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三黃瀉心湯濃縮方劑與水煎劑中活性指標成分

相對生可用率之研究

Relative Bioavailability of Bioactive Constituents between  
Commercial Extract and Traditional Herbal Decoction of  
San Huang Shel Shin Tang

研究報告

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# 三黃瀉心湯濃縮方劑與水煎劑中 活性指標成分相對生可用率之研究

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## 摘要

目前臺灣中醫臨床上使用之中藥劑型主要為傳統水煎劑及濃縮製劑。鮮見有評估濃縮製劑與傳統水煎劑在療效上相等性之研究。本研究以三黃瀉心湯為模型，探討濃縮方劑與水煎方劑中活性指標成分相對生可用率，研究濃縮方劑是否可達與傳統水煎劑相等的口服吸收率。

本研究隨機收集市售 GMP 藥廠三黃瀉心湯濃縮散劑檢品 11 種，另依據標準湯劑方式製備三黃瀉心湯之水煎劑，並以高效液相層析儀(HPLC)建立定量兩類劑型中，其所含黃連鹼、棕櫚鹼、小檗鹼、黃芩苷、黃芩苷元、漢黃芩素、大黃素、蘆薈大黃素、大黃酸、大黃酚等指標成分含量之方法，結果顯示，各藥廠三黃瀉心湯濃縮散劑每克所含成分彼此差異甚大。

在大鼠口服兩種劑型之相對生可用率研究部分，我們以水煎劑為基準，投予動物相當於化學相當量之濃縮散劑，實驗採交叉設計，血清檢品於水解前及經 sulfatase 和  $\beta$ -glucuronidase 分別水解後，以自行開發之 HPLC 方法，定量各成分原型和結合態代謝物，藉以比較兩種劑型活性指標成分之生可用率，結果顯示，口服濃縮散劑組，除黃芩苷元外，蘆薈大黃素、漢黃芩素、大黃酸、大黃素、大黃酚等結合態代謝物之曲線下面積低於口服水煎劑分別達 83.6%、42.2%、35.5%、46.0%、25.2%。總之，由本動物實驗血藥濃度之結果顯示，三黃瀉心湯水煎劑型比濃縮劑型有較佳之生可用率，此一現象顯示中藥濃縮製劑之賦型劑配方對其藥物指標成分之吸收應具一定程度之影響。

關鍵詞：三黃瀉心湯、濃縮方劑、結合態代謝物

**Relative Bioavailability of Bioactive Constituents between  
Commercial Extract and Traditional Herbal Decoction of San  
Huang Shel Shin Tang**

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**ABSTRACT**

The commercial extract of San Huang Shel Shin Tang (SHSST) in market were randomly collected in this study. The quantitation method of coptisine, palmatine, berberine, baicalin, baicalein, wogonin, emodin, aloe-emodin, rhein, chrysophanol using HPLC was developed and validated in this study. Samples were extracted by 70% methanol, and the aglycones and glycosides of each constituent were determined prior to and after hydrolysis. The results showed that the contents of the bioactive constituents in commercial extract of SHSST among manufactures were quite different.

Rats were administered with traditional decoction and a commercial extract of SHSST in crossover design. The aglycones and conjugates metabolites were measured by HPLC prior to and after hydrolysis with sulfatase and  $\beta$ -glucuronidase, respectively. The results showed that the AUC of the conjugated metabolites of aloe-emodin, wogonin, rhein and emodin from commercial extract were significantly lower than those from decoction by 83.6%, 42.2%, 35.5% and 46.0%, respectively. These results indicated that the dosage form of decoction confers better bioavailability than the commercial extract of SHSST.

Keywords : San Huang Shel Shin Tang, conjugated metabolites, Bioavailability

## 壹、前言

目前臺灣中醫臨床上使用之中藥劑型主要為傳統水煎劑及濃縮製劑。其中濃縮製劑為全民健保給付的劑型，因此中藥濃縮製劑之應用佔健保費支出之比率相當可觀。中藥科學濃縮製劑為臺灣臨床應用之特有劑型，其療效攸關民眾醫療品質之良窳，然而自從中醫界使用濃縮製劑以來，鮮見有評估濃縮製劑與傳統水煎劑在療效上相等性之研究。故臨床中醫師在使用濃縮製劑時之劑量決策，除了參考藥廠建議的劑量外，通常會依自由心証加減劑量，因此對於濃縮製劑使用劑量似無一定的準則與客觀依據。藥廠所建議的劑量係根據其水煎劑濃縮的倍數而決定。然而濃縮製劑與傳統水煎劑之劑型不同，傳統水煎劑不須溶解的步驟即可吸收；但濃縮製劑為固體劑型，又添加了賦形劑，其吸收速率、程度與傳統水煎劑應不相等。因此濃縮製劑之劑量，依據藥廠之建議劑量是否可達與傳統水煎劑相等的口服吸收率與療效，是一個值得關注的研究課題。

由於中藥成分組成複雜且多數不明，因此研究其口服吸收具相當之挑戰性，單味中藥如是，中藥方劑更是複雜。但有鑒於本土問題必須靠國內自行研發解決。因此本實驗室過去曾以兩年的時間，針對使用頻率較高或活性指標成分明確之單味中藥如枳殼、陳皮、甘草與槐花等，進行了濃縮製劑與傳統水煎劑中活性指標成分相對生體可用率等基礎研究。建立了中藥水煎劑與濃縮劑相對生體可用率之研究模式，可提供傳統水煎劑與濃縮製劑在臨床應用上是否相等之基礎資訊。未來將進一步評估中藥方劑中活性指標成分之生體可用率，提供臨床應用與

政策制訂之參考。中藥方劑之成分複雜度更高，期能克服分析技術上之困難。三黃瀉心湯出於金匱要略，包含大黃(2)、黃連(1)及黃芩(1)，以水三升，煮取一升，頓服之<sup>(1)</sup>。三黃瀉心湯苦寒瀉火，為治療熱盛吐衄証要方，其以黃連瀉心清胃、燥濕解毒，黃芩瀉肺燥濕解毒，大黃瀉火通腑瀉毒，引火下行，故全方能清瀉三焦火、解毒、燥濕。為一常用之重要方劑。

近代藥理學研究顯示，三黃瀉心湯具抑制葡萄球菌、痢疾桿菌、大腸桿菌等抗病原微生物之作用<sup>(2)</sup>，可用於急性肺炎、急性化膿性扁桃腺炎及眼部炎症等疾病<sup>(3-6)</sup>，另外尚有治療缺血性腦血管疾病<sup>(7)</sup>，鎮靜、降壓、降血脂<sup>(8)</sup>、抑制大鼠胃潰瘍形成<sup>(9-10)</sup>，保護胃黏膜<sup>(11)</sup>、抑制胃酸分泌<sup>(8)</sup>、抗發炎<sup>(12)</sup>等作用。且此方目前亦於台灣進行抗癌之臨床試驗中。另外，組成此方之藥物大黃及其成分在現代藥理研究中亦顯示其具抗菌、抗腫瘤、抗病毒、抗發炎等活性<sup>(13-18)</sup>。黃芩及其成分則具抗腫瘤、抗菌、抗病毒、抗真菌、抗發炎、抗氧化<sup>(19-21)</sup>等活性。黃連及其成分具抗菌、抗腫瘤、抗發炎等活性<sup>(22-25)</sup>。若從化學與現代藥理學的角度觀之，富含多酚類成分之中藥多具抗腫瘤、抗病毒、抗發炎、抗氧化等活性<sup>(26-44)</sup>，三黃瀉心湯之組成藥物，均為富含多酚類成分之中藥，因此三黃瀉心湯應具開發成為治療癌症、心血管疾病與抗 SARS 等相關疾病藥物之潛力。

基於本研究室已累積的單味中藥代謝動力學研究之基礎上<sup>(45-46)</sup>，本計畫擬進行濃縮方劑與水煎方劑中活性指標成分相對生可用率之研究，並擬以三黃瀉心湯為模型方劑進行此一研究。

本計畫將製備三黃瀉心湯水煎劑，並收集市售濃縮製劑產品及，分析其中所含之 aloe-emodin、emodin、chrysophanol、rhein、baicalin、baicalein、wogonin、berberine 及其配醣體等活性成分含量。從中選擇大黃黃芩黃連比例為(2:1:1)及上述活性指標成分含量較高之市售製劑，以大鼠為實驗動物，分析動物口服水煎劑與濃縮製劑後，藥物吸收之藥物動力學參數，藉以比較水煎劑與濃縮方劑中

活性指標成分相對生可用率，本年度之實驗結果，除可作為製劑品管與臨床療效之參考資訊外，亦可提供後續進行人體口服水煎劑與濃縮方劑中活性指標成分相對生可用率研究之基礎資訊。同時亦可提供中藥製劑與臨床療效相關性更高之評估方法，並檢討中藥濃縮製劑之賦型劑配方，期能加速中醫藥現代化之腳步。

## 貳、材料與方法

### 一、材料

#### (一) 標準品

- |                            |   |
|----------------------------|---|
| 1. Coptisine hydrochloride | Wako (osaka, Japan)                                 |
| 2. Palmatine hydrochloride | Aldrich Chemical Co. (Milwaukee, WI, U.S.A)         |
| 3. Baicalin                | Wako (osaka, Japan)                                 |
| 4. Berberine hydrochloride | Tokyo Pure chemical Industries, Ltd. (Tokyo, Japan) |
| 5. Baicalein               | Wako (osaka, Japan)                                 |
| 6. aloe-emodin             | Sigma Chemical Co. (St. Louis, MO, U.S.A)           |
| 7. wogonin                 | Wako (osaka, Japan)                                 |
| 8. rhein                   | Aldrich Chemical Co. (Milwaukee, WI, U.S.A)         |
| 9. emodin                  | Sigma Chemical Co. (St. Louis, MO, U.S.A)           |
| 10. chrysophanol           | Aldrich Chemical Co. (Milwaukee, WI, U.S.A)         |
| 11. 2-methylanthraquinone  | Aldrich Chemical Co. (Milwaukee, WI, U.S.A)         |

#### (二) 試劑

- |                             |  |
|-----------------------------|--|
| 1. $\beta$ -glucosidase     | Sigma Chemical Co. (St. Louis, MO, U.S.A)  |
| 2. $\beta$ -glucuronidase   | Sigma Chemical Co. (St. Louis, MO, U.S.A)  |
| 3. sulfatase                | Sigma Chemical Co. (St. Louis, MO, U.S.A)  |
| 4. L(+)-ascorbic acid       | Riedel-deHean AG (Seelze, Germany)         |
| 5. acetonitrile( LC Grade)  | J.T. Baker, Inc. (Phillipsburg, NJ, U.S.A) |
| 6. methanol(LC Grade )      | J.T. Baker, Inc. (Phillipsburg, NJ, U.S.A) |
| 7. Ethyl acetate(LC Grade ) | J.T. Baker, Inc. (Phillipsburg, NJ, U.S.A) |
| 8. Acetic acid, glacial     | J.T. Baker, Inc. (Phillipsburg, NJ, U.S.A) |

- |                          |                                    |
|--------------------------|------------------------------------|
| 9. Ortho-phosphoric acid | Riedel-deHean AG (Seelze, Germany) |
| 10. Hydrochloric acid    | Wako (osaka, Japan)                |
| 11. 大黃、黃芩、黃連             | 台中市欣隆藥行 (已進行生藥學組織鑑定)               |
| 12. 三黃瀉心湯濃縮製劑            | 隨機採購 GMP 藥廠製濃縮散劑，共 11 家。           |

### (三) 儀器設備

#### 1. 高效液相層析儀(HPLC)包括:

- |              |  |
|--------------|--|
| (1) 幫浦       | Shimadzu (Tokyo, Japan)                                  |
| (2) 層析管      | Apollo C18 5u 250mm x 4.6mm, Alltech                     |
| (3) 管柱前濾膜    | P.J. Cobert Associates, Inc. (St.Louis, Missouri, U.S.A) |
| (4) 紫外光偵測器   | Shimadzu (Tokyo, Japan)                                  |
| (5) 系統控制儀    | Shimadzu (Tokyo, Japan)                                  |
| (6) 自動注射器    | Perkin Elmer Series 200                                  |
| 2. 酸鹼測定儀     | Microprocessor pH-mV meter                               |
| 3. 高速離心機     | Denver Instrument (Force -14)                            |
| 4. 渦旋振盪器     | Scientific Industries                                    |
| 5. 超音波振盪器    | Branson 5200   |
| 6. 控溫往復式振盪水槽 | Shaker ZC4000  |
| 7. 吹氮氣濃縮裝置   | OA-SYSEVAP-112   |
| 8. Milli-Q   | Millipore corporation(Billerica, MA,USA)                 |
| 9. 電子分析天平    | Mettler AJ100  |



10. 微量移液管

11. 注射針及針筒

2.5 ml syringe ( 0.55 × 25 mm )

1.0 ml syringe ( 0.45 × 13 mm )

12. 過濾膜 ( 0.22  $\mu$  m , 13 mm ) Alltech Associates Inc.

13. 混合氣體 (95% O<sub>2</sub>, 5%CO<sub>2</sub>) 源吉行有限公司

#### (四) 溶液製備

1. 0.1N acetate buffer solution ( pH 5.0 )

取 0.1N 醋酸鈉溶液 67.8 ml，加入 0.1N 醋酸至 100.0 ml，再加 1N 氫氧化鈉調至 pH = 5.0 ± 0.1。

2.  $\beta$ -glucosidase 溶液

取  $\beta$ -glucosidase 35.6 mg (14 units/mg)，加 pH 5.0 buffer 定容至 10.0 ml，貯存在-30°C 備用。

3.  $\beta$ -glucuronidase 溶液

取  $\beta$ -glucuronidase 41.1 mg (2440 units/mg)，加 pH 5.0 buffer 至 50 ml，貯存在-30°C 備用。

4. sulfatase 溶液

取 sulfatase 331.1 mg (15.1 units/mg)，加 pH 5.0 buffer 至 50 ml，貯存在-30°C 備用。

## 5. 維生素 C 溶液

取維生素 C 150.0 mg，加水至 1.0 ml 即得 150.0 mg/mL 之維生素 C 溶液，使用前新鮮製備。

## 6. 內部標準品溶液

精確稱取 2-methylanthraquinone 5.0 mg，加甲醇定容至 5.0 mL，即 2-methylanthraquinone 儲存溶液。取適量儲存溶液，分別以甲醇和乙酸乙酯稀釋成濃度為  $10 \mu\text{g/mL}$  之 2-methylanthraquinone 甲醇溶液和  $0.125 \mu\text{g/mL}$  之 2-methylanthraquinone 乙酸乙酯溶液。

## 二、市售三黃瀉心湯濃縮製劑與水煎劑中活性指標成分之定量

### (一) 三黃瀉心湯水煎劑檢品之製備

分別精確稱取大黃、黃芩、黃連原藥材 100.0 g、50.0 g、50.0 g，(依金匱要略組成比例 (2:1:1))，並依標準湯劑製備方式，加入 4.0 L 之蒸餾水，室溫下浸泡 30 分鐘後，直火加熱，沸騰後以小火煎煮至體積約 2.0 L，趁熱過濾後，減壓濃縮至體積略少於 400.0 mL，加水定容至 400.0 mL。置於-20°C 冷凍櫃貯存備用。

### (二) 三黃瀉心湯濃縮製劑檢品之製備

精確稱取濃縮散劑 0.2 g，加入 70% 之甲醇溶液 20.0 mL，以超音波振盪器振盪 30 分鐘，再經熔砂漏斗過濾，取濾液，再將過濾後之濾渣置於原萃取容器，同上述之法萃取第二次，合併兩次濾液後定容至 50.0 mL，置於-20°C 冷凍櫃貯存備用。

### (三) 三黃瀉心湯水煎劑中指標成分之定量

取三黃瀉心湯水煎劑檢品 300.0  $\mu$ L 加甲醇 700.0  $\mu$ L 振盪混合，經 9860 g 高速離心 15 分鐘，取上清液 200.0  $\mu$ L，再以甲醇 700.0  $\mu$ L 稀釋，取 100.0  $\mu$ L 稀釋液，用氮氣吹乾，依序加入 pH 5.0 之緩衝溶液 300.0  $\mu$ L、內標準溶液 2-methylantraquinone (10 $\mu$ g/mL in methanol) 300.0  $\mu$ L、甲醇溶液 300.0  $\mu$ L，並混合均勻後，高速離心(9860 g) 5 分鐘，取上清液 50.0  $\mu$ L 注入 HPLC 分析。

### (四) 三黃瀉心湯濃縮製劑中指標成分之定量

取三黃瀉心湯濃縮製劑萃取檢品 200.0  $\mu$ L，用氮氣吹乾，依序加入 pH 5.0 之緩衝溶液 300  $\mu$ L、內標準溶液 2-methylantraquinone ( 10.0  $\mu$ g/mL in

methanol) 300.0  $\mu\text{L}$ 、甲醇溶液 300.0  $\mu\text{L}$ ，並混合均勻後，高速離心(9860 g) 5 分鐘，取上清液 50.0  $\mu\text{L}$  注入 HPLC 分析。

#### (五) 三黃瀉心湯水煎劑中 glucosides 之定量

取三黃瀉心湯水煎劑檢品 300.0  $\mu\text{L}$  加甲醇 700.0  $\mu\text{L}$  振盪混合，經 9860 g 高速離心 15 分鐘，取上清液 200.0  $\mu\text{L}$ ，再以甲醇 700.0  $\mu\text{L}$  稀釋，取 100.0  $\mu\text{L}$  稀釋液，用氮氣吹乾，加入  $\beta$ -glucucosidase 溶液(溶於 pH 5.0 之緩衝溶液，含  $\beta$ -glucucosidase 50.0 units/mL)，以振盪器充分混合均勻後，置於 37°C 之恆溫水槽反應 3 小時；反應後加入內標準溶液(2-methylanthraquinone (10 $\mu\text{g}/\text{mL}$  in methanol) 300.0  $\mu\text{L}$ 、甲醇溶液 300.0  $\mu\text{L}$ ，混合均勻後，高速離心(9860 g) 5 分鐘，取上清液 50.0  $\mu\text{L}$  注入 HPLC 分析。

