

# **Potential antifibrotic effect of honokiol in an experimental model of liver fibrosis**

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*(Pharmacology and Toxicology)*

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Liver fibrosis represents a massive global health burden with limited therapeutic options. Therefore, the need for development of novel treatment is such an urge. Honokiol, a natural biphenolic compound, possesses multiple biological activities, such as antioxidant, anti-inflammatory and anti-cancer. The present study was divided into two parts. The first part aimed at screening the hepatoprotective dose of honokiol where male Sprague-Dawley rats received honokiol once daily for 5 consecutive days at doses of 5, 10 & 20 mg/kg orally followed by a single dose of Con A (20 mg/kg, IV) on the fifth day. Con A induced significant liver damage as proved by the significant elevation in aminotransferases activities with marked histopathological damage. This was almost prevented by pre-treatment with honokiol at dose 10 mg/kg. The selected dose was further used in the second part of the study that designed to assess the potential antifibrotic effect of honokiol in a rat model Con A-induced liver fibrosis as well as to study the possible molecular underlying mechanisms. Male Sprague-Dawley adult rats were treated with either Con A (20 mg/kg, once a week) and/or honokiol (10 mg/kg, five times a week) for four consecutive weeks. Con A induced a significant increase in liver index and serum aminotransferases, while induced a significant decrease in albumin level. Honokiol co-treatment protected against Con A-induced alterations with preservation of normal hepatic architecture. Liver fibrosis induced by Con A was evidenced by increased  $\alpha$ -SMA expression and collagen deposition as indicated by Masson's trichome staining and significant elevation of hydroxyproline content. Honokiol significantly reduced the aforementioned liver fibrosis markers. To elucidate the underlying

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molecular mechanisms, the effect of honokiol on oxidative stress markers, inflammatory markers and fibrosis markers was assessed. Con A significantly elevated lipid peroxides, while significantly depleted glutathione as well as superoxide dismutase enzyme. In addition, Con A increased NF- $\kappa$ B expression with subsequent downstream of inflammatory cytokines such as TNF- $\alpha$  and INF- $\gamma$  as well as inflammatory enzymes such as iNOS. Moreover, Con A significantly activated TGF- $\beta$ 1 signaling pathway confirmed by significant up regulation of TGF- $\beta$ 1 level as well as marked elevated expression of p.samd2/3. Indeed, honokiol co-treatment significantly attenuated the aforementioned oxidative stress, inflammatory and fibrosis markers. In conclusion, these findings indicate that honokiol possesses a promising antifibrotic effect which may be attributed to its antioxidant and anti-inflammatory activities as well as its inhibitory effect on the TGF- $\beta$ /SMAD signaling pathway.

**Key words:** Liver fibrosis, Concanavalin A, Honokiol, TGF- $\beta$ , p.samd2/3.

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<i><b>Subject</b></i>	<i><b>Page No.</b></i>
<i><b>1- List of abbreviations-----</b></i>	<i><b>I</b></i>
<i><b>2- List of tables-----</b></i>	<i><b>IV</b></i>
<i><b>3- List of figures -----</b></i>	<i><b>V</b></i>
<i><b>4- Review of Literature-----</b></i>	<i><b>1</b></i>
<i><b>-Liver fibrosis -----</b></i>	<i><b>1</b></i>
<i><b>-Background-----</b></i>	<i><b>1</b></i>
<i><b>-Etiology-----</b></i>	<i><b>2</b></i>
<i><b>-Diagnosis-----</b></i>	<i><b>6</b></i>
<i><b>- Pathophysiology of liver fibrosis-----</b></i>	<i><b>8</b></i>
<i><b>- TGF-<math>\beta</math>1 pathways; insights into liver fibrosis---</b></i>	<i><b>25</b></i>
<i><b>-Therapeutic approaches for liver fibrosis-----</b></i>	<i><b>31</b></i>
<i><b>- Concanavalin A-----</b></i>	<i><b>38</b></i>
<i><b>- Honokiol-----</b></i>	<i><b>40</b></i>
<i><b>5-Aim of the work-----</b></i>	<i><b>49</b></i>
<i><b>6- Materials and Methods-----</b></i>	<i><b>50</b></i>
<i><b>7-Results-----</b></i>	<i><b>100</b></i>
<i><b>8- Discussion -----</b></i>	<i><b>140</b></i>
<i><b>9- Summary and Conclusions -----</b></i>	<i><b>150</b></i>
<i><b>10- References-----</b></i>	<i><b>157</b></i>
<i><b>11- Arabic summary-----</b></i>	<i><b>A</b></i>

