
**Pharmacokinetic Characterization of the Main
Flavonoids in the Extract of Scutellariae Radix**

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PHARMCOKINETIC CHARACTERIZATION OF THE MAIN
FLAVONOIDS IN THE EXTRACT OF SCUTELLATIAE RADIX

By

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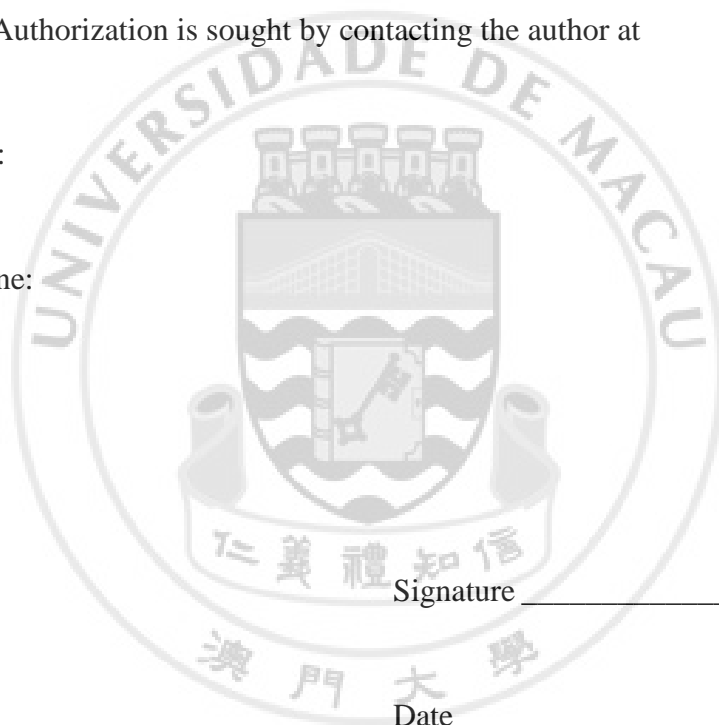
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碩士學位論文

黃芩水提物中主要黃酮類成份的藥代動力學研究

UNIVERSIDADE DE MACAU
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本論文是在我的導師—燕茹博士的悉心指導下完成的。在選題及研究過程中，燕博士為我指點迷津，幫助我開拓研究思路，傾注了大量心血。導師淵博的專業知識、嚴謹的治學態度、精益求精的工作風格、誨人不倦的高尚師德對我影響深遠。在燕博士的教導下，我不僅樹立了遠大的學術目標、掌握了基本的研究方法，還明白了許多為人處世的道理。在此，謹向燕博士表達最崇高的敬意與衷心的感謝！

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University of Macau

Abstract

PHARMCOKINETIC CHARACTERIZATION OF THE MAIN
FLAVONOIDS IN THE EXTRACT OF SCUTELLARIAE RADIX

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Chinese Medicinal Sciences

Keywords:

Scutellariae Radix; flavonoids; baicalin; baicalein; wogonoside; wogonin; oroxylin-A-glucuronide; oroxylin-A; *in vivo*; pharmacokinetics; intestinal bacteria; Caco-2 cell monolayer; glucuronidation; metabolism

Background:

Scutellariae Radix (SR) has been extensively prescribed due to notable medicinal benefits. Flavonoids, including glucuronides baicalin, wogonoside, oroxylin-A-glucuronide and their aglycones baicalein, wogonin and oroxylin-A, were considered as the main active components of the herb. So far, *in vivo* fates of all six flavonoids in SR extract and the determinant factors were not yet fully characterized.

Methods:

Kinetics of six flavonoids in plasma and their excretion profiles in urine and feces

within 48h after oral administration of SR extract (800 mg/kg) to rats were simultaneously determined using LC-MS/MS. Glucuronide isomers formed *in vivo* were also semi-quantitated. Intestinal bacterial conversion and transepithelial permeability of the flavonoids in the extract and hepatic metabolism of the glucuronides were characterized *in vitro*.