#### (六) 三黃瀉心湯濃縮製劑中 glucosides 之定量

取三黃瀉心湯濃縮製劑萃取檢品 200.0  $\mu\text{L}$ ，用氮氣吹乾，加入  $\beta$ -glucucosidase 溶液(溶於 pH 5.0 之緩衝溶液，含  $\beta$ -glucucosidase 50 units/mL)，以振盪器充分混合均勻後，置於 37°C 之恆溫水槽反應 3 小時；反應後加入內標準溶液(2-methylanthraquinone (10 $\mu\text{g}/\text{mL}$  in methanol) 300.0  $\mu\text{L}$ 、甲醇溶液 300.0  $\mu\text{L}$ ，混合均勻後，高速離心(9860 g) 5 分鐘，取上清液 50.0  $\mu\text{L}$  注入 HPLC 分析。

#### (七) 高效液相層析之分析條件

層析管柱採用 Apollo C18 5u 250mm x 4.6mm。移動相由 0.1%  $\text{H}_3\text{PO}_4$  磷

酸水溶液和氬甲烷組合而成，0.1% H<sub>3</sub>PO<sub>4</sub> 磷酸水溶液比例隨時間改變，第 0 分鐘時為 77%、第 10 分鐘時為 77%、第 15 分鐘時為 80%、第 25 分鐘時為 80%、第 30 分鐘時為 78%、第 45 分鐘時為 78%、第 50 分鐘時為 62%、第 60 分鐘時為 62%、第 65 分鐘時為 30%、第 95 分鐘時為 30%、第 100 分鐘時為 77%。流速亦隨時間改變，第 0 分鐘時為 1.0 mL/min、第 68 分鐘時為 0.2 mL/min、第 70 分鐘時為 0.2 mL/min、第 78 分鐘時為 1.0 mL/min、第 100 分鐘時為 1.0 mL/min。檢測波長為 250 nm。

#### (八) 檢量線之繪製

精確稱取適量指標成分標準品，以甲醇系列稀釋成標準溶液。

指標成分	標準溶液濃度( $\mu$ g/mL)
Coptisine	3.1、6.3、12.5、25.0、50.0、100.0
Baicalin	12.5、25.0、50.0、100.0、200.0、400.0
Palmatine	3.1、6.3、12.5、25.0、50.0、100.0
Berberine	15.6、31.2、62.5、125.0、250.0、500.0
Baicalein	7.8、15.6、31.2、62.5、125.0、250.0
Aloe-emodin	0.8、1.6、3.1、6.3、12.5、25.0
Wogonin	3.1、6.3、12.5、25.0、50.0、100.0
Rhein	3.1、6.3、12.5、25.0、50.0、100.0
Emodin	0.3、0.6、1.3、2.5、5.0、10.0
Chrysophanol	0.3、0.6、1.3、2.5、5.0、10.0

取標準溶液 200.0  $\mu\text{L}$ ，依序加入內標準溶液 2-methylanthraquinone ( 10.0  $\mu\text{g}/\text{mL}$  in methanol) 300.0  $\mu\text{L}$ 、甲醇溶液 400.0  $\mu\text{L}$ ，以震盪器混合均勻後，高速離心(9860 g ) 5 分鐘，取上清液 50.0  $\mu\text{L}$  注入 HPLC 分析。分析後，以標準品與內標準波峰面積比值和各標準品濃度進行直線迴歸分析，得各標準品之檢量線。

#### (九) 分析方法之確效

##### 1. 精密度 (precision)

將各種濃度之標準溶液，分別於同日內晨、午、晚及連續三日之異日間各進行一次層析，並以獲得的直線迴歸方程式，求得每次實驗濃度值。以三次同日內和三次異日間分析值，分別求得平均值 (mean)、標準差 (standard deviation, S.D.) 及變異係數 (coefficient of variation, C.V.)。

##### 2. 準確度 (accuracy)

以三次同日內及三次異日間實驗所得平均濃度與理論濃度之相對誤差 (relative error, R.E.)表示之。

##### 3. 靈敏度 (sensitivity)

將標準溶液一再稀釋，直至其波峰與雜訊之比值為 3/1 時之濃度為其偵測極限 (limits of detection)。

##### 4. 回收率 (recovery)

將各濃度指標成分標準溶液，用氮氣吹乾，依序加入已知指標濃度之三黃瀉心湯濃縮製劑檢品 200.0  $\mu\text{L}$ 、內標準溶液 2-methylanthraquinone ( 10  $\mu\text{g}/\text{mL}$  in methanol) 300.0  $\mu\text{L}$ 、甲醇溶液 400.0  $\mu\text{L}$ ，以震盪器混合均勻後，

高速離心(9860 g) 5 分鐘，取上清液注入 HPLC 分析。經檢量線之方程式，  
求出各指標成分含量，扣除原檢品含量，所得之增加量除以已知的標準品  
添加量，求其百分比即為回收率。

## 二、大鼠口服兩種劑型三黃瀉心湯之相對生可用率

### (一) 口服三黃瀉心湯水煎劑之製備

與本章二、(一) 三黃瀉心湯水煎劑檢品之製備方法相同。

### (二) 口服三黃瀉心湯濃縮散劑溶液之製備

從收集並經定量後之三黃瀉心湯濃縮散劑中選擇其中組成為大黃、黃芩、黃連為 2:1:1 與指標成分含量較高之一市售品，精秤 1.0 g，加入 9.0 mL 溫水，混合均勻備用。

### (三) 動物

雄性 Sprague-Dawley 大白鼠十二隻，體重介於 320 ~ 450 g，出生 16~20 週，實驗前禁食 12 小時，自由給水，給藥後 3 小時再進食。

### (四) 給藥方法與採血

#### 1. 給藥

採取交叉試驗設計，隨機將大白鼠分為兩組，每組各六隻，兩種劑型藥物以投予約化學等量之 rhein 為基準，一組給予 5 g/10 mL/kg 三黃瀉心湯水煎劑，另一組給予 1.1 g/10 mL/kg 三黃瀉心湯濃縮散劑溶液，俟藥物於體內代謝完畢後一周後交換給藥。三黃瀉心湯水煎劑和濃縮散劑溶液之各成分含量如 Table 1 所示。

#### 2. 採血

經由胃管給藥後於 10、30、60、240、480、720、1440、1440 及 2880 分鐘從心臟採血。每次採血量約為 1.0 mL，將血液檢品以 9860 g 高速離心 10 分鐘，取上層血清，並貯存於-30°C 冰櫃，俟後分析。



## (五) 血清檢品中指標成分結態代謝物之定量

### 1. 體內自由態指標成分之定量

取 150.0  $\mu\text{L}$  血清檢品，依序加入 150.0  $\mu\text{L}$  pH 5.0 buffer、維生素 C 溶液 50.0  $\mu\text{L}$  (150 mg/mL)、50.0  $\mu\text{L}$  0.1N HCl 後，充分混合，以 400.0  $\mu\text{L}$  乙酸乙酯萃取 (含 0.125  $\mu\text{g/mL}$  2-methylantraquinone 為內標準)，用振盪器振盪 30 秒後，經 9860 g 高速離心 5 分鐘，取乙酸乙酯層，用氮氣吹乾後，以 50.0  $\mu\text{L}$  甲醇溶解，取 20.0  $\mu\text{L}$  供 HPLC 分析。檢品中指標成分與內標準之波峰面積比值，代入檢量線方程式，求得濃度。

### 2. 體內指標成分結合態代謝物之定量

#### (1) 葡萄糖醛酸結合態代謝物之定量

取 150.0  $\mu\text{L}$  血清檢品，加入 150.0  $\mu\text{L}$   $\beta$ -glucuronidase 溶液 (2000 units/mL 溶於 pH 5.0 之 acetate buffer) 和維生素 C 溶液 50  $\mu\text{L}$  (150 mg/mL)，充分混合，置於 37°C 之恆溫水槽反應 4 小時。反應後，加 50.0  $\mu\text{L}$  0.1N HCl 後，以 400.0  $\mu\text{L}$  乙酸乙酯萃取 (含 0.125  $\mu\text{g/mL}$  2-methylantraquinone 為內標準)，用振盪器振盪 30 秒後，經 9860 g 高速離心 5 分鐘，取乙酸乙酯層，用氮氣吹乾後，以 50.0  $\mu\text{L}$  甲醇溶解，取 20.0  $\mu\text{L}$  供 HPLC 分析。檢品中指標成分與內標準之波峰面積比值，代入檢量線方程式，求得濃度。

將水解後之各濃度扣除自由態部分即為 glucuronides。

#### (2) 硫酸結合態代謝物之定量

取 150.0  $\mu\text{L}$  血清檢品，加入 150.0  $\mu\text{L}$  sulfatase 溶液 (1000 units/mL 溶於 pH 5.0 之 acetate buffer) 和維生素 C 溶液 50.0  $\mu\text{L}$  (150 mg/mL)，充分混合，置於 37°C 之恆溫水槽反應 4 小時。反應後，加 50.0  $\mu\text{L}$  0.1N HCl 後，以 400.0  $\mu\text{L}$  乙酸乙酯萃取 (含 0.125  $\mu\text{g/mL}$  2-methylantraquinone 為內標

準),用振盪器振盪 30 秒後,經 9860 g 高速離心 5 分鐘,取乙酸乙酯層,用氮氣吹乾後,以 50.0  $\mu\text{L}$  甲醇溶解,取 20.0  $\mu\text{L}$  供 HPLC 分析。檢品中指標成分與內標準之波峰面積比值,代入檢量線方程式,求得濃度。

將水解後之各濃度扣除自由態部分即為 sulfates。

#### (六) 血清檢品高效液相層析之分析條件

層析管柱採用 Apollo C18 5u 250mm x 4.6mm。移動相由 0.1%  $\text{H}_3\text{PO}_4$  磷酸水溶液和氬甲烷組合而成,0.1%  $\text{H}_3\text{PO}_4$  磷酸水溶液比例隨時間改變,第 0 分鐘時為 70%、第 10 分鐘時為 30%、第 15 分鐘時為 20%、第 25 分鐘時為 20%、第 30 分鐘時為 70%、第 45 分鐘時為 70%。流速為 0.8 mL/min,檢測波長為 250 nm。

#### (七) 檢量線繪製

將各指標成分以甲醇釋成一系列濃度,取指標成分以甲醇溶液,加九倍體積空白血清,血清標準溶液,系列濃度下

指標成分	標準溶液濃度( $\mu\text{g/mL}$ )
Baicalein	0.3、0.6、1.3、2.5、5.0、10.0、20.0
Aloe-emodin	0.2、0.3、0.6、1.3、2.5、5.0、10.0
Wogonin	0.2、0.3、0.6、1.25、2.5、5.0
Rhein	0.2、0.3、0.6、1.3、2.5、5.0、10.0
Emodin	0.2、0.3、0.6、1.3、2.5、5.0、10.0
Chrysophanol	0.2、0.3、0.6、1.25、2.5、5.0

取 150.0  $\mu\text{L}$  血清標準溶液，依序加入 150.0  $\mu\text{L}$  pH 5.0 buffer、維生素 C 溶液 50.0  $\mu\text{L}$  (150 mg/mL)、50.0  $\mu\text{L}$  0.1N HCl 後，充分混合，以 400.0  $\mu\text{L}$  乙酸乙酯萃取 (含 0.125  $\mu\text{g/mL}$  2-methylanthraquinone 為內標準)，用振盪器振盪 30 秒後，經 9860 g 高速離心 5 分鐘，取乙酸乙酯層，用氮氣吹乾後，以 50.0  $\mu\text{L}$  甲醇溶解，取 20.0  $\mu\text{L}$  供 HPLC 分析。檢品中指標成分與內標準之波峰面積比值，代入檢量線方程式，求得濃度。

所得各指標成分與內標準之波峰面積比值與各已知濃度進行直線迴歸，求得檢量線之方程式。

#### (八) 分析方法之確效

##### 1. 精密度 (precision)

將各種濃度之標準溶液，分別於同日內晨、午、晚及連續三日之異日間各進行一次層析，並以獲得的直線迴歸方程式，求得每次實驗濃度值。以三次同日內和三次異日間分析值，分別求得平均值 (mean)、標準差 (standard deviation, S.D.) 及變異係數 (coefficient of variation, C.V.)。

##### 2. 準確度 (accuracy)

以三次同日內及三次異日間實驗所得平均濃度與理論濃度之相對誤差 (relative error, R.E.) 表示之。

##### 3. 靈敏度 (sensitivity)

將標準溶液一再稀釋，直至其波峰與雜訊之比值為 3/1 時之濃度為其偵測極限 (limits of detection)。

#### 4. 回收率 (recovery)

將各指標成分甲醇溶液，分別加入空白血清及水中，各濃度如下

指標成分	標準溶液濃度( $\mu\text{g/mL}$ )
baicalein	0.6、2.5、10.0
Aloe-emodin	0.3、1.3、5.0
Wogonin	0.2、0.6、2.5
Rhein	0.3、1.3、5.0
Emodin	0.3、1.3、5.0
chrysophanol	0.2、0.6、2.5

分析方法同 二、5.(a) 自由態指標成分之定量，HPLC 定量，所測得血清標準溶液中各指標成分濃度除以水標準溶液中各指標成分濃度比值，即為回收率。

#### (九) 數據處理及統計分析

利用 WINNONLIN (version 1.1, SCI Software, Statistical Consulting, Inc., Apex, NC) 軟體之非室體模式處理血中濃度數據，計算動力學參數。並以 paired Student's t-test 分析組間之差異。

## 參、結果

### 一、市售三黃瀉心湯濃縮製劑與水煎劑中活性指標成分之定量

本實驗利用高效液相層析法(HPLC)定量三黃瀉心湯濃縮製劑與水煎劑中活性指標成分，包含 coptisine、palmatine、berberine、baicalin、baicalein、wogonin、emodin、aloe-emodin、rhein、chrysophanol，由於成分間極性差異大，因此移動相採取梯度沖提方式進行分析，依據本實驗室先前對於三黃瀉心湯研究，以 250 nm 為偵測波長，2-methylanthraquinone 為內標準，檢品於 100 分鐘內分析完成，層析圖如 Fig. 1。

以各指標成分標準品與內標準之波峰面積比值與指標成分濃度直線迴歸所製作之檢量線顯示，coptisine 於 3.1 ~ 100.0  $\mu\text{g/mL}$ 、palmatine 3.1 ~ 100.0  $\mu\text{g/mL}$ 、berberine 15.6 ~ 500.0  $\mu\text{g/mL}$ 、baicalin 12.5 ~ 400.0  $\mu\text{g/mL}$ 、baicalein 7.8 ~ 250.0  $\mu\text{g/mL}$ 、wogonin 3.1 ~ 100.0  $\mu\text{g/mL}$ 、emodin 0.3 ~ 10.0  $\mu\text{g/mL}$ 、aloe-emodin 3.1 ~ 100.0  $\mu\text{g/mL}$ 、rhein 3.1 ~ 100.0  $\mu\text{g/mL}$ 、chrysophanol 0.3 ~ 10.0  $\mu\text{g/mL}$  均有良好之線性關係( $r = 0.999$ )，結果如 Table 2 所示。

對於此方法之確效，進行 intra-run 及 inter-run 之試驗，各指標成分於 intra-run 及 inter-run 之變異係數(C.V.)、相對誤差(relative error)與回收率均在可接受之範圍內，結果見於 Table 3~Table 13。

本研究隨機收集市售三黃瀉心湯濃縮製劑，並利用上述自行開發之 HPLC 條件進行指標成分定量，我們抽樣收集 11 家 GMP 藥廠所生產的三黃瀉心湯濃縮製

劑檢品，定量結果顯示，不同廠商所製造的三黃瀉心湯濃縮散劑每公克分別含 coptisine  $2.3\pm 0.3\sim 11.6\pm 1.8$  mg、palmatine  $3.0\pm 0.4\sim 11.7\pm 2.5$  mg、berberine  $7.5\pm 1.0\sim 39.0\pm 10.0$  mg、baicalin  $4.2\pm 1.0\sim 57.6\pm 14.5$  mg、baicalein  $0.4\pm 0.2\sim 24.7\pm 4.3$  mg、wogonin  $0.6\pm 0.2\sim 7.8\pm 1.3$  mg、emodin  $0.2\pm 0.1\sim 1.3\pm 0.2$  mg、aloe-emodin  $0.2\pm 0.0$  至  $0.8\pm 0.4$  mg、rhein  $0.8\pm 0.1\sim 4.9\pm 0.9$  mg、chrysophanol  $0.1\pm 0.0\sim 0.4\pm 0.0$  mg，對這些指標成分而言，各家檢品成分含量差異極大。上述定量結果如 Table 14~Table 16 所示。