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## *List of Abbreviations*

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<b>ALT</b>	Alanine aminotransferase.
<b>AST</b>	Aspartate aminotransferase.
<b>AUC</b>	Area under the curve.
<b>CCl<sub>4</sub></b>	Carbon tetrachloride.
<b>C<sub>max</sub></b>	Peak plasma concentration.
<b>Con A</b>	Concanavalin A.
<b>COX-II</b>	Cyclooxygenase-II.
<b>CTGF</b>	Connective tissue growth factor.
<b>CYP 450</b>	Cytochrome P450.
<b>DMSO</b>	Dimethyl sulfoxide.
<b>DTNB</b>	Ellman's reagent [5,5'-dithiobis (2-nitrobenzoic acid)].
<b>ECM</b>	Extracellular matrix.
<b>EGF</b>	Epidermal growth factor.
<b>ET-1</b>	Endothelin-1.
<b>GSH</b>	Reduced glutathione.
<b>H<sub>2</sub>O<sub>2</sub></b>	hydrogen peroxide.
<b>HBV</b>	Hepatitis B virus.
<b>HCV</b>	Hepatitis C virus.
<b>HCC</b>	Hepatocellular carcinoma.
<b>HDV</b>	Hepatitis delta virus.
<b>H &amp; E</b>	Hematoxylin and Eosin.
<b>HSCs</b>	Hepatic stellate cells.
<b>IL-1</b>	Interleukin-1.
<b>INF-<math>\gamma</math></b>	Interferon gamma.

## *List of Abbreviations*

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<b>iNOS</b>	Inducible nitric oxide synthase.
<b>IV</b>	Intravenous.
<b>KCs</b>	Kupffer cells.
<b>LAP</b>	Latency-Associated Protein.
<b>LPS</b>	Lipopolysaccharide.
<b>LTBP</b>	Latent TGF- $\beta$ binding proteins.
<b>MCP-1</b>	Monocyte chemoattractant protein 1.
<b>MDA</b>	Malondialdehyde.
<b>MMPs</b>	Matrix metalloproteinases.
<b>NAFLD</b>	Non-alcoholic fatty liver disease.
<b>NF-<math>\kappa</math>B</b>	Nuclear factor kappa B.
<b>NMDA</b>	N-methyl-d-aspartic acid.
<b>NO</b>	Nitric oxide.
<b>PDGF</b>	Platelet-derived growth factor.
<b>ROS</b>	Reactive oxygen species.
<b>siRNA</b>	Small interfering RNA.
<b>SOD</b>	Superoxide dismutase.
<b>SP-Conjugate</b>	Streptavidin-Peroxidase Conjugate.
<b>t<sub>1/2</sub></b>	Elimination with half-life.
<b>TBA</b>	Thiobarbituric acid.
<b>TBARS</b>	Thiobarbituric acid reactive substances.
<b>TCA</b>	Trichloroacetic acid.
<b>TGF-<math>\beta</math></b>	Transforming growth factor beta.
<b>Th cells</b>	T helper cells.
<b>TIMPs</b>	Tissue inhibitors of metalloproteinases.

## *List of Abbreviations*

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<b>TNF-<math>\alpha</math></b>	Tumor necrosis factor alpha.
<b>T<math>\beta</math>RI</b>	TGF- $\beta$ type I receptor kinase.
<b>T<math>\beta</math>RII</b>	TGF- $\beta$ type II receptor kinase.
<b>VEGF</b>	Vascular endothelial growth factor.
<b>WHO</b>	World Health Organization.
<b><math>\alpha</math>-SMA</b>	Alpha smooth muscle actin.

<b>Table No.</b>	<b>Table Title</b>	<b>Page No.</b>
<b>1</b>	Effect of different doses of honokiol on serum ALT and AST levels in acute Con A-induced hepatotoxicity model.	<b>101</b>
<b>2</b>	Histopathological grading of liver sections taken from different groups of rat subjected to acute Con A-induced hepatotoxicity.	<b>104</b>
<b>3</b>	Effect of honokiol on liver index, serum ALT, AST and albumin levels in rats subjected to chronic Con A injection.	<b>109</b>
<b>4</b>	Effect of honokiol on liver reduced glutathione (GSH), liver lipid peroxides (MDA) contents and superoxide dismutase (SOD) activity in rats subjected to chronic Con A injection.	<b>116</b>
<b>5</b>	Effect of honokiol on liver tumor necrosis factor alpha (TNF- $\alpha$ ) and interferon gamma (INF- $\gamma$ ) contents in rats subjected to chronic Con A injection.	<b>121</b>
<b>6</b>	Effect of honokiol on liver hydroxyproline content in rats subjected to chronic Con A injection.	<b>132</b>
<b>7</b>	Effect of honokiol on liver transforming growth factor beta 1 (TGF- $\beta$ 1) content in rats subjected to chronic Con A injection.	<b>136</b>

