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 biphenoilic 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 fifith 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 α-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

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-κB expression with subsequent downstream of inflammatory cytokines such as TNF-α and INF-γ as well as inflammatory enzymes such as iNOS. Moreover, Con A significantly activated TGF-β1 signaling pathway confirmed by significant up regulation of TGF-β1 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-β/SMAD signaling pathway.

Key words: Liver fibrosis, Concanavalin A, Honokiol, TGF-β, p.samd2/3.

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ALT	Alanine aminotransferase.
AST	Aspartate aminotransferase.
AUC	Area under the curve.
CCl_4	Carbon tetrachloride.
C_{max}	Peak plasma concentration.
Con A	Concanavalin A.
COX-II	Cyclooxygenase-II.
CTGF	Connective tissue growth factor.
CYP 450	Cytochrome P450.
DMSO	Dimethyl sulfoxide.
DTNB	Ellman's reagent [5,5'-dithiobis (2-nitrobenzoic acid)].
ECM	Extracellular matrix.
EGF	Epidermal growth factor.
ET-1	Endothelin-1.
GSH	Reduced glutathione.
H_2O_2	hydrogen peroxide.
HBV	Hepatitis B virus.
HCV	Hepatitis C virus.
HCC	Hepatocellular carcinoma.
HDV	Hepatitis delta virus.
H & E	Hematoxylin and Eosin.
HSCs	Hepatic stellate cells.
IL-1	Interleukin-1.
INF-γ	Interferon gamma.

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

TNF-α	Tumor necrosis factor alpha.
TβRI	TGF-β type I receptor kinase.
TβRII	TGF-β type II receptor kinase.
VEGF	Vascular endothelial growth factor.
WHO	World Health Organization.
α-SMA	Alpha smooth muscle actin.