由於三黃瀉心湯中尚有許多配醣體成分，口服進入人體後，透過腸胃道細菌的水解，仍有機會為生物體所吸收利用。因此本研究將各濃縮製劑檢品以 glucosidase 進行水解後，定量上述成分之 glucosides 含量，其結果如 Table 1 所示。水解後，每克濃縮製劑中，含 coptisine  $2.7\pm 0.1\sim 14.8\pm 0.9$  mg、palmatine  $2.9\pm 0.4\sim 11.8\pm 1.0$  mg、berberine  $8.6\pm 0.3\sim 42.0\pm 1.4$  mg、baicalein  $9.2\pm 1.5\sim 47.7\pm 13.7$  mg、wogonin  $3.1\pm 0.5\sim 13.4\pm 1.2$  mg、emodin  $0.8\pm 0.1\sim 2.1\pm 0.1$  mg、aloe-emodin  $1.4\pm 0.2\sim 5.3\pm 0.1$  mg、rhein  $3.7\pm 0.4\sim 11.7\pm 1.1$  mg、chrysophanol  $0.2\pm 0.0\sim 0.5\pm 0.2$  mg。各家濃縮製劑水解前後各指標成分之含量比較如 Fig. 2~Fig. 10 所示

## 二、大鼠口服兩種劑型三黃瀉心湯之相對生可用率

為進行大鼠口服兩種不同劑型間之相對生可用率研究，本研究室以 HPLC 法自行開發了定量大鼠口服三黃瀉心湯後，血清中活性指標成分原型與結合態代謝物之分析條件。由於成分間極性差異頗大，因此移動相採取梯度沖提方式進行分析，每一檢品可於 45 分鐘內分析完成，層析圖如 Fig. 11 所示。各成分於血清中可定量之濃度範圍及其檢量線如 Table 17 所示，均有良好之線性關係( $r > 0.999$ )，分析方法之精密度於 intra-run 與 inter-run 之確效結果如 Table 18~Table 23 所示，變異係數 (C.V.) 與相對誤差均在可接受範圍內，顯示再現性和準確度良好。定量極限 (limit of quantitation, LOQ) 與偵測極限 (limit of detection, LOD)(S/N=3) 如 Table 24. 所示。各成分之回收率評估，結果如 Table 25 所示，各檢品回收率介於 76.9~111.5%。綜合上述之結果，此分析方法精確可行。

在動物給藥實驗部分，利用交叉設計，分別投予水煎劑，及與水煎劑含等量 rhein 為基準之濃縮散劑後，各成分及其結合態代謝物 (sulfates/glucuronides) 於血清中濃度經時變化圖如 Fig. 12~Fig. 23 所示，以 WINNONLIN 軟體，利用非室體模式處理計算動力學參數，如 Table 26~Table 31 所示。大鼠口服水煎劑和濃縮散劑後，baicalein sulfates/baicalein glucuronides 之  $T_{max}$  分別為  $185.5 \pm 246.7 / 36.7 \pm 47.7$  及  $44.4 \pm 45.9 / 40.0 \pm 48.2$  min,  $C_{max}$  分別為  $5.7 \pm 2.8/5.0 \pm 2.2$  及  $10.9 \pm 2.5/11.3 \pm 5.1$  nmol/mL,  $AUC_{0-2880}$  分別為  $4733.9 \pm 1850.2/4553.1 \pm 2062.2$  及  $6615.9 \pm 2369.8/7798.2 \pm 4059.1$  nmol · min/mL,  $MRT_{0-2880}$  分別為  $920.8 \pm 331.9/1081.0 \pm 542.2$  及  $942.3 \pm 221.0/1034.1 \pm 330.1$  min。Aloe-emodin sulfates/aloe-emodin

glucuronides 之  $T_{max}$  分別為  $43.3 \pm 44.4/31.1 \pm 34.8$  及  $20.0 \pm 17.3/30.0 \pm 37.7$  min,  $C_{max}$  分別為  $3.0 \pm 1.3/2.7 \pm 1.4$  及  $0.7 \pm 0.2/0.8 \pm 0.4$  nmol/mL,  $AUC_{0-2880}$  分別為  $340.4 \pm 176.1 \pm /294.6 \pm 165.4$  及  $41.3 \pm 14.3/62.6 \pm 60.5$  nmol · min/mL,  $MRT_{0-2880}$  分別為  $104.4 \pm 65.72/207.8 \pm 216.0$  及  $45.4 \pm 13.6/48.8 \pm 24.1$  min。Wogonin sulfates/wogonin glucuronides 之  $T_{max}$  分別為  $561.1 \pm 443.4/1202.2 \pm 1077.2$  及  $675.5 \pm 726.3/357.8 \pm 615.2$  min,  $C_{max}$  分別為  $1.1 \pm 0.4/1.3 \pm 0.8$  及  $1.4 \pm 0.9/1.1 \pm 0.4$  nmol/mL,  $AUC_{0-2880}$  分別為  $1045.5 \pm 491.1/1479.8 \pm 1142.7$  及  $746.3 \pm 524.5/712.6 \pm 609.8$  nmol · min/mL,  $MRT_{0-2880}$  分別為  $960.7 \pm 296.3/1165.0 \pm 403.9$  及  $765.9 \pm 308.9/850.2 \pm 384.0$  min。Rhein free form/rhein sulfates/rhein glucuronides 之  $T_{max}$  分別為  $16.7 \pm 10.3/75.0 \pm 49.3/291.6 \pm 564.6$  及  $21.7 \pm 20.4/48.3 \pm 41.7/40.0 \pm 43.8$  min,  $C_{max}$  分別為  $17.5 \pm 4.6/27.8 \pm 15.2/22.7 \pm 9.0$  及  $13.4 \pm 3.5/12.6 \pm 6.3/10.9 \pm 4.5$  nmol/mL,  $AUC_{0-2880}$  分別為  $5249.5 \pm 3838.8/9348.9 \pm 5510.3/8736.1 \pm 5299.0$  及  $8212.7 \pm 6682.0/4184.5 \pm 4432.1/2642.6 \pm 3128.9$  nmol · min/mL,  $MRT_{0-2880}$  分別為  $692.0 \pm 293.9/632.0 \pm 226.6/779.8 \pm 616.4$  及  $999.9 \pm 480.4/536.1 \pm 418.8/727.2 \pm 747.0$  min。Emodin sulfates/emodin glucuronides 之  $T_{max}$  分別為  $57.8 \pm 47.1/66.7 \pm 78.6$  及  $21.1 \pm 10.5/30.0 \pm 37.7$  min,  $C_{max}$  分別為  $5.0 \pm 2.2/4.6 \pm 2.0$  及  $2.4 \pm 0.6/2.4 \pm 0.8$  nmol/mL,  $AUC_{0-2880}$  分別為  $1929.4 \pm 1012.6 \pm /1221.8 \pm 379.3$  及  $1224.6 \pm 1116.6/475.7 \pm 345.8$  nmol · min/mL,  $MRT_{0-2880}$  分別為  $570.9 \pm 198.0/503.3 \pm 388.1$  及  $731.2 \pm 520.6/390.9 \pm 279.0$  min。Chrysophanol sulfates/chrysophanol glucuronides 之  $T_{max}$  分別為  $18.9 \pm 10.5/60.0 \pm 76.0$  及  $14.4 \pm 8.8/14.4 \pm 8.8$  min,  $C_{max}$  分別為  $0.7$



$\pm 0.3/0.6 \pm 0.3$  及  $0.5 \pm 0.2/0.4 \pm 0.2$  nmol/mL,  $AUC_{0-2880}$  分別為  $169.1 \pm 79.6/221.1 \pm 209.5$  及  $160.4 \pm 107.4/131.3 \pm 125.5$  nmol · min/mL,  $MRT_{0-2880}$  分別為  $629.4 \pm 502.5/624.3 \pm 604.1$  及  $636.4 \pm 520.3/765.6 \pm 489.4$  min。

綜合以上數據結果顯示，baicalein、aloe-emodin、wogonin、emodin、chrysophanol 幾全以結合態形式 (sulfates/glucuronides) 存在於血清中，僅 rhein 以原型及結合態代謝物 (sulfates/glucuronides) 並存，但仍以結合態代謝物為主，而 coptisine、palmatine、berberine 則未出現於血清中。

## 肆、討論

市售之三黃瀉心湯濃縮劑型包含濃縮顆粒、散劑、濃縮散、濃縮細粒，另有濃縮錠之劑型(錠劑未列入本次生可用率之研究)，這些濃縮製劑由生藥製成浸膏加上各種賦型劑組成，賦型劑多為澱粉，比重約佔製劑之 1/3 至 1/2，少數產品並無賦型劑為何物之標示。浸膏組成部分，則有大黃、黃連及黃芩以 2:1:1 及 1:1:1 兩種比例。使用方法上，所有產品皆建議日服三次，與古方有異，關於服用時間，包括有食前一小時、食前兩小時、食後服藥以及十五歲以下兒童可服用劑量等，各產品敘述不盡相同。

依據廠商所標示之生藥與浸膏比例，其濃縮倍數約在 2~6 倍，若計算每克濃縮製劑所含原生藥克數，則每克含 1~4 克不等之原生藥，如再考慮個別建議之每日劑量，則成人每日服用約 6~24 克原生藥，其中約 36%(4/11) 的產品建議劑量低於古方用量(15~16 克/日)，而有 64%(7/11) 高於或等於古方用量。

本實驗利用高效液相層析法定量十一個三黃瀉心湯濃縮製劑中指標成分含量，每克濃縮製劑中，coptisine 含量相差 5~6 倍、palmatine 含量相差 3~4 倍、berberine 含量相差 5~6 倍、baicalin 含量相差 13~14 倍、baicalein 含量相差 61~62 倍、wogonin 含量相差 13~14 倍、emodin 含量相差 6~7 倍、aloe-emodin 含量相差 3~4 倍、rhein 含量相差 6~7 倍、chrysophanol 含量相差 3~4 倍。

若各濃縮製劑檢品以 $\beta$ -glucucosidase 進行水解後定量，結果顯示，每克濃縮製劑中，coptisine 含量相差 5~6 倍、palmatine 含量相差 4~5 倍、berberine 含量相差 4~5 倍、baicalein 含量相差 5~6 倍、wogonin 含量相差 4~5 倍、emodin 含量相

差 2~3 倍、aloe-emodin 含量相差 3~4 倍、rhein 含量相差 3~4 倍、chrysophanol 含量相差 2~3 倍。

此結果顯示各家三黃瀉心湯濃縮製劑之指標成分差異頗大，黃芩之指標成分 (baicalin、baicalein、wogonin) 均可於酶解前測得，酶解後則未測得 baicalin，而 baicalein 和 wogonin 含量大幅增加，顯示 baicalin 完全被酶解成 baicalein，而 wogonin 亦有配糖體存在。大黃之指標成分 (emodin、aloe-emodin、rhein、chrysophanol) 在藥材中亦以非糖體和配糖體兩種形式存在，酶解後含量皆增加，成分差異於酶解前為 3~7 倍，酶解後為 2~4 倍，當評估黃芩和大黃之指標成分含量時，若以非糖體和配糖體之總含量做為指標，應可更客觀合理標示出製劑中可供人體吸收利用的指標成分含量。

本研究基於上述定量分析之基礎，我們餵予含 rhein 等量的水煎劑與濃縮散劑，濃縮散劑 1.1g 的 rhein 含量相當於本實驗以 5 克原生藥所製成之水煎劑，進而以大鼠比較傳統水煎劑與現代濃縮劑間，指標成分的相對生可用率。

實驗結果顯示血清中並未檢出 coptisine、palmatine、berberine，顯示此三成分幾乎不吸收，其生可用率為零，此結果與藥物吸收理論一致，因為它們皆為帶正電的胺陽離子，吸收不易。Baicalein、wogonin、emodin、aloe-emodin、rhein、chrysophanol 則以結合態代謝物 (sulfates/glucuronides) 存在於血清中、僅 rhein 有原形分子存在，此結果與本實驗室先前對於黃連、黃芩、大黃等單味中藥代謝動力學的研究結果相符。

在個別成分的吸收比較部分，指標成分如 aloe-emodin sulfates/glucuronides、

wogonin sulfates/glucuronides、rhein free from/sulfates/glucuronides、chrysophanol sulfates/glucuronides，服用水煎劑時  $C_{max}$  高於濃縮散劑，但是服用水煎劑後 baicalein sulfates/glucuronides 之  $C_{max}$  低於服用濃縮散劑，此係因水煎劑中 baicalin/baicalein 之含量遠低於本實驗室所用的濃縮散劑。

另外以 AUC 之參數評估各個成分之吸收情形，AUC 若以 sulfates 與 glucuronides 之總和計算，如 Table 32 所示，服用濃縮散劑比水煎劑之吸收，aloe-emodin conjugates 顯著低了  $83.6\pm 59.8\%$ 、wogonin conjugates 低了  $42.2\pm 70.9\%$ 、rhein free from and conjugates 低了  $35.5\pm 69.1\%$ 、emodin conjugates 低了  $46.0\pm 59.7\%$ 、chrysophanol conjugates 低了  $25.2\pm 87.7\%$ ，僅 baicalein conjugates 比服用水煎劑組高出  $55.2\pm 69.1\%$ 。

綜合上述數據，若以三黃瀉心湯為例，在動物實驗中，投予以水煎劑之劑型比投予濃縮劑型有較佳的吸收率。因此，傳統水煎劑之生可用率，似乎較優於目前的濃縮製劑，因此濃縮散劑的療效是否能與水煎劑相當，值得進一步探討。

## 伍、結論與建議

### 結論

本研究建立三黃瀉心湯中 coptisine、palmatine、berberine、baicalin、baicalein、wogonin、emodin、aloe-emodin、rhein、chrysophanol 等活性成分之原型與其配醣體型式含量之分析方法。並以此為基礎進一步開發含藥血清的分析方法。

定量市售三黃瀉心湯濃縮散劑指標活性成分之結果顯示，各產品之間成分差異頗大。

以主成分 rhein 相當含量之三黃瀉心湯水煎劑與濃縮製劑投予大白鼠。定量血清中指標成分及其結合態代謝物 (sulfates/glucuronide)，實驗結果顯示，服用濃縮製劑比水煎劑組有較好的生可用率。而 coptisine、palmatine 與 berberine 此三成分無法吸收進入動物體內； baicalein、wogonin、emodin、aloe-emodin、rhein、chrysophanol 等非醣體指標成分僅能以結合態代謝物之形式存在於血液中；僅有 rhein 能在血液中檢測出原型與結合態代謝物。

### 建議

1. 在中藥製劑如三黃瀉心湯濃縮製劑之品質管制部份，建議除定量其活性指標成分外，應考慮加入其配醣體之定量檢測。
2. 中藥傳統水劑與現代濃縮劑間對疾病症狀之療效是否相等，實應進行更多之研究來觀察與驗證，同時一般使用濃縮製劑時多數強調濃縮倍率，卻嚴重忽略該製劑於體內之吸收現況，實為一觀念上極大之誤差。為中藥現代製劑能為更多人所接受，在其品質品管之部份，我們應考慮建立並提供此些現代劑型與傳統水藥於生體內吸收代謝上差異之資訊，而讓臨床醫師選擇使用時有參考之依據，而期使現代製劑能具有與水煎劑相等之療效表現。

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Table 1. Comparison of contents ( $\mu\text{mol}$ ) in decoction (5 g/mL) and commercial extract (1.1 g/mL) of SHSST

Constituente	decoction ( $\mu\text{mol}/10\text{mL}$ )	Commercial extract ( $\text{nmol}/10\text{mL}$ )
Coptisine	21434.4	18115.3
Baicalin	0.0	0.0
Palmatine	16613.9	18726.5
Berberine	59994.4	65509.4
Baicalein	118760.2	160722.9
Aloe-emodin	16522.8	18363.6
Wogonin	32779.1	52338.8
Rhein	49520.0	45816.8
Emodin	9815.4	7461.4
chrysophanol	1977.9	1662.7

Table 2. The regression equations, concentration ranges and correlation coefficients of constituent in San-Huang-Shel-Shin-Tang.