Results:

The main ingredients in SR extract were baicalin, baicalein, wogonoside, wogonin, oroxylin-A-glucuronide and oroxylin-A. Both glucuronides and aglycones stayed in plasma over 48h with AUCs of glucuronides 10-100 times that of respective aglycones after the SR extract was oral administrated to S.D. rats. AUPs (area under peak area ratio-time curve) of baicalin isomers were ~12 times that of baicalin yet AUP of wogonoside isomer only one seventh of wogonoside. Glucuronides were the main forms in urine, whereas aglycones predominated in feces, throughout the experiment. Glucuronides were rapidly converted by rat intestinal bacteria (RIB) to respective aglycones. Both glucuronides and aglycones traversed Caco-2 cell monolayers via passive diffusion with the latter bearing >10 times higher permeability. There were remarkable (baicalein), minor (wogonin) and no (oroxylin-A) isomerization in rat liver microsomes.

Conclusion:

Oral kinetics of six flavonoid glucuronides and aglycones in SR were simultaneously obtained, for the first time. Intestinal bacterial conversion, transepithelial permeation and hepatic isomerization are determinant factors for their systemic exposures.

澳門大學

摘要

黃芩水提物中主要黃酮類成份的藥代動力學研究

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關鍵詞：

黃芩；黃酮；黃芩苷；黃芩素；漢黃芩苷；漢黃芩素；千層紙素 A 苷；千層紙素 A；藥代動力學；腸內菌；Caco-2 單層細胞吸收模型；葡萄糖醛酸化；代謝

背景：

黃芩是一味常用中藥，具有清熱燥濕、瀉火解毒和止血安胎等功效，在臨床上使用較多。其主要成份為黃酮，包括：黃芩苷、黃芩素、漢黃芩苷、漢黃芩素、千層紙素 A 苷和千層紙素 A。目前，關於黃芩提取物中主要黃酮類成份的體內藥代動力學及其影響因素尚未解釋清楚。本研究結合體內、體外模型，揭示了黃芩提取物經口服后，其中的主要黃酮類成份在體內過程及吸收、代謝機理。

方法：

本文從黃芩藥材中提取出主要黃酮類成份，並將此提取物灌胃 SD 大鼠后，對原有的成份及代謝產物進行測定，研究提取物在體內存在的形式。此外，利用體外腸內菌代謝模型、Caco-2 單層細胞模型和體外肝代謝模型對黃芩中主要黃酮類成份的吸收、代謝機理進行了研究，闡明了影響黃芩主要黃酮類成份體內系統暴露水平的主要影響因素。

結果:

黃芩提取物中主要含有黃芩苷、黃芩素、漢黃芩苷、漢黃芩素、千層紙素 A 苷和千層紙素 A。在黃芩提取物灌胃給予 SD 大鼠后，于 48 小時內黃芩黃酮類成份葡萄糖醛酸苷的 AUC 分別為其對應苷元 AUC 的 10—100 倍。黃芩苷異構體的 AUP(峰面積-時間曲線下的面積)約為黃芩苷的 12 倍，而漢黃芩苷的 AUP 為其異構體的 7 倍。在尿液中的主要黃酮成份以葡萄糖醛酸苷存在，而糞便中主要存在形式為苷元。在與大鼠腸內菌共孵育下，漢黃芩苷和千層紙素 A 苷迅速轉化為其對應苷元；黃芩苷在轉化成黃芩素的同時，有其異構體 BM4 先生成、后水解。葡萄糖醛酸苷與苷元均以被動擴散的形式透過 Caco-2 單層細胞，並且苷元的透過能力為葡萄糖醛酸苷的 10 倍以上。在大鼠肝代謝中，發現顯著的黃芩苷異構化產物、少量的漢黃芩苷異構化產物。

結論:

从黃芩藥材中得到提取物，并確定其中主要為黃酮類成份。首次通過 LC-MS/MS 同時得到提取物中六個成份的口服藥代動力學。體內實驗表明六個成份在藥時曲線中表現出雙峰現象，在葡萄糖醛酸苷類成份中千層紙素 A 苷的系統暴露量最高；在苷元類成份中，漢黃芩素的系統暴露量最高。在尿和糞便中的主要存在形式分別為葡萄糖醛酸苷和苷元。体外实验表明：黃芩提取物被服用后，首先在腸內菌的作用下，葡萄糖醛酸苷轉化為其對應的苷元；隨後苷元以被動擴散的形式被快速吸收入血；在肝臟中，苷元發生葡萄糖醛酸化—黃芩素形成黃芩苷及其異構體，漢黃芩素形成漢黃芩苷及其異構體。由此解釋提取物在體內的過程。