<b>Figure No.</b>	<b>Figure Title</b>	<b>Page No.</b>
<b>1</b>	Changes in hepatic architecture (A) associated with advanced liver fibrosis (B).	<b>2</b>
<b>2</b>	Key events involved in the molecular pathogenesis of liver fibrosis.	<b>9</b>
<b>3</b>	Mechanisms of oxidative stress induced by various factors on liver disease.	<b>11</b>
<b>4</b>	Summary of the inflammatory cascade.	<b>14</b>
<b>5</b>	Pathways of hepatic stellate cell activation during liver injury and resolution.	<b>18</b>
<b>6</b>	TGF- $\beta$ 1 signaling pathways.	<b>28</b>
<b>7</b>	Chemical structure of honokiol.	<b>41</b>
<b>8</b>	Standard calibration curve of ALT.	<b>62</b>
<b>9</b>	Standard calibration curve of AST.	<b>65</b>
<b>10</b>	Standard calibration curve of MDA.	<b>72</b>
<b>11</b>	Standard calibration curve of TNF- $\alpha$ .	<b>81</b>
<b>12</b>	Standard calibration curve of INF- $\gamma$ .	<b>86</b>
<b>13</b>	Standard calibration curve of TGF- $\beta$ 1.	<b>91</b>
<b>14</b>	Standard calibration curve of hydroxyproline.	<b>93</b>
<b>15</b>	Effect of different doses of honokiol on serum level of (A) ALT and (B) AST in rats subjected to acute Con A-induced hepatotoxicity expressed as percentage of control group.	<b>102</b>
<b>16</b>	Representative photomicrographs of liver sections taken from different groups of rats subjected to acute Con A-induced hepatotoxicity stained with H & E ( $\times 400$ ).	<b>105</b>

## *List of Figures*

<b>17</b>	Effect of honokiol on serum level of (A) ALT and (B) AST in rats subjected to Con A injection expressed as percentage of control group.	<b>110</b>
<b>18</b>	Effect of honokiol on (A) liver index and (B) serum albumin in rats subjected to Con A injection expressed as percentage of control group.	<b>111</b>
<b>19</b>	Representative photomicrographs of liver sections stained with H&E ( $\times 200$ ).	<b>113</b>
<b>20</b>	Effect of honokiol on liver (A) reduced glutathione (GSH) and (B) lipid peroxides (MDA) content in rats subjected to Con A injection expressed as percentage of control group.	<b>117</b>
<b>21</b>	Effect of honokiol on liver superoxide dismutase (SOD) activity in rats subjected to Con A injection expressed as percentage of control group.	<b>118</b>
<b>22</b>	Effect of honokiol on liver (A) tumor necrosis factor alpha (TNF- $\alpha$ ) and (B) interferon gamma (INF- $\gamma$ ) content in rats subjected to Con A injection expressed as percentage of control group .	<b>122</b>
<b>23</b>	Expression of nuclear factor kappa B (NF- $\kappa$ B) by immunohistochemical staining (x400).	<b>123</b>
<b>24</b>	Expression of inducible nitric oxide synthase (iNOS) by immunohistochemical staining (x400).	<b>125</b>
<b>25</b>	Representative photomicrographs of liver sections stained with Masson's trichrome ( $\times 200$ ).	<b>130</b>
<b>26</b>	Effect of honokiol on liver hydroxyproline content in rats subjected to Con A injection expressed as percentage of control group.	<b>133</b>
<b>27</b>	Expression of alpha smooth muscle actin ( $\alpha$ -SMA) by immunohistochemical staining (x400).	<b>134</b>

## *List of Figures*

---

<b>28</b>	Effect of honokiol on liver transforming growth factor beta 1 (TGF- $\beta$ 1) content in rats subjected to Con A injection expressed as percentage of control group .	<b>137</b>
<b>29</b>	Expression of p.smad2/3 by immunohistochemical staining (x400).	<b>138</b>
<b>30</b>	Graphical abstract.	<b>156</b>