Constituent	Regression equations	Con.c range ( $\mu\text{g/mL}$ )	r
coptisine	$Y = 0.0164 X - 0.0174$	3.1 ~ 100.0	0.9999
palmatin	$Y = 0.0187 X - 0.0165$	3.1 ~ 100.0	0.9999
berberine	$Y = 0.0207 X + 0.00734$	15.6 ~ 500.0	0.9999
baicalin	$Y = 0.0114 X + 0.0006$	12.5 ~ 400.0	0.9998
baicalein	$Y = 0.0223 X + 0.0077$	7.8 ~ 250.0	0.9999
wogonin	$Y = 0.0969 X + 0.0398$	3.1 ~ 100.0	0.9999
emodin	$Y = 0.0303 X + 0.00234$	0.3 ~ 10.0	0.9996
aloe-emodin	$Y = 0.0968 X + 0.0058$	0.8 ~ 25.0	0.9996
rhein	$Y = 0.1469 X + 0.0800$	3.1 ~ 100.0	0.9998
chrysophanol	$Y = 0.0707 X + 0.0007$	0.3 ~ 10.0	0.9999

Table 3. Intra-run and inter-run analytical precision and accuracy of coptisine

Conc ( $\mu\text{g/mL}$ )	Intra-run		Inter-run	
	Precision	Accuracy	Precision	Accuracy
	Mean $\pm$ S.D. (C.V.%)	(%)	Mean $\pm$ S.D. (C.V.%)	(%)
100.0	96.2 $\pm$ 3.2 (3.4)	-3.9	97.2 $\pm$ 4.4 (4.6)	-2.8
50.0	47.8 $\pm$ 2.9 (6.1)	-4.4	49.4 $\pm$ 2.2 (4.4)	-1.3
25.0	23.4 $\pm$ 2.1 (9.0)	-6.3	24.7 $\pm$ 0.7 (2.9)	-1.1
12.5	11.7 $\pm$ 0.5 (4.5)	-6.6	11.5 $\pm$ 0.2 (1.9)	-8.1
6.3	5.4 $\pm$ 0.3 (3.1)	-13.1	5.6 $\pm$ 0.2 (2.7)	-9.8
3.1	3.2 $\pm$ 0.1 (4.0)	4.0	3.5 $\pm$ 0.4 (9.9)	12.6

n=3

Table 4. Intra-run and inter-run analytical precision and accuracy of palmatine

Conc ( $\mu\text{g/mL}$ )	Intra-run		Inter-run	
	Precision	Accuracy	Precision	Accuracy
	Mean $\pm$ S.D. (C.V.%)	(%)	Mean $\pm$ S.D. (C.V.%)	(%)
100.0	101.8 $\pm$ 3.0 (2.9)	1.8	104.6 $\pm$ 1.9 (1.9)	4.6
50.0	51.3 $\pm$ 3.9 (7.7)	2.6	52.6 $\pm$ 3.4 (6.4)	5.1
25.0	25.9 $\pm$ 0.8 (3.3)	3.6	26.1 $\pm$ 0.7 (2.7)	4.2
12.5	12.7 $\pm$ 0.1 (1.0)	1.7	12.8 $\pm$ 0.2 (1.2)	2.7
6.3	5.4 $\pm$ 0.3 (5.3)	-13.9	5.4 $\pm$ 0.3 (5.1)	-13.7
3.1	3.5 $\pm$ 0.08 (2.2)	12.2	3.6 $\pm$ 0.1 (3.4)	15.4

n=3

Table 5. Intra-run and inter-run analytical precision and accuracy of berberine

Conc ( $\mu\text{g/mL}$ )	Intra-run		Inter-run	
	Precision	Accuracy	Precision	Accuracy
	Mean $\pm$ S.D. (C.V.%)	(%)	Mean $\pm$ S.D. (C.V.%)	(%)
500.0	509.0 $\pm$ 14.5 (2.8)	1.8	515.7 $\pm$ 20.4 (4.0)	3.1
250.0	257.6 $\pm$ 18.5 (7.2)	3.1	263.9 $\pm$ 15.9 (6.0)	5.5
125.0	130.3 $\pm$ 6.0 (4.6)	4.2	132.6 $\pm$ 3.3 (2.5)	6.1
62.5	64.5 $\pm$ 2.1 (3.2)	3.2	64.7 $\pm$ 2.5 (3.8)	3.5
31.3	29.0 $\pm$ 0.8 (2.8)	-7.4	29.1 $\pm$ 0.6 (1.9)	-6.8
15.6	14.7 $\pm$ 0.8 (5.2)	-5.7	14.5 $\pm$ 0.9 (6.1)	-7.4

n=3

Table 6. Intra-run and inter-run analytical precision and accuracy of baicalin

Conc ( $\mu\text{g/mL}$ )	Intra-run		Inter-run	
	Precision	Accuracy	Precision	Accuracy
	Mean $\pm$ S.D. (C.V.%)	(%)	Mean $\pm$ S.D. (C.V.%)	(%)
400.0	406.9 $\pm$ 11.3 (0.6)	1.7	411.9 $\pm$ 15.7 (3.8)	3.0
200.0	205.9 $\pm$ 14.4 (0.9)	2.9	210.5 $\pm$ 12.4 (5.9)	5.2
100.0	102.7 $\pm$ 7.0 (0.8)	2.7	106.0 $\pm$ 2.9 (2.7)	6.0
50.0	51.8 $\pm$ 1.5 (1.6)	3.6	51.4 $\pm$ 0.9 (1.8)	2.9
25.0	22.6 $\pm$ 0.4 (3.1)	-9.5	23.0 $\pm$ 0.2 (0.9)	-8.1
12.5	10.5 $\pm$ 1.1 (0.5)	-15.7	12.3 $\pm$ 0.6 (4.7)	-1.3

n=3

Table 7. Intra-run and inter-run analytical precision and accuracy of baicalein

Conc ( $\mu\text{g/mL}$ )	Intra-run		Inter-run	
	Precision	Accuracy	Precision	Accuracy
	Mean $\pm$ S.D. (C.V.%)	(%)	Mean $\pm$ S.D. (C.V.%)	(%)
250.0	250.6 $\pm$ 5.5 (2.2)	0.2	249.4 $\pm$ 5.4 (2.2)	-0.2
125.0	126.7 $\pm$ 3.3 (2.6)	1.4	127.7 $\pm$ 3.7 (2.9)	2.2
62.5	62.6 $\pm$ 1.7 (2.7)	0.1	64.0 $\pm$ 1.7 (2.6)	2.4
31.3	32.0 $\pm$ 1.6 (5.0)	2.3	31.8 $\pm$ 1.1 (3.4)	1.8
15.6	14.1 $\pm$ 0.5(3.9)	-9.9	15.1 $\pm$ 1.4 (9.1)	-3.6
7.8	6.6 $\pm$ 0.4 (5.8)	-15.3	6.5 $\pm$ 0.2 (3.6)	-16.5

n=3

Table 8. Intra-run and inter-run analytical precision and accuracy of wogonin

Conc ( $\mu\text{g/mL}$ )	Intra-run		Inter-run	
	Precision	Accuracy	Precision	Accuracy
	Mean $\pm$ S.D. (C.V.%)	(%)	Mean $\pm$ S.D. (C.V.%)	(%)
100.0	100.7 $\pm$ 2.8 (2.8)	0.7	99.6 $\pm$ 3.6 (3.6)	-0.5
50.0	51.5 $\pm$ 4.0 (7.7)	3.0	52.7 $\pm$ 3.5 (6.6)	5.4
25.0	27.0 $\pm$ 0.7 (2.4)	7.8	26.4 $\pm$ 1.0 (3.9)	5.4
12.5	13.1 $\pm$ 0.5 (3.5)	4.7	12.9 $\pm$ 0.4 (3.1)	3.5
6.3	5.8 $\pm$ 0.2 (4.1)	-7.2	6.1 $\pm$ 0.4 (5.9)	-2.4
3.1	2.7 $\pm$ 0.2(7.0)	-13.7	2.8 $\pm$ 0.3 (9.8)	-9.8

n=3

Table 9. Intra-run and inter-run analytical precision and accuracy of emodin

Conc ( $\mu\text{g/mL}$ )	Intra-run		Inter-run	
	Precision	Accuracy	Precision	Accuracy
	Mean $\pm$ S.D. (C.V.%)	(%)	Mean $\pm$ S.D. (C.V.%)	(%)
10.0	10.4 $\pm$ 0.3 (2.8)	3.5	10.2 $\pm$ 0.4 (3.4)	2.3
5.0	5.3 $\pm$ 0.3 (5.6)	6.4	5.3 $\pm$ 0.3 (4.9)	6.8
2.5	2.5 $\pm$ 0.1 (3.7)	1.5	2.7 $\pm$ 0.2 (8.1)	10.5
1.3	1.3 $\pm$ 0.1 (3.9)	-0.3	1.3 $\pm$ 0.1 (3.8)	0.4
0.6	0.6 $\pm$ 0.1 (8.1)	-9.1	0.6 $\pm$ 0.1 (13.0)	-6.5
0.3	0.3 $\pm$ 0.01 (4.9)	-20.0	0.3 $\pm$ 0.01 (5.0)	-21.0

n=3

Table 10. Intra-run and inter-run analytical precision and accuracy of aloe-emodin

Conc ( $\mu\text{g/mL}$ )	Intra-run		Inter-run	
	Precision	Accuracy	Precision	Accuracy
	Mean $\pm$ S.D. (C.V.%)	(%)	Mean $\pm$ S.D. (C.V.%)	(%)
25.0	24.2 $\pm$ 2.9 (12.1)	-3.2	26.1 $\pm$ 1.1 (4.4)	4.5
12.5	13.0 $\pm$ 0.9 (6.6)	4.1	12.7 $\pm$ 1.2 (9.4)	1.4
6.3	6.8 $\pm$ 0.4 (6.4)	9.3	6.7 $\pm$ 0.6 (8.8)	7.7
3.1	2.8 $\pm$ 0.2 (7.8)	-10.6	2.9 $\pm$ 0.1 (4.0)	-7.1
1.6	1.5 $\pm$ 0.04 (2.4)	-2.5	1.5 $\pm$ 0.1 (4.2)	-3.6
0.8	0.9 $\pm$ 0.1 (11.4)	9.7	0.8 $\pm$ 0.01 (1.8)	1.5

n=3

Table 11. Intra-run and inter-run analytical precision and accuracy of rhein

Conc ( $\mu\text{g/mL}$ )	Intra-run		Inter-run	
	Precision	Accuracy	Precision	Accuracy
	Mean $\pm$ S.D. (C.V.%)	(%)	Mean $\pm$ S.D. (C.V.%)	(%)
100.0	98.7 $\pm$ 2.3 (2.3)	-1.3	96.9 $\pm$ 4.2(4.3)	-3.1
50.0	50.7 $\pm$ 3.1 (6.1)	1.3	51.6 $\pm$ 5.0 (9.7)	3.1
25.0	25.6 $\pm$ 1.1 (4.4)	2.3	25.5 $\pm$ 1.2 (4.8)	2.1
12.5	12.8 $\pm$ 0.4 (3.1)	2.1	12.5 $\pm$ 0.4 (3.1)	0.2
6.3	5.6 $\pm$ 0.4 (6.3)	-10.3	6.0 $\pm$ 0.4 (6.3)	-4.6
3.1	2.7 $\pm$ 0.2 (9.0)	-13.8	2.7 $\pm$ 0.2 (8.1)	-15.0

n=3

Table 12. Intra-run and inter-run analytical precision and accuracy of chrysophanol

Conc ( $\mu\text{g/mL}$ )	Intra-run		Inter-run	
	Precision	Accuracy	Precision	Accuracy
	Mean $\pm$ S.D. (C.V.%)	(%)	Mean $\pm$ S.D. (C.V.%)	(%)
10.0	9.9 $\pm$ 0.2 (1.6)	-0.9	10.1 $\pm$ 0.4 (3.7)	1.3
5.0	5.1 $\pm$ 0.3 (5.2)	1.9	4.9 $\pm$ 0.1 (2.4)	-2.1
2.5	2.5 $\pm$ 0.2 (7.9)	-2.0	2.4 $\pm$ 0.1 (4.0)	-4.4
1.3	1.3 $\pm$ 0.1 (8.9)	-0.2	1.2 $\pm$ 0.1 (6.3)	-6.3
0.6	0.6 $\pm$ 0.04 (7.3)	-3.3	0.6 $\pm$ 0.03 (4.5)	-4.9
0.3	0.3 $\pm$ 0.02 (7.0)	3.2	0.3 $\pm$ 0.02 (5.3)	-3.2

n=3

Table 13-1. Recoveries (%) of coptisine, palmatine, berberine, baicalin and baicalein from commercial extracts of SHSST

Constituent	Conc. Spiked ( $\mu\text{g/mL}$ )				Recoveries (%)
		1	2	3	Mean $\pm$ S.D.
Coptisine	50.0	95.2	79.5	97.4	90.7 $\pm$ 9.8
	18.8	114.4	101.9	115.8	110.7 $\pm$ 7.7
	6.3	120.1	124.5	146.7	130.5 $\pm$ 14.2
Palmatine	50.0	97.0	82.9	102.6	94.2 $\pm$ 10.1
	18.8	109.6	111.0	128.3	116.3 $\pm$ 10.4
	6.3	106.0	114.0	139.4	119.8 $\pm$ 17.4
Berberine	250.0	95.1	82.1	100.5	92.6 $\pm$ 9.4
	93.8	111.0	104.7	124.4	113.4 $\pm$ 10.1
	31.3	112.7	116.1	139.0	122.6 $\pm$ 14.3
Baicalin	200.0	93.8	75.0	100.1	89.7 $\pm$ 13.1
	75.0	123.1	106.2	146.1	125.1 $\pm$ 20.0
	25.0	126.0	133.9		130.0 $\pm$ 5.5
Baicalein	125.0	96.6	98.1	101.2	98.6 $\pm$ 2.3
	46.9	103.4	101.0	111.9	105.4 $\pm$ 5.8
	15.6	97.4	98.1	101.0	98.8 $\pm$ 1.9

n=3



Table 13-2. Recoveries (%) of wogonin, emodin, aloe-emodin, rhein, and chrysophanol from commercial extracts of SHSST

Constituent	Conc. Spiked ( $\mu\text{g/mL}$ )				Recoveries (%)
		1	2	3	Mean $\pm$ S.D.
Wogonin	50.0	94.6	97.3	98.5	96.8 $\pm$ 2.0
	18.8	104.8	102.6	118.4	108.6 $\pm$ 8.5
	6.3	102.7	106.3	128.9	112.7 $\pm$ 14.2
Emodin	5.0	99.0	102.1	105.3	102.1 $\pm$ 3.2
	1.9	118.6	110.3	135.3	121.4 $\pm$ 12.8
	0.6	124.2	128.1	N.D.	126.2 $\pm$ 2.8
Aloe-emodin	12.5	69.9	73.9	68.1	70.7 $\pm$ 3.0
	4.7	70.4	74.3	81.4	75.4 $\pm$ 5.6
	1.6	70.4	72.9	91.2	78.2 $\pm$ 11.4
Rhein	50.0	104.4	100.0	104.8	103.1 $\pm$ 2.7
	18.8	118.6	111.2	134.3	121.4 $\pm$ 11.8
	6.3	122.3	125.8	145.3	131.2 $\pm$ 12.4
chrysophanol	5.0	105.5	106.0	100.5	104.0 $\pm$ 3.0
	1.9	108.5	98.3	89.3	98.7 $\pm$ 9.6
	0.6	85.2	82.7	74.8	80.9 $\pm$ 5.4

n=3

Table 14. Contents (mg) of coptine, palmatine and berberine in each gram commercial extracts of SHSST

Samples	Coptisine	Palmatine	Berberine
a	6.0 ± 0.8	5.4 ± 0.7	19.6 ± 2.0
b	7.0 ± 0.7	6.1 ± 0.6	22.9 ± 3.7
c	5.3 ± 0.4	5.7 ± 0.4	20.9 ± 1.0
d	7.4 ± 1.6	5.3 ± 1.1	19.9 ± 4.4
e	3.9 ± 1.4	3.1 ± 1.1	11.1 ± 2.8
f	11.6 ± 1.8	11.7 ± 2.5	39.1 ± 10.9
g	2.3 ± 0.3	3.0 ± 0.4	7.5 ± 1.1
h	3.7 ± 0.5	3.4 ± 0.3	11.6 ± 0.8
i	6.0 ± 0.8	7.2 ± 1.3	23.0 ± 1.2
j	3.3 ± 0.3	3.6 ± 0.3	13.0 ± 1.1
k	10.8 ± 0.1	8.9 ± 0.5	33.0 ± 2.2

n=11 triplicate

Table 15. Contents (mg) of baicalin, baicalein and wogonin in each gram commercial extracts of SHSST

Samples	Baicalin	Baicalein	Wogonin
a	57.7 ± 14.5	1.3 ± 0.3	1.4 ± 0.3
b	52.0 ± 8.5	24.7 ± 4.3	7.8 ± 1.3
c	40.5 ± 2.8	1.8 ± 0.3	2.6 ± 0.3
d	25.4 ± 7.0	11.1 ± 1.1	3.3 ± 0.8
e	25.6 ± 12.0	2.4 ± 0.4	1.2 ± 0.3
f	45.6 ± 6.1	5.7 ± 3.9	2.5 ± 1.6
g	4.2 ± 1.0	0.4 ± 0.2	0.6 ± 0.2
h	30.5 ± 2.9	7.3 ± 1.9	2.3 ± 0.3
i	49.0 ± 10.2	2.1 ± 0.9	0.8 ± 0.3
j	25.3 ± 1.9	2.4 ± 0.9	1.3 ± 0.3
k	25.6 ± 6.3	2.0 ± 0.05	1.3 ± 0.2

n=11 triplicate

Table 16. Contents (mg) of emodin, aloe-emodin, rhein and chrysophanol in each gram commercial extracts of SHSST

Samples	Emodin	Aloe-emodin	Rhein	Chrysophanol
a	0.5 ± 0.1	0.7 ± 0.1	4.5 ± 1.0	0.3 ± 0.02
b	1.3 ± 0.2	0.6 ± 0.2	4.9 ± 0.9	0.4 ± 0.04
c	0.8 ± 0.04	0.7 ± 0.1	3.9 ± 0.2	0.3 ± 0.04
d	0.3 ± 0.1	0.2 ± 0.1	1.4 ± 0.3	0.4 ± 0.01
e	0.2 ± 0.1	0.2 ± 0.1	1.2 ± 0.3	0.1 ± 0.02
f	0.6 ± 0.2	0.8 ± 0.4	2.7 ± 0.8	0.2 ± 0.01
g	0.3 ± 0.04	0.2 ± 0.02	0.8 ± 0.1	0.2 ± 0.08
h	0.4 ± 0.1	0.5 ± 0.1	2.2 ± 0.3	0.2 ± 0.03
i	0.3 ± 0.02	0.4 ± 0.1	1.3 ± 0.1	0.1 ± 0.02
j	0.8 ± 0.2	0.5 ± 0.3	2.0 ± 0.5	0.2 ± 0.04
k	0.6 ± 0.1	0.5 ± 0.1	1.9 ± 0.02	0.2 ± 0.09

n=11 triplicate

Table 17. The regression equations, concentration ranges and correlation coefficients of constituent in rat serum of San-Huang-Shel-Shin-Tang.