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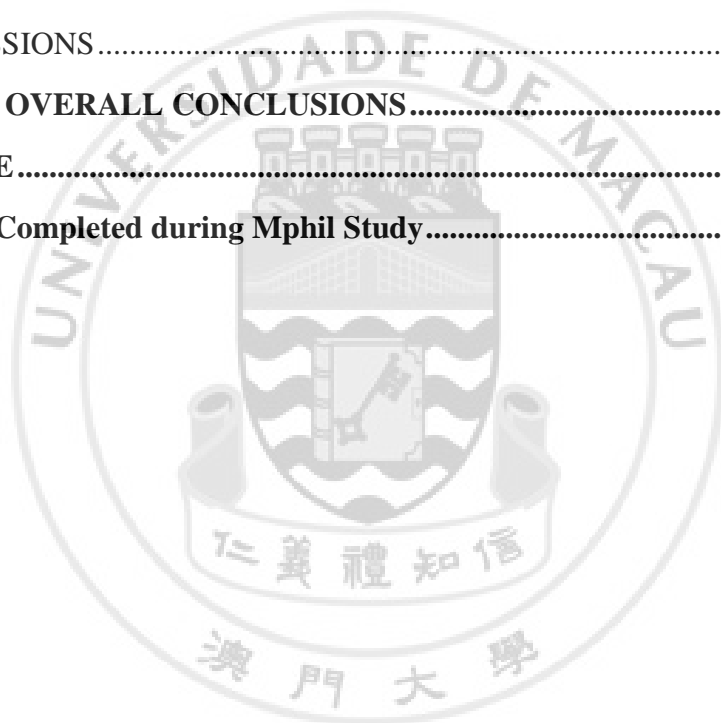
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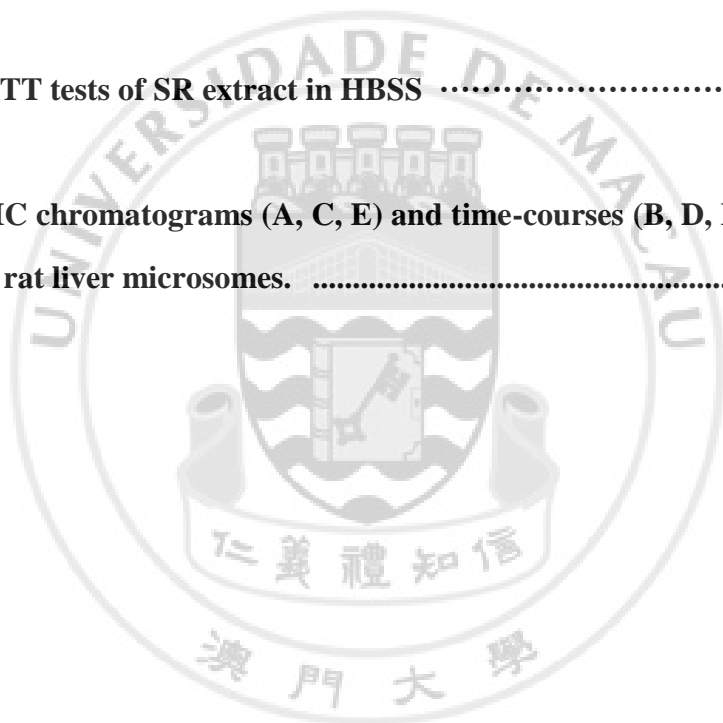
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LIST OF ABBREVIATIONS

HPLC	High performance liquid chromatography
IS	Internal standard
LOD	Limit of detection
LOQ	Limit of quantification
HBSS	Hanks' balanced salt solution
TEER	Trans epithelial electrical resistance
P_{app}	Apparent permeability coefficient
P-gp	P-glycoprotein
RLM	Rat liver microsome
UGT	UDP-glucuronosyl transferases
UDPGA	Uridine 5'-diphosphoglucuronic acid

