the other hand, increased 53.5% lipid accumulation, 21.8% extracellular LDH activity and 68.5% DNA fragmentation. The results were in agreement with previous study $^{19}$. CCl$_4$'s biotransformation compounds, CCl$_3$• and CCl$_3$OO•, are capable of binding to proteins and other macromolecules of the lipid membranes with simultaneous attack on polyunsaturated fatty acids to produce lipid peroxidation $^{20}$. As a result, fats from the adipose tissue are translocated and accumulated in the hepatocytes $^{21}$. GSH, a key component of the overall antioxidant defense system, decreased in the CCl$_4$ treated group might be due to its utilization by the excessively generated quantity of free radicals in the hepatocytes $^{22}$. When the GSH stores were markedly in deplete state, necrosis begins, causing significant increase in the activities of LDH $^{23}$. The oxidative stress triggered by CCl$_4$ also led to apoptotic death as noted herein the morphological analysis of HepG2 cells exposed to these xenobiotics $^{24}$. 

Figure 3. Images of DNA fragmentation using agarose gel electrophoresis. Upper: sample control and quercetin control; Lower: sample treatment and quercetin treatment. IQ1, IQ2, IQ3: isoquercitrin at C1, C2, C3; EC1, EC2, EC3: EM at C1, C2, C3; CC1, CC2, CC3: AM at C1, C2, C3
Those changes in CCl₄-injured HepG2 cells were shown to be minimal or absent with 10 μM quercetin treatment or AM, EM and IQ, dose-dependently. This might be due to lower fat accumulation and re-establishment of the antioxidant defense system in the liver tissue through the antioxidant and hepatoprotective effects of quercetin and M. oleifera leaf extracts. Quercetin was known as a strong antioxidant compound possessing hepatoprotective effect 25,26.

Isoquercitrin, a compound in EM, AM, IQ, was proved to regulate AMPK activation, thereby enhancing AdipoR expression, suppressing SREBP-1 and FAS expressions. AdipoRs and AMPK signaling suppress fatty acid synthesis by inactivating ACC and FAS and stimulate fatty acid oxidation. SREBP-1, a key lipogenic transcription factor, plays a major role in regulating hepatic lipid metabolism, including lipolysis and fatty acid synthesis. Inhibition of SREBP-1 expression by activating AMPK reduces lipid accumulation in hepatocytes and resulting in the regulation of lipid accumulation 27.

In other study, 80% ethanolic extract of Moringa oleifera leaves showed the ability to downregulate the expression of the HMG-CoAR, PPARα1, and PPARγ genes of HepG2 cells, hereby inhibited lipid synthesis 28.

GSH is a tripeptide acting as an antioxidant. The intracellular GSH level is therefore often used as an indicator of the cellular redox imbalance state that is associated with oxidative stress or cellular toxicity. In the present study it was clearly shown that reduced GSH levels caused by CCl₄ were significantly counteracted by isoquercitrin. The subsequent recovery in HepG2 cells treated with M. oleifera leaf extracts might be explained due to the antioxidant properties in EM, AM, IQ or de-novo GSH synthesis or GSH regeneration (GSSG to GSH) 22.

As isoquercitrin prevented lipid accumulation as well as GSH depletion, it hereby increased HepG2 cell viability against the oxidative metabolites of CCl₄ 29. Necrosis and apoptosis process in CCl₄-injured HepG2 cells were minimized, leading to the significant reduction in LDH leakage, cell morphological alterations and DNA fragmentation.

In conclusion, the results showed that hepatoprotective effect in HepG2 cells injured by CCl₄ were found in AM, EM and especially highest in IQ. This effect could be associated with the antioxidant activity. The result encourages further studies on evaluation of liver protective activity of M. oleifera leaves using animal models and clinical trials. From that, isoquercitrin and M. oleifera leaves can be formulated as dietary supplements and/or drugs for liver diseases.

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