Constituent	Regression equations	Con.c range ( $\mu\text{g/mL}$ )	r
baicalein	$Y = 0.7442 X + 0.1122$	0.3 ~ 20.0	0.9997
wogonin	$Y = 0.5826 X + 0.0248$	0.2 ~ 5.0	0.9999
emodin	$Y = 1.0670 X + 0.0274$	0.2 ~ 10.0	0.9999
aloe-emodin	$Y = 1.6732 X + 0.1045$	0.2 ~ 10.0	0.9999
rhein	$Y = 0.9073 X + 0.0962$	0.2 ~ 10.0	0.9999
chrysophanol	$Y = 3.0880 X + 0.0723$	0.1 ~ 5.0	0.9999

Table 18. Intra-run and inter-run analytical precision and accuracy of baicalein in rat serum

Conc ( $\mu\text{g/mL}$ )	Intra-run		Inter-run	
	Precision	Accuracy	Precision	Accuracy
	Mean $\pm$ S.D. (C.V.%)	(%)	Mean $\pm$ S.D. (C.V.%)	(%)
20.0	20.4 $\pm$ 2.7(0.0)	2.2	19.4 $\pm$ 0.4(2.3)	-3.2
10.0	10.7 $\pm$ 2.7(0.0)	7.2	9.8 $\pm$ 0.6(5.8)	-2.1
5.0	5.2 $\pm$ 2.7(0.0)	4.0	5.2 $\pm$ 0.1(1.2)	3.6
2.5	2.5 $\pm$ 2.7(0.0)	0.6	2.2 $\pm$ 0.1 (5.6)	-12.1
1.3	1.3 $\pm$ 2.7(0.0)	0.8	1.1 $\pm$ 0.08(6.8)	-8.2
0.6	0.6 $\pm$ 2.7(0.0)	1.8	0.7 $\pm$ 0.07(10.5)	7.4
0.3	0.4 $\pm$ 2.7(0.0)	24.3	0.4 $\pm$ 0.03(6.8)	30.9

n=3

Table 19. Intra-run and inter-run analytical precision and accuracy of wogonin in rat serum

Conc ( $\mu\text{g/mL}$ )	Intra-run		Inter-run	
	Precision	Accuracy	Precision	Accuracy
	Mean $\pm$ S.D. (C.V.%)	(%)	Mean $\pm$ S.D. (C.V.%)	(%)
5.0	5.0 $\pm$ 0.5(9.3)	0.3	4.6 $\pm$ 0.4(7.6)	-7.4
2.5	2.6 $\pm$ 0.2(6.8)	4.9	2.4 $\pm$ 0.2(6.8)	-2.3
1.3	1.4 $\pm$ 0.2(16.5)	8.7	1.3 $\pm$ 0.1(5.1)	4.1
0.6	0.6 $\pm$ 0.02(2.8)	-6.6	0.6 $\pm$ 0.01(1.6)	-5.6
0.3	0.3 $\pm$ 0.01(4.0)	-7.7	0.3 $\pm$ 0.01(3.4)	-11.9
0.2	0.2 $\pm$ 0.01(9.3)	2.1	0.1 $\pm$ 0.02(17.7)	-21.8

n=3

Table 20. Intra-run and inter-run analytical precision and accuracy of emodin in rat serum

Conc ( $\mu\text{g/mL}$ )	Intra-run		Inter-run	
	Precision	Accuracy	Precision	Accuracy
	Mean $\pm$ S.D. (C.V.%)	(%)	Mean $\pm$ S.D. (C.V.%)	(%)
10.0	10.4 $\pm$ 1.0(2.3)	3.7	10.4 $\pm$ 1.1(10.6)	4.2
5.0	5.2 $\pm$ 0.5(5.8)	3.5	5.1 $\pm$ 0.3(5.8)	2.3
2.5	2.5 $\pm$ 0.1(1.2)	1.4	2.8 $\pm$ 0.1(4.8)	10.6
1.3	1.2 $\pm$ 0.1 (5.6)	-1.3	1.3 $\pm$ 0.1 (7.4)	4.6
0.6	0.6 $\pm$ 0.01(6.8)	-4.7	0.6 $\pm$ 0.03(5.1)	3.0
0.3	0.3 $\pm$ 0.02(10.5)	-16.1	0.3 $\pm$ 0.02(8.0)	-1.1
0.2	0.1 $\pm$ 0.00(6.8)	-19.8	0.1 $\pm$ 0.01(4.7)	-17.6

n=3

Table 21. Intra-run and inter-run analytical precision and accuracy of aloe-emodin in rat serum

Conc ( $\mu\text{g/mL}$ )	Intra-run		Inter-run	
	Precision	Accuracy	Precision	Accuracy
	Mean $\pm$ S.D. (C.V.%)	(%)	Mean $\pm$ S.D. (C.V.%)	(%)
10.0	10.0 $\pm$ 1.0(9.1)	-0.5	10.3 $\pm$ 0.2(2.1)	0.3
5.0	4.8 $\pm$ 0.5(3.1)	-3.7	4.9 $\pm$ 0.2(4.6)	-1.8
2.5	2.5 $\pm$ 0.1(6.1)	-1.0	2.5 $\pm$ 0.1(5.6)	0.3
1.3	1.2 $\pm$ 0.1 (1.7)	-1.7	1.3 $\pm$ 0.1 (10.1)	5.4
0.6	0.6 $\pm$ 0.01(6.3)	-3.4	0.6 $\pm$ 0.02(4.0)	-7.5
0.3	0.3 $\pm$ 0.02(12.2)	-1.1	0.4 $\pm$ 0.02(4.8)	10.9
0.2	0.2 $\pm$ 0.00(12.2)	2.9	0.2 $\pm$ 0.01(3.9)	10.7

n=3

Table 22. Intra-run and inter-run analytical precision and accuracy of rhein in rat

serum				
Conc	Intra-run		Inter-run	
	Precision	Accuracy	Precision	Accuracy
( $\mu\text{g/mL}$ )	Mean $\pm$ S.D. (C.V.%)	(%)	Mean $\pm$ S.D. (C.V.%)	(%)
10.0	9.6 $\pm$ 0.6(6.1)	-3.7	10.3 $\pm$ 0.8(7.6)	3.1
5.0	5.4 $\pm$ 0.6(10.3)	7.0	5.2 $\pm$ 0.5(9.9)	3.6
2.5	2.5 $\pm$ 0.3(10.0)	-1.6	2.4 $\pm$ 0.1(3.0)	-3.0
1.3	1.1 $\pm$ 0.1 (7.4)	-10.2	1.3 $\pm$ 0.1 (4.6)	1.7
0.6	0.8 $\pm$ 0.1(9.6)	19.3	0.8 $\pm$ 0.1(14.6)	22.6
0.3	0.4 $\pm$ 0.2(16.6)	27.8	0.3 $\pm$ 0.02(7.5)	-19.0
0.2	0.1 $\pm$ 0.02(21.5)	-42.8	0.2 $\pm$ 0.08(41.8)	22.5

n=3

Table 23. Intra-run and inter-run analytical precision and accuracy of chrysophanol in

rat serum				
Conc	Intra-run		Inter-run	
	Precision	Accuracy	Precision	Accuracy
( $\mu\text{g/mL}$ )	Mean $\pm$ S.D. (C.V.%)	(%)	Mean $\pm$ S.D. (C.V.%)	(%)
5.0	50 $\pm$ 0.4(8.3)	0.76	5.6 $\pm$ 0.1(2.1)	11.7
2.5	2.8 $\pm$ 0.2(7.1)	12.75	2.4 $\pm$ 0.2(9.3)	-2.5
1.3	1.5 $\pm$ 0.04(2.5)	18.24	1.4 $\pm$ 0.04(3.2)	12.6
0.6	0.6 $\pm$ 0.03 (4.3)	-0.68	0.6 $\pm$ 0.04 (6.3)	-9.8
0.3	0.3 $\pm$ 0.03(9.5)	9.08	0.3 $\pm$ 0.03(8.4)	5.8
0.2	0.2 $\pm$ 0.02(9.3)	11.27	0.1 $\pm$ 0.01(8.7)	-10.3
0.1	0.1 $\pm$ 0.00(2.8)	10.59	0.1 $\pm$ 0.01(7.0)	15.9

n=3



Table 24. Limit of detection (LOD) of various constituent in methanol, and limit of quantitation (LOQ) of various constituent in decoction of SHSST and rat serum

Constituent	LOD ( $\mu\text{g/mL}$ )	LOQ( $\mu\text{g/mL}$ )	LOQ ( $\mu\text{g/mL}$ )
	in methanol	in decoction	in serum
Coptisine	1.0	3.1	-
Baicalin	0.04	12.5	-
Palmatine	1.0	3.1	-
Berberine	0.7	15.6	-
Baicalein	0.04	7.8	0.6
Aloe-emodin	0.03	0.8	0.3
Wogonin	0.02	3.1	0.2
Rhein	0.06	3.1	0.3
Emodin	0.04	0.3	0.3
chrysophanol	0.01	0.3	0.2

Table 25. Recoveries (%) of constituent of SHSST from rat serum

Constituent	Conc. Spiked ( $\mu\text{g/mL}$ )				Recoveries (%)
		1	2	3	Mean $\pm$ S.D.
Baicalein	10.0	76.6	79.0	74.8	76.8 $\pm$ 2.1
	2.5	80.5	76.8	77.3	78.2 $\pm$ 2.0
	0.6	80.9	78.6	73.0	77.5 $\pm$ 4.0
Wogonin	2.5	108.9	101.1	102.8	104.3 $\pm$ 4.1
	0.6	93.1	93.2	91.4	92.6 $\pm$ 1.0
	0.2	73.7	79.7	83.1	78.8 $\pm$ 4.8
Emodin	5.0	91.7	89.6	95.0	92.1 $\pm$ 2.7
	1.3	97.4	98.4	98.2	98.0 $\pm$ 0.5
	0.3	106.2	102.6	100.8	103.2 $\pm$ 2.8
Aloe-emodin	5.0	94.4	93.9	101.6	96.6 $\pm$ 4.4
	1.3	93.2	92.3	94.7	93.4 $\pm$ 1.2
	0.3	82.5	85.2	81.1	82.9 $\pm$ 2.1
Rhein	5.0	89.5	90.6	89.7	89.9 $\pm$ 0.6
	1.3	85.4	88.7	88.3	87.5 $\pm$ 1.8
	0.3	80.4	71.1	79.2	76.9 $\pm$ 5.1
chrysophanol	2.5	90.9	92.3	94.6	92.6 $\pm$ 1.8
	0.6	92.9	93.5	94.2	93.5 $\pm$ 0.6
	0.2	118.5	111.3	104.5	111.5 $\pm$ 7.0

n=3

Table 26. Pharmacokinetic parameters of baicalein sulfates and glucuronides in serum after oral administration of decoctions (5.0 g/kg) of and commercial extract (1.1 g/kg) of SHSST to rats (n = 9)

Parameters	T <sub>max</sub> (min)	C <sub>max</sub> (nmol/mL)	AUC <sub>0-2880</sub> (nmol · min/mL)	MRT <sub>0-2880</sub> (min)
Decoction				
sulfates	185.5 ± 246.7	5.7 ± 2.8	4733.9 ± 1850.2	920.8 ± 331.9
glucuronides	36.7 ± 47.7	5.0 ± 2.2	4553.1 ± 2062.2	1081.0 ± 542.2
Commercial extract				
sulfates	44.4 ± 45.9	10.9 ± 2.5	6615.9 ± 2369.8	942.3 ± 221.0
glucuronides	40.0 ± 48.2	11.3 ± 5.1	7798.2 ± 4059.1	1034.1 ± 330.1

Table 27. Pharmacokinetic parameters of aloe-emodin sulfates and glucuronides in serum after oral administration of decoctions (5.0 g/kg) of and commercial extract (1.1 g/kg) of SHSST to rats (n = 9)

Parameters	T <sub>max</sub> (min)	C <sub>max</sub> (nmol/mL)	AUC <sub>0-2880</sub> (nmol · min/mL)	MRT <sub>0-2880</sub> (min)
Decoction				
sulfates	43.3 ± 44.4	3.0 ± 1.3	340.4 ± 176.1	104.4 ± 65.72
glucuronides	31.1 ± 34.8	2.7 ± 1.4	294.6 ± 165.4	207.8 ± 216.0
Commercial extract				
sulfates	20.0 ± 17.3	0.7 ± 0.2	41.3 ± 14.3	45.4 ± 13.6
glucuronides	30.0 ± 37.7	0.8 ± 0.4	62.6 ± 60.5	48.8 ± 24.1

Table 28. Pharmacokinetic parameters of wogonin sulfates and glucuronides in serum after oral administration of decoctions (5.0 g/kg) of and commercial extract (1.1 g/kg) of SHSST to rats (n = 9)

Parameters	T <sub>max</sub> (min)	C <sub>max</sub> (nmol/mL)	AUC <sub>0-2880</sub> (nmol · min/mL)	MRT <sub>0-2880</sub> (min)
Decoction				
sulfates	561.1 ± 443.4	1.1 ± 0.4	1045.5 ± 491.1	960.7 ± 296.3
glucuronides	1202.2 ± 1077.2	1.3 ± 0.8	1479.8 ± 1142.7	1165.0 ± 403.9
Commercial extract				
sulfates	675.5 ± 726.3	1.4 ± 0.9	746.3 ± 524.5	765.9 ± 308.9
glucuronides	357.8 ± 615.2	1.1 ± 0.4	712.6 ± 609.8	850.2 ± 384.0

Table 29. Pharmacokinetic parameters of rhein free form, sulfates and glucuronides in serum after oral administration of decoctions (5.0 g/kg) of and commercial extract (1.1 g/kg) of SHSST to rats (n = 6)

Parameters	T <sub>max</sub> (min)	C <sub>max</sub> (nmol/mL)	AUC <sub>0-2880</sub> (nmol · min/mL)	MRT <sub>0-2880</sub> (min)
Decoction				
free form	16.7 ± 10.3	17.5 ± 4.6	5249.5 ± 3838.8	692.0 ± 293.9
sulfates	75.0 ± 49.3	27.8 ± 15.2	9348.9 ± 5510.3	632.0 ± 226.6
glucuronides	291.6 ± 564.6	22.7 ± 9.0	8736.1 ± 5299.0	779.8 ± 616.4
Commercial extract				
free form	21.7 ± 20.4	13.4 ± 3.5	8212.7 ± 6682.0	999.9 ± 480.4
sulfates	48.3 ± 41.7	12.6 ± 6.3	4184.5 ± 4432.1	536.1 ± 418.8
glucuronides	40.0 ± 43.8	10.9 ± 4.5	2642.6 ± 3128.9	727.2 ± 747.0

Table 30. Pharmacokinetic parameters of emodin sulfates and glucuronides in serum after oral administration of decoctions (5.0 g/kg) of and commercial extract (1.1 g/kg) of SHSST to rats (n = 9)

Parameters	T <sub>max</sub> (min)	C <sub>max</sub> (nmol/mL)	AUC <sub>0-2880</sub> (nmol · min/mL)	MRT <sub>0-32880</sub> (min)
Decoction				
sulfates	57.8 ± 47.1	5.0 ± 2.2	1929.4 ± 1012.6	570.9 ± 198.0
glucuronides	66.7 ± 78.6	4.6 ± 2.0	1221.8 ± 379.3	503.3 ± 388.1
Commercial extract				
sulfates	21.1 ± 10.5	2.4 ± 0.6	1224.6 ± 1116.6	731.2 ± 520.6
glucuronides	30.0 ± 37.7	2.4 ± 0.8	475.7 ± 345.8	390.9 ± 279.0

Table 31. Pharmacokinetic parameters of chrysophanol sulfates and glucuronides in serum after oral administration of decoctions (5.0 g/kg) of and commercial extract (1.1 g/kg) of SHSST to rats (n = 9)

Parameters	T <sub>max</sub> (min)	C <sub>max</sub> (nmol/mL)	AUC <sub>0-2880</sub> (nmol · min/mL)	MRT <sub>0-2880</sub> (min)
Decoction				
sulfates	18.9 ± 10.5	0.7 ± 0.3	169.1 ± 79.6	629.4 ± 502.5
glucuronides	60.0 ± 76.0	0.6 ± 0.3	221.1 ± 209.5	624.3 ± 604.1
Commercial extract				
sulfates	14.4 ± 8.8	0.5 ± 0.2	160.4 ± 107.4	636.4 ± 520.3
glucuronides	14.4 ± 8.8	0.4 ± 0.2	131.3 ± 125.5	765.6 ± 489.4

Table 32. Comparison of AUC<sub>0-2880</sub> of various constituent in serum after oral administration of decoctions (5.0 g/kg) and commercial extract (1.1 g/kg) of SHSST to rats (Rhein n=6, Baicalein , Aloe-emodin , Wogonin , Emodin , Chrysophanol n=9 )

Constituent	Commercial extract	Decoction	Difference(%)
Baicalein	14414.1 ± 5243.7	9287.1 ± 3243.9	55.2 ± 69.1
Aloe-emodin	104.0 ± 70.5	635.0 ± 286.4	-83.6 ± 59.8
Wogonin	1458.9 ± 796.8	2525.3 ± 1476.9	-42.2 ± 70.9
Rhein	15039.7 ± 10206.3	23334.5 ± 11764.6	-35.5 ± 69.1
Emodin	1700.3 ± 1452.6	3151.2 ± 1087.8	-46.0 ± 59.7
Chrysophanol	291.7 ± 185.5	390.2 ± 278.6	-25.2 ± 87.7

The AUC of each constituent include its sulfates and glucuronides except rhein, which includes free from, sulfate and glucuronide conjugates.

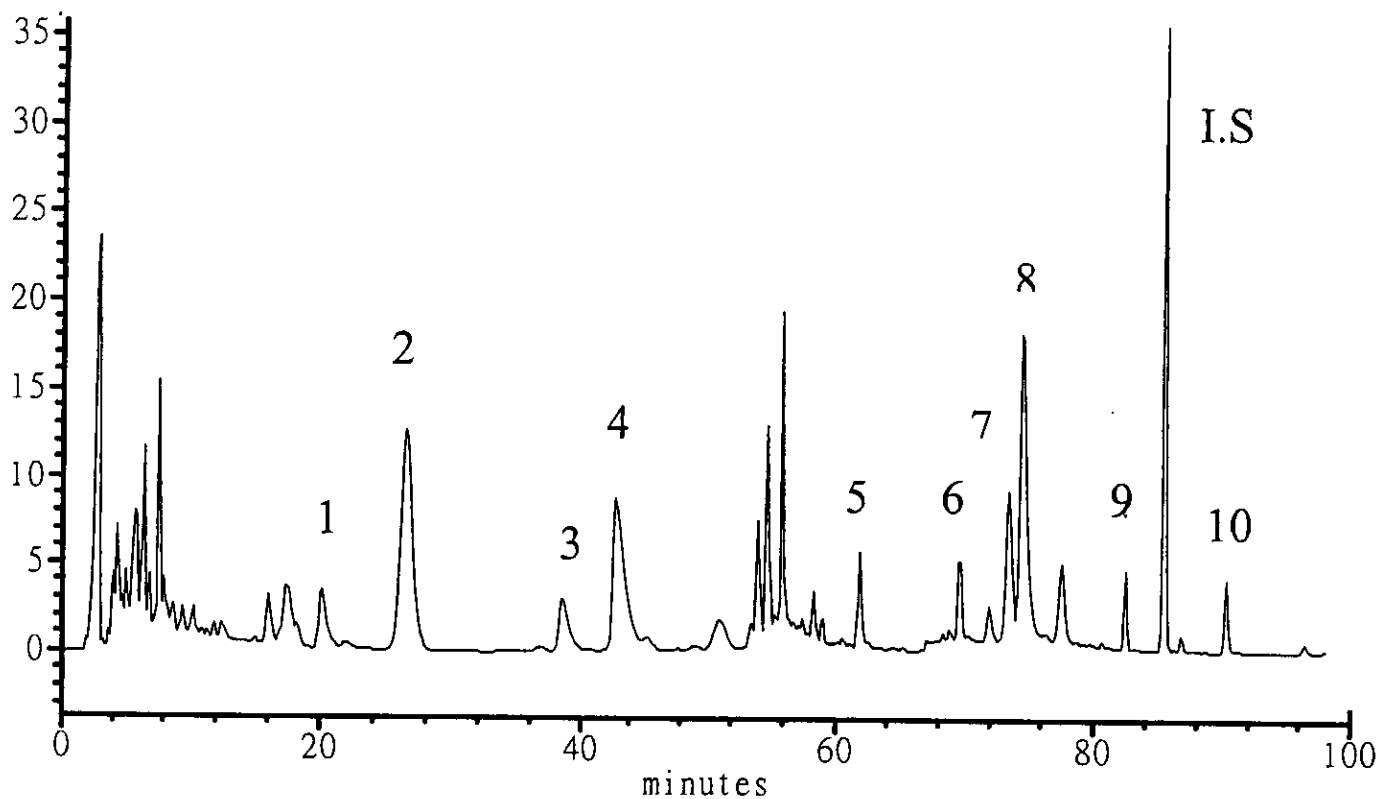


Fig. 1. The HPLC chromatogram of commercial extract of SHSST

1. coptisine 2. baicalin 3. palmatine 4. berberine 5. baicalein

6. aloe-emodin 7. wogonin 8. rhein 9. emodin 10. chrysophanol

I.S. : 2-methylantraquinone

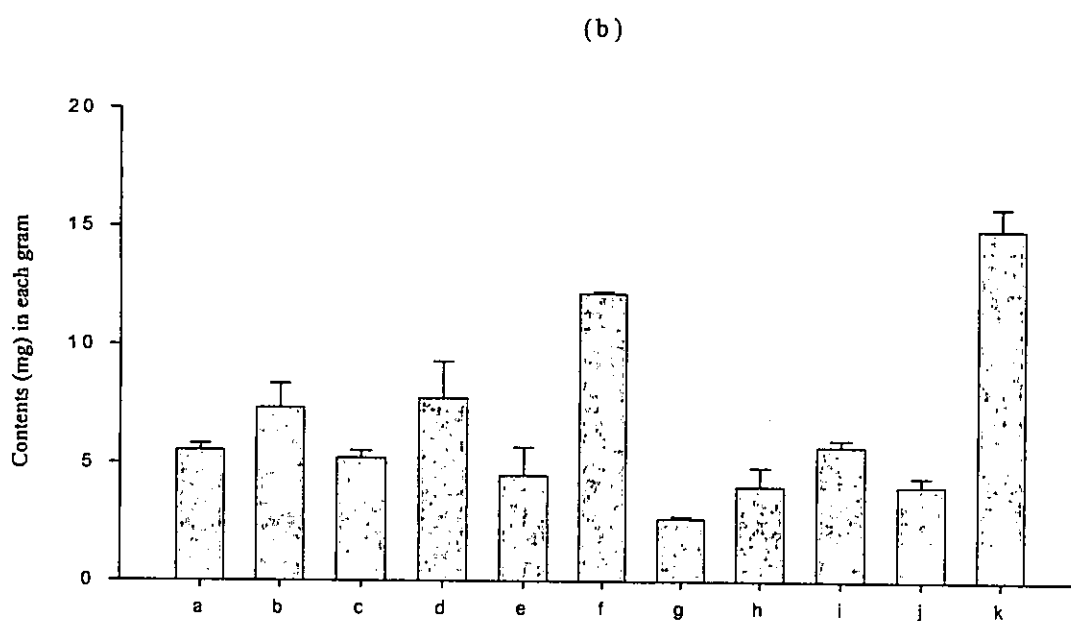
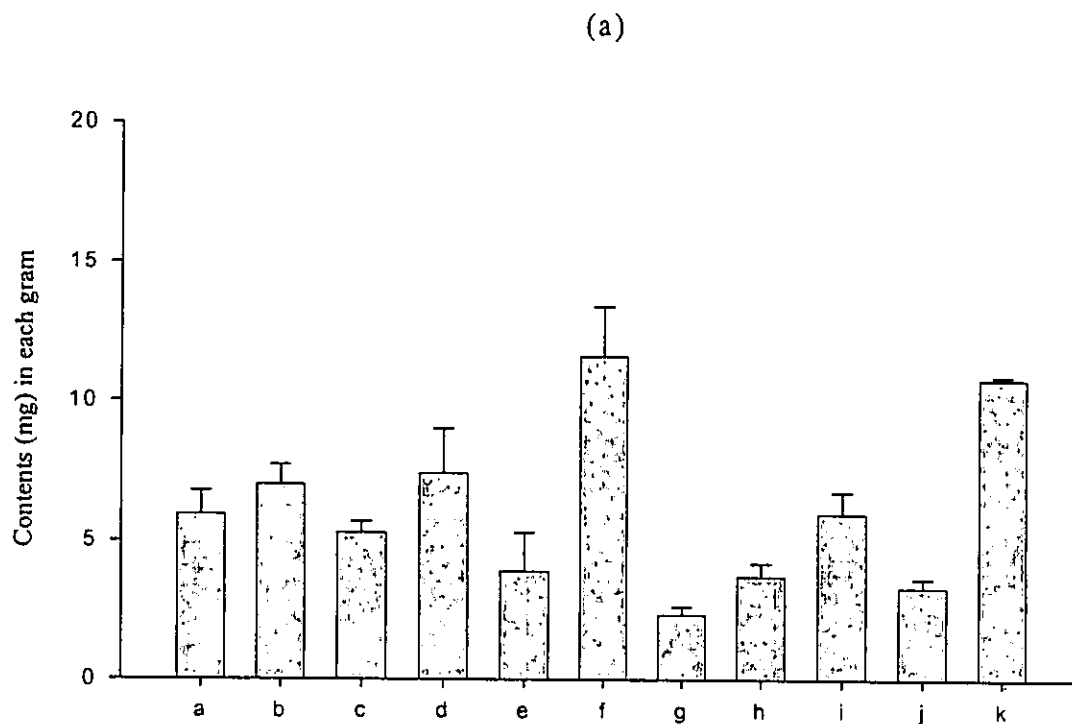


Fig. 2. Comparison of mean ( $\pm$ S.D.) contents (mg) of coptisine in each gram of commercial extract of SHSST prior to (a) and after (b) hydrolysis with  $\beta$ -glucosidase.



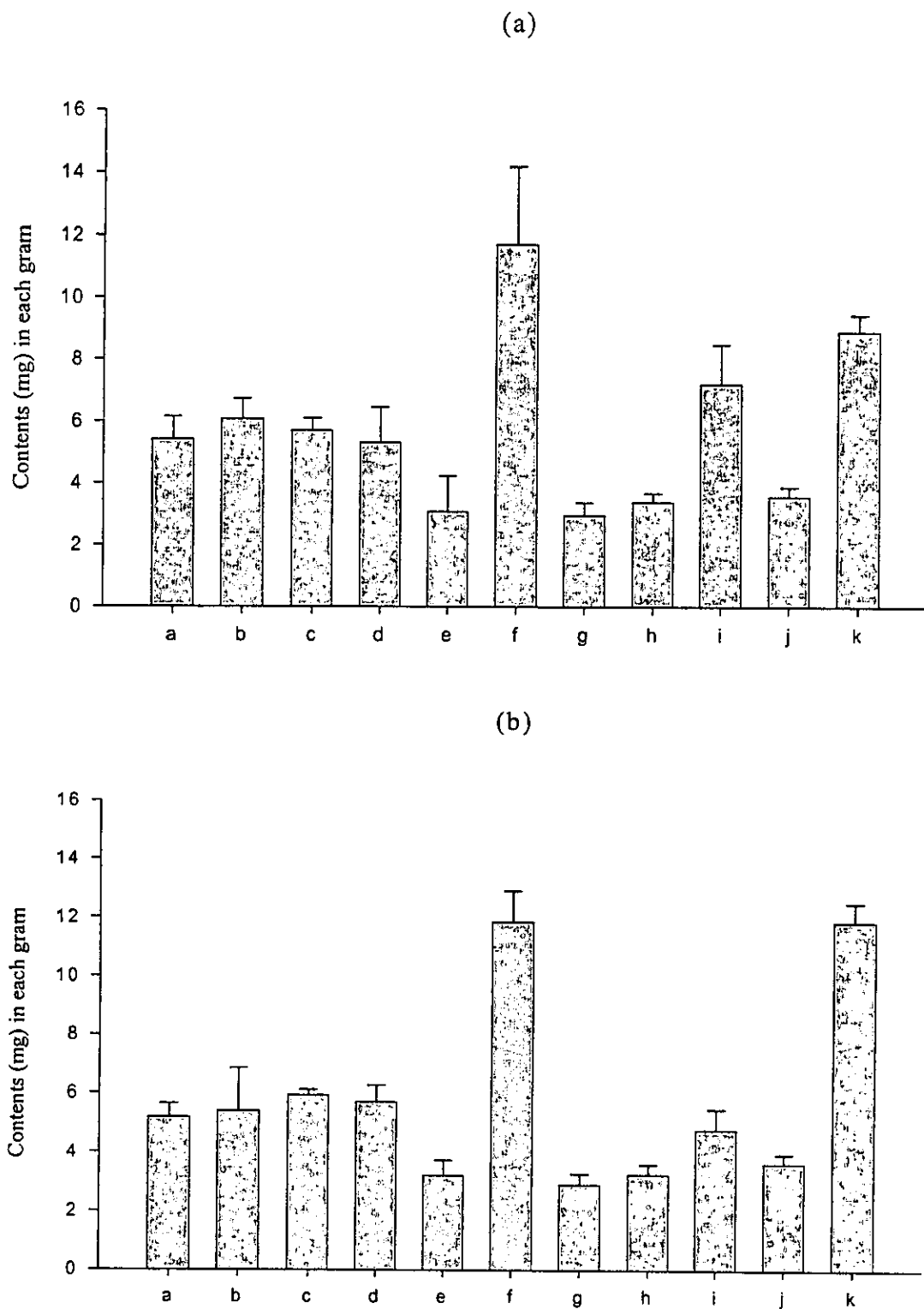


Fig. 3. Comparison of mean ( $\pm$ S.D.) contents (mg) of palmatine in each gram of commercial extract of SHSST prior to (a) and after (b) hydrolysis with  $\beta$ -glucosidase.

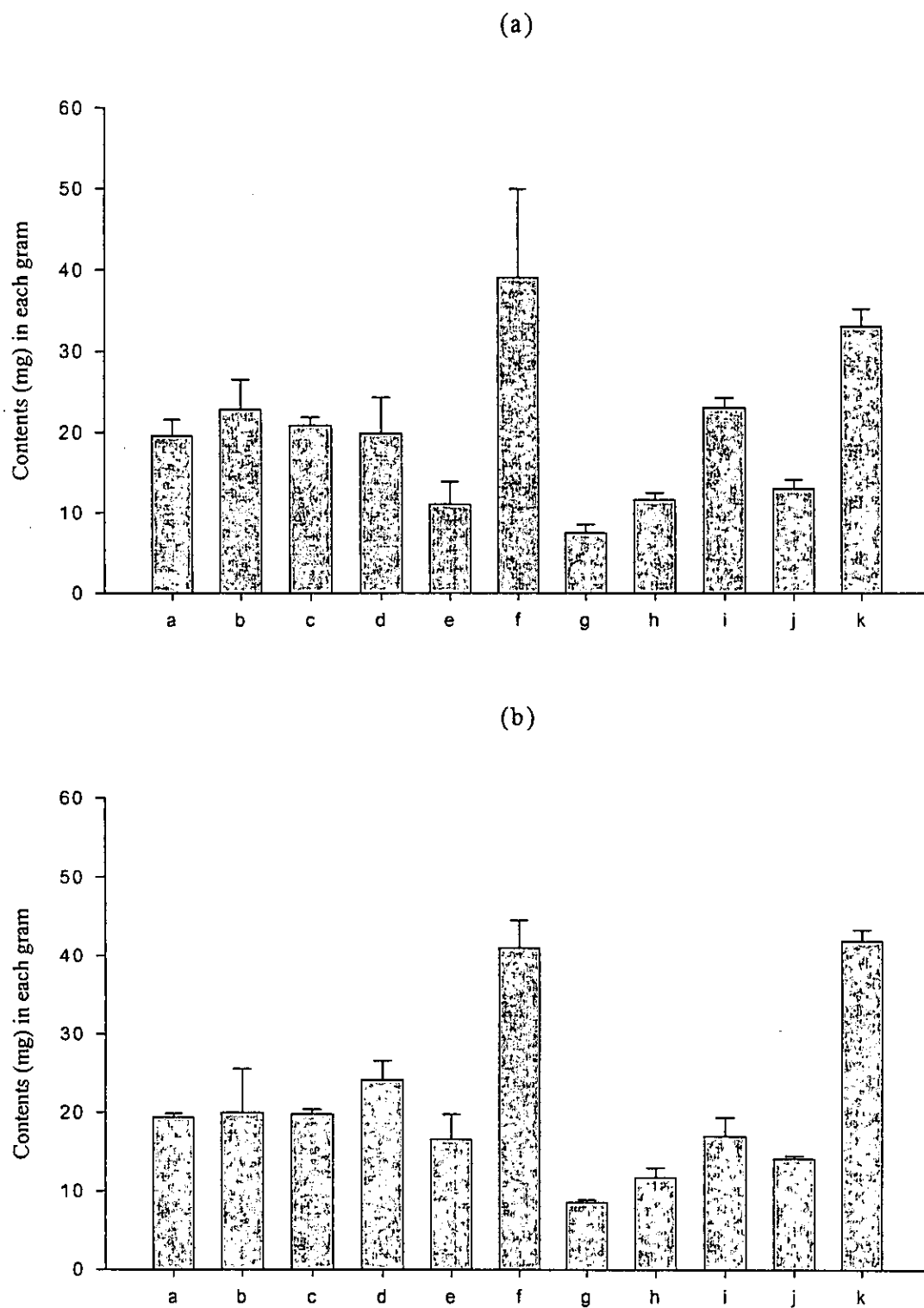


Fig. 4. Comparison of mean ( $\pm$ S.D.) contents (mg) of berberine in each gram of commercial extract of SHSST prior to (a) and after (b) hydrolysis with  $\beta$ -glucosidase.

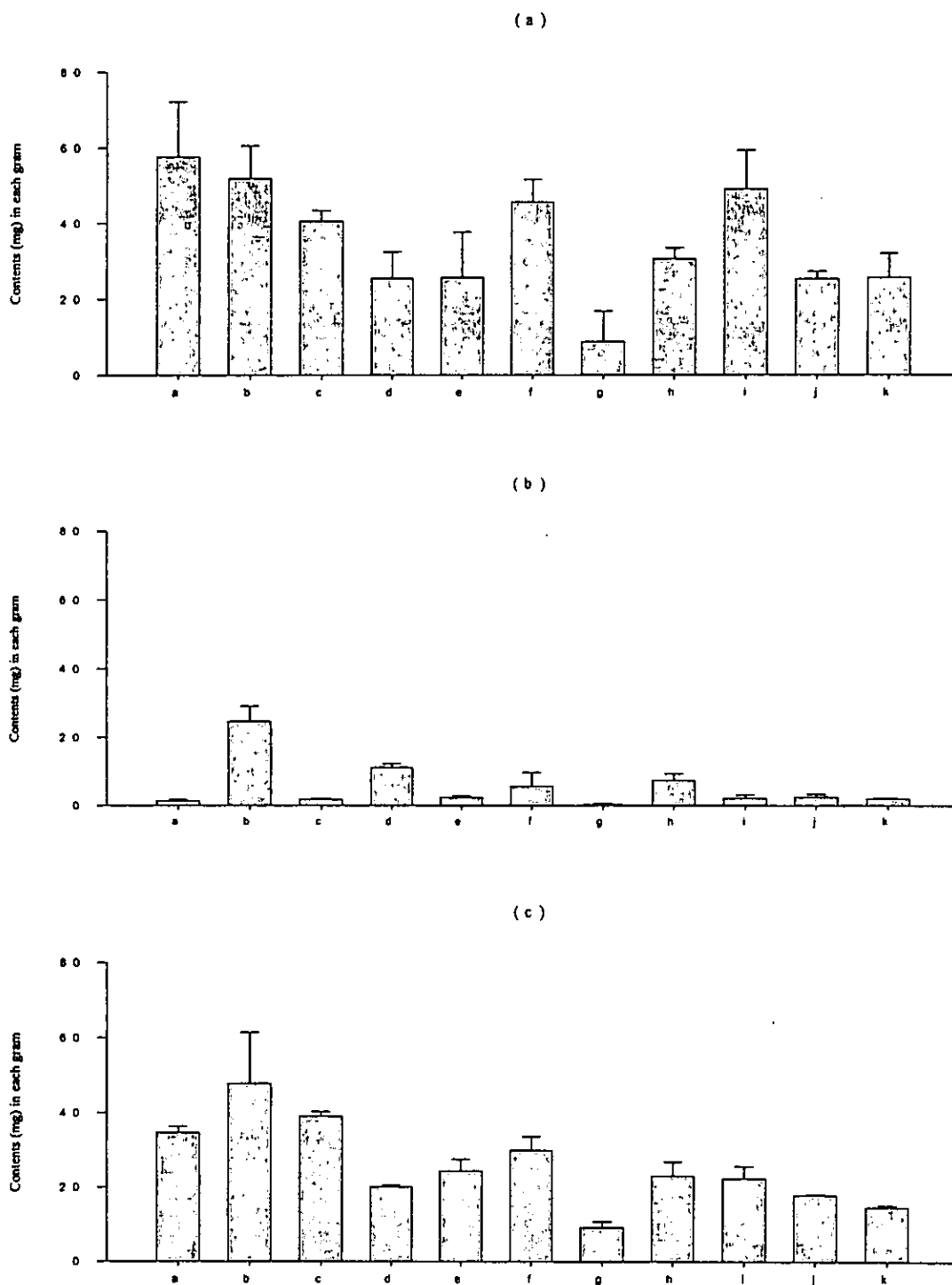


Fig. 5. Comparison of mean ( $\pm$ S.D.) contents (mg) of baicalin and baicalein in each gram of commercial extract. Sample were hydrolyzed with and without  $\beta$ -glucosidase. (a) expressed baicalin before hydrolysis. (b) expressed baicalein before hydrolysis. (c) expressed baicalein after hydrolysis.

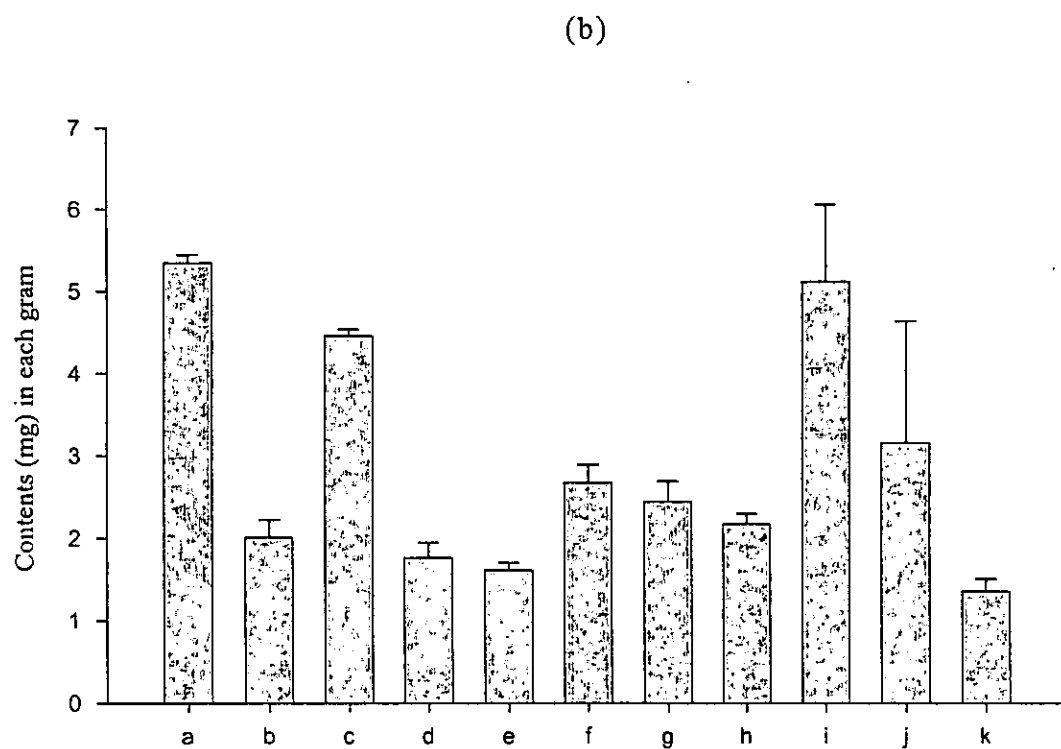
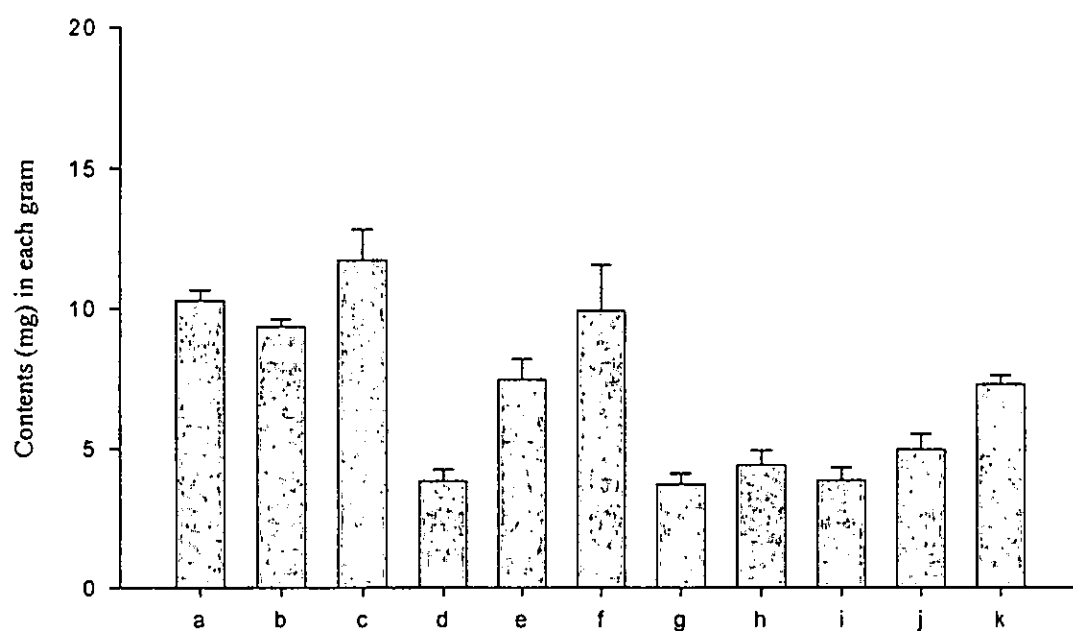


Fig. 6. Comparison of mean ( $\pm$ S.D.) contents (mg) of aloe-emodin in each gram of commercial extract of SHSST prior to (a) and after (b) hydrolysis with  $\beta$ -glucosidase.

(a)



(b)

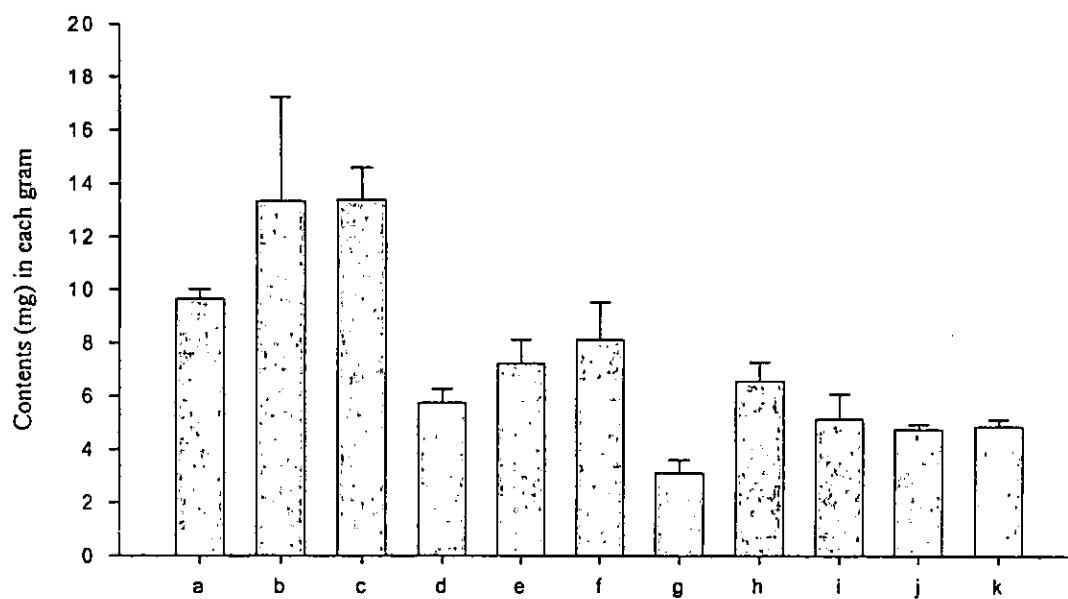


Fig. 7. Comparison of mean ( $\pm$ S.D.) contents (mg) of wogonin in each gram of commercial extract of SHSST prior to (a) and after (b) hydrolysis with  $\beta$ -glucosidase.

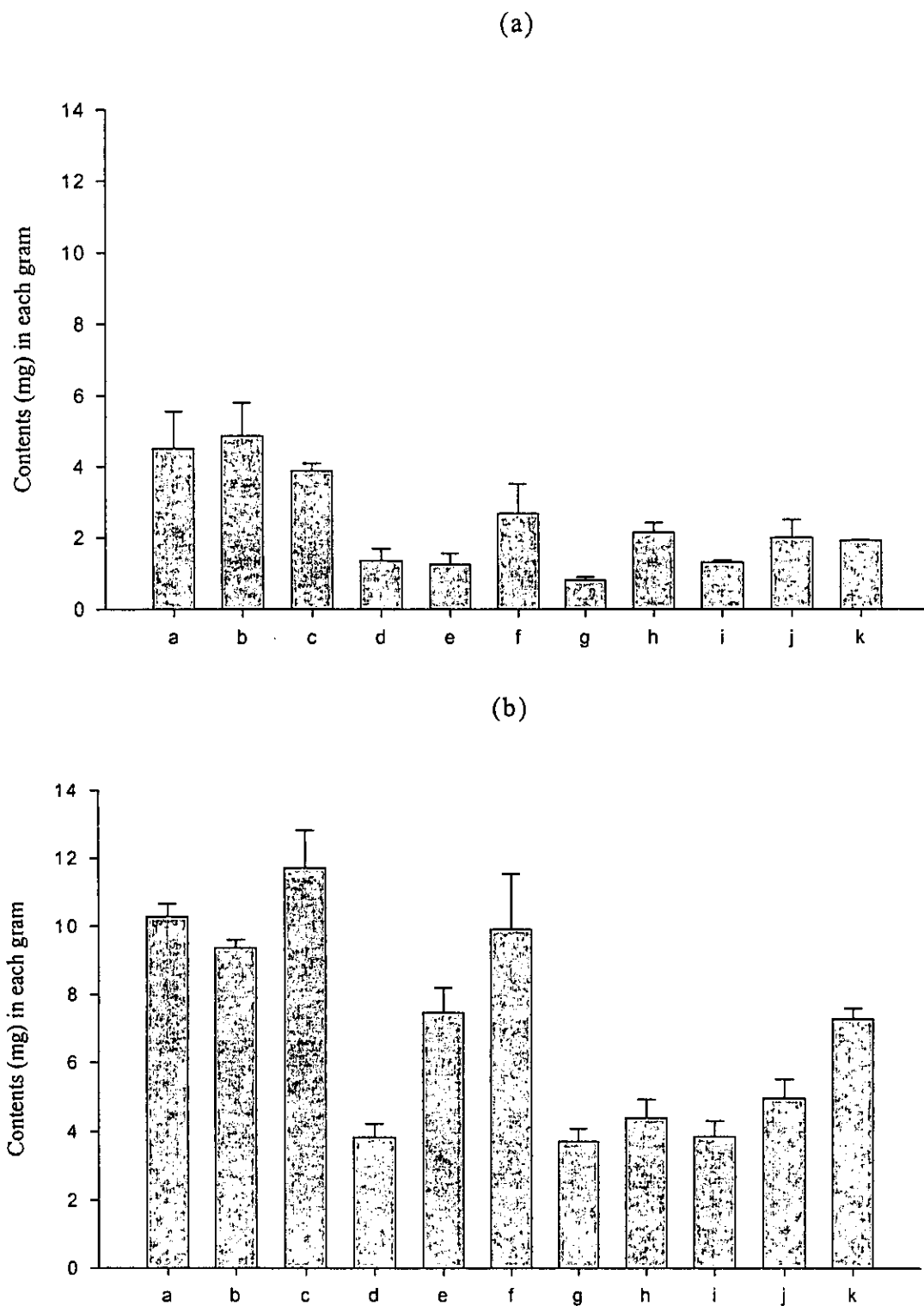
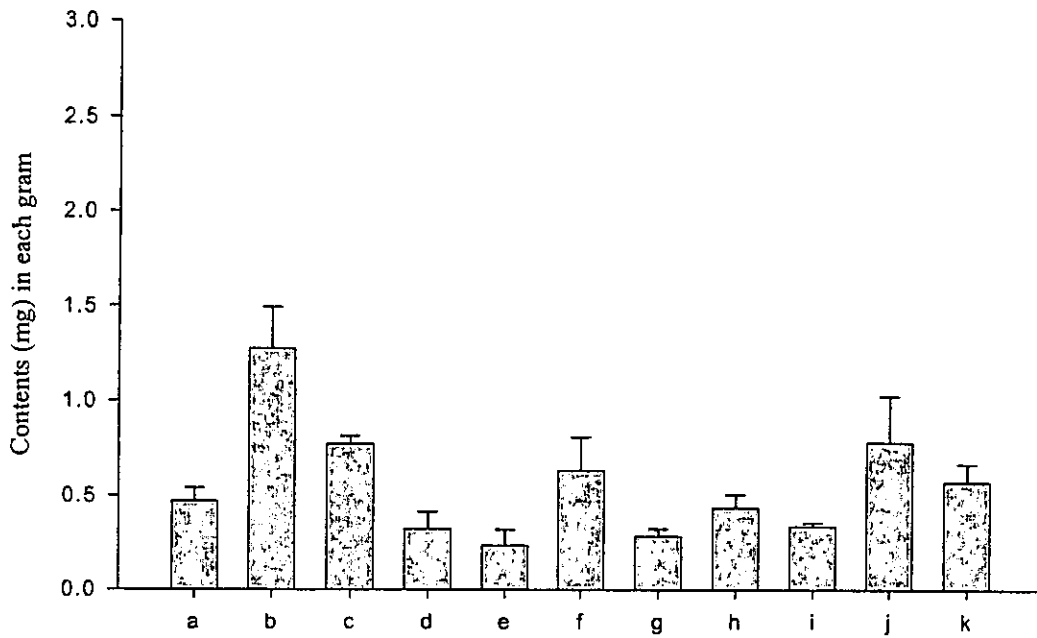


Fig. 8. Comparison of mean ( $\pm$ S.D.) contents (mg) of rhein in each gram of commercial extract of SHSST prior to (a) and after (b) hydrolysis with  $\beta$ -glucosidase.

(a)



(b)

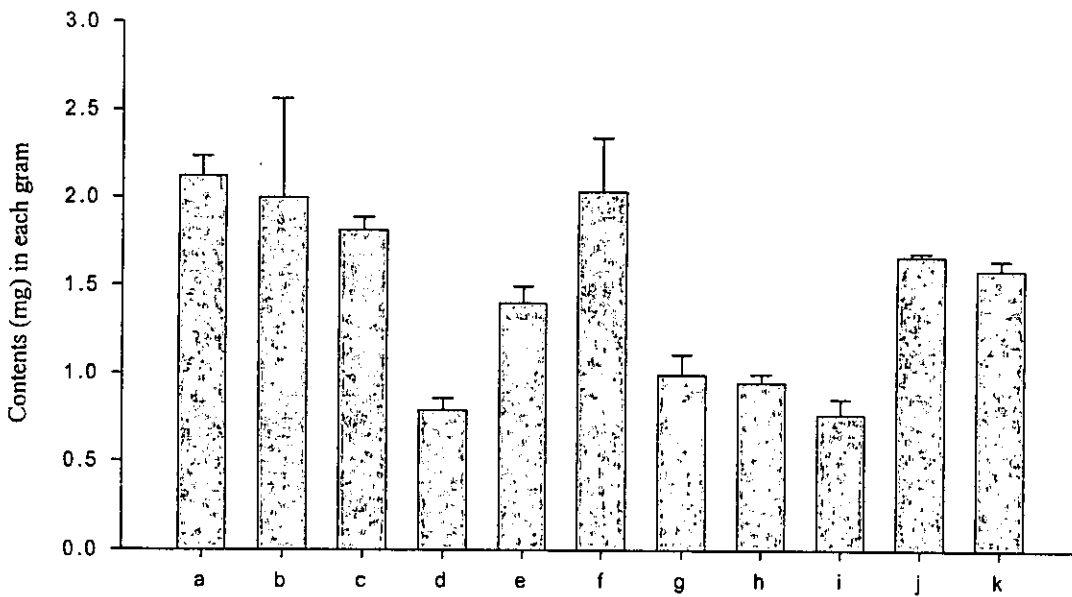
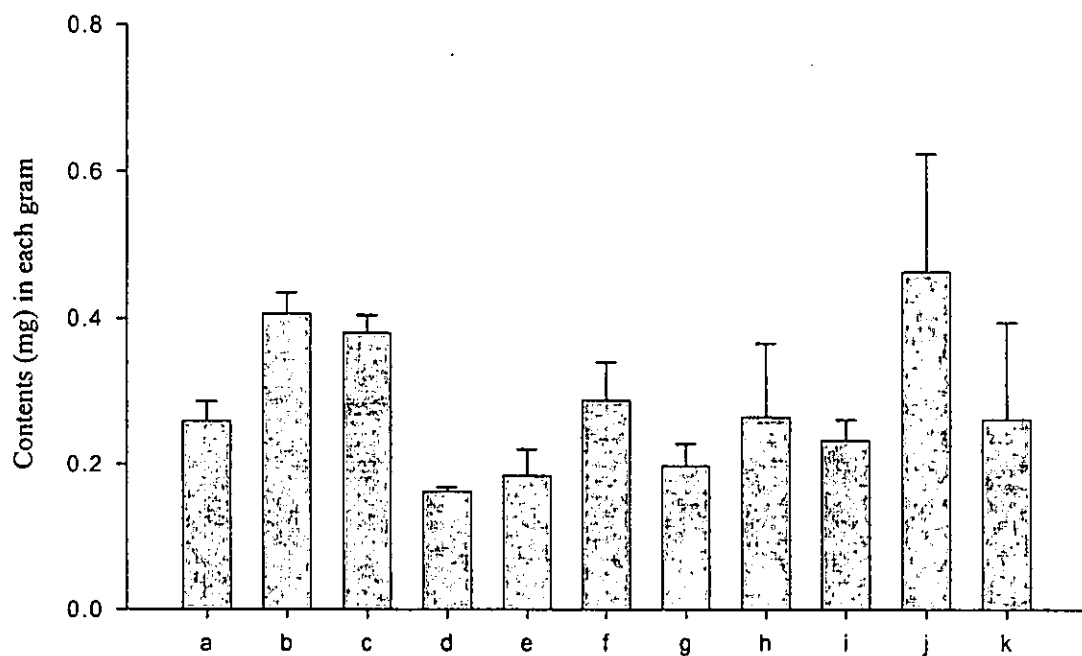


Fig. 9. Comparison of mean ( $\pm$ S.D.) contents (mg) of emodin in each gram of commercial extract of SHSST prior to (a) and after (b) hydrolysis with  $\beta$ -glucosidase.

(a)



(b)

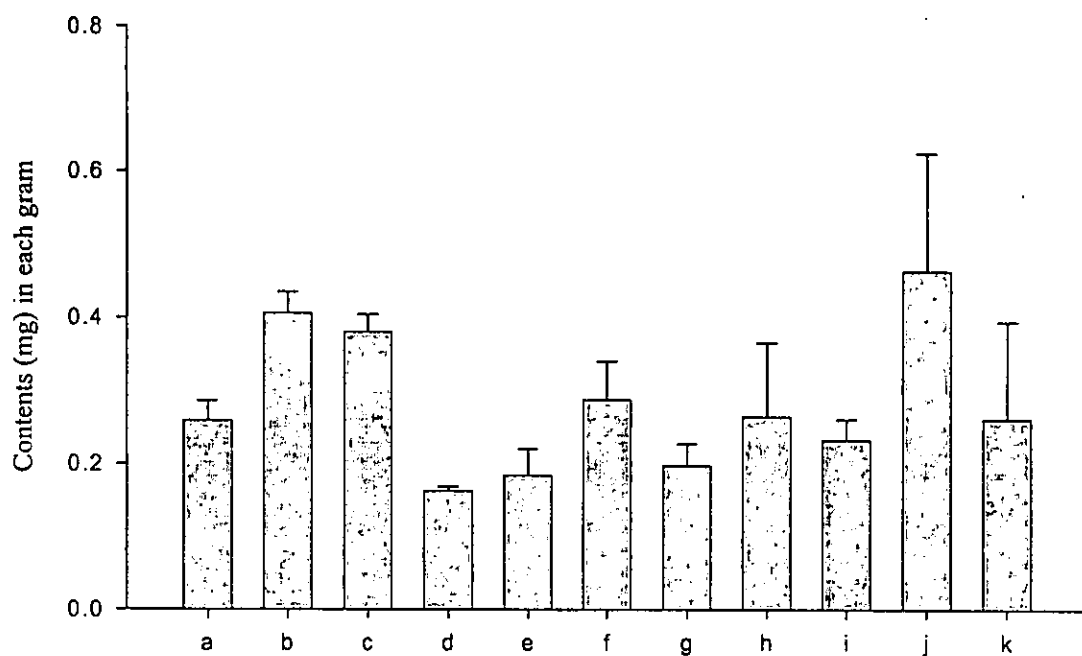


Fig. 10. Comparison of mean ( $\pm$ S.D.) contents (mg) of chrysophanol in each gram of commercial extract of SHSST prior to (a) and after (b) hydrolysis with  $\beta$ -glucosidase.



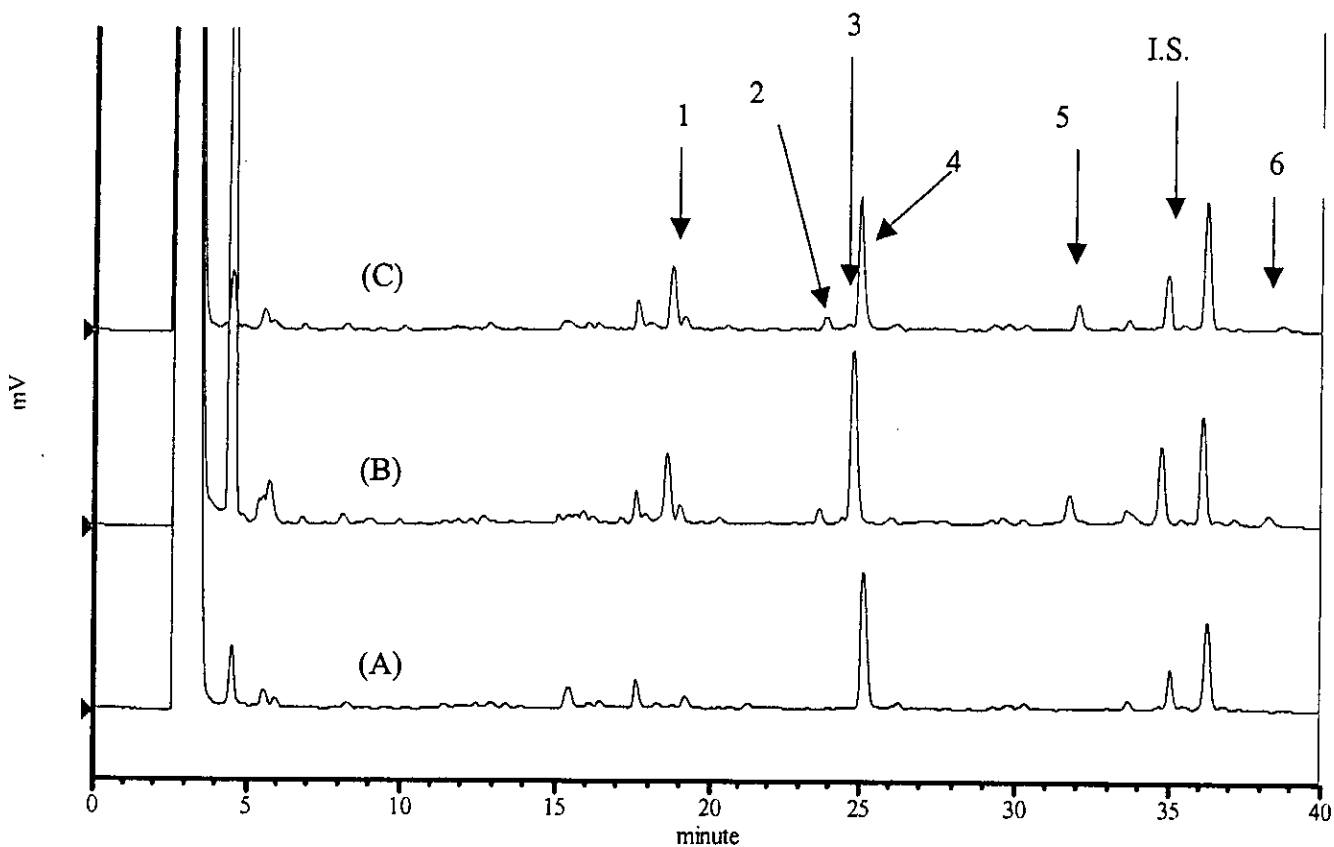


Fig. 11. HPLC chromatogram of rat serum after dosing with SHSST. (A) serum sample ;(B) serum sample hydrolyzed with sulfatase ; (C) serum sample hydrolyzed with glucuronidase

1. baicalein    2. aloe-emodin    3. wogonin    4. rhein    5. emodin

6. chrysophanol

I.S. : 2-methylanthraquinone

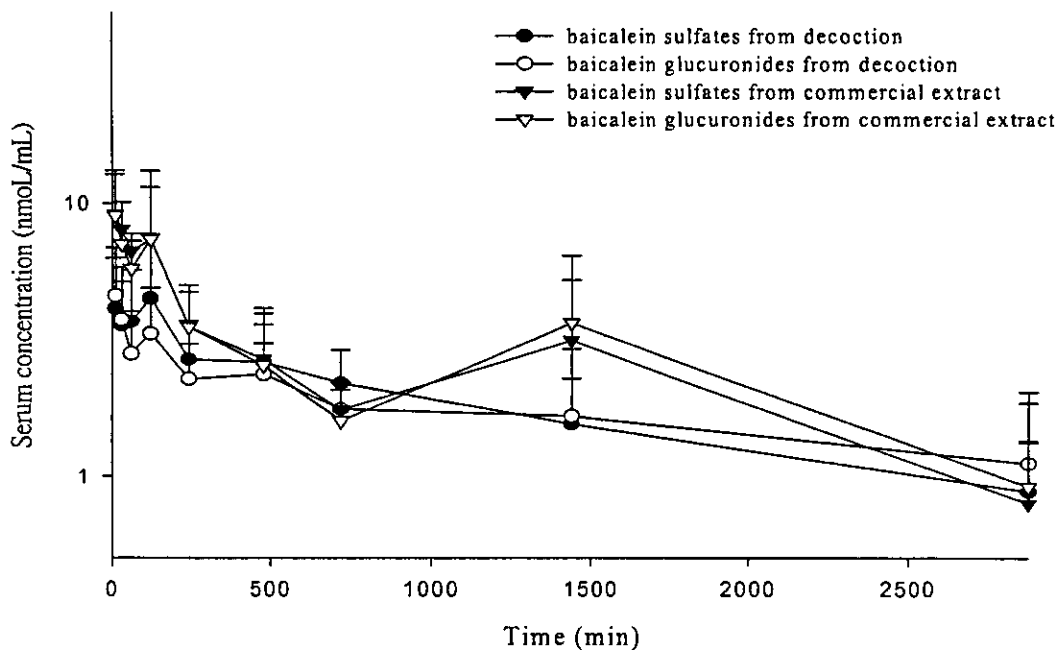
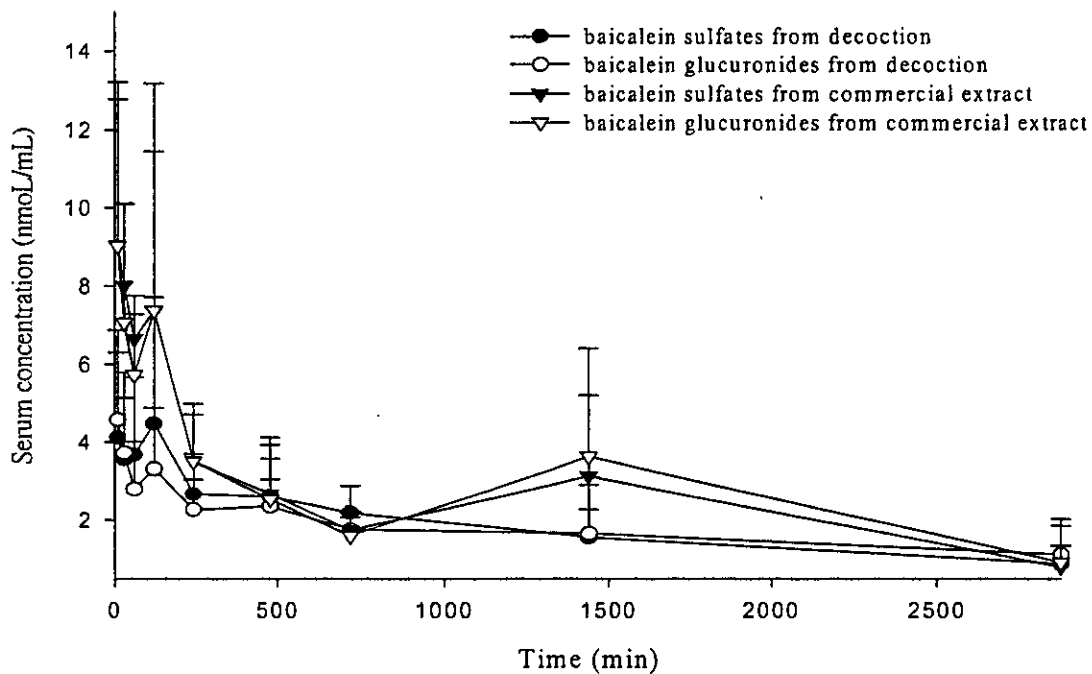


Fig. 12 (a) Comparison of mean ( $\pm$ S.D.) serum concentration-time profiles of baicalein conjugates (sulfates or glucuronides) after oral administration of decoction and commercial extract of SHSST in rats ( $n = 9$ ); (b) the semi-log diagram of (a).

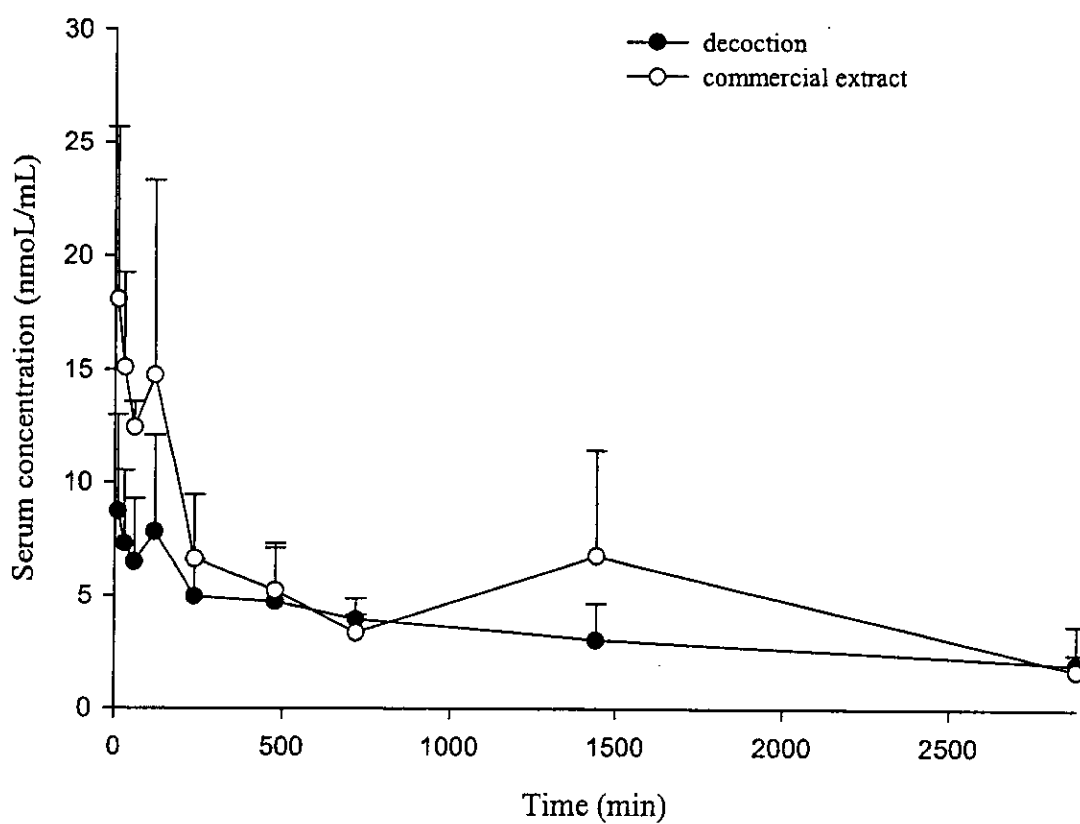


Fig. 13 Comparison of mean ( $\pm$ S.D.) serum concentration-time profiles of baicalein conjugates (sulfates and glucuronides) after administration oral of decoction and commercial extract of SHSST. Conjugates represent the sum of sulfates and glucuronides. (n = 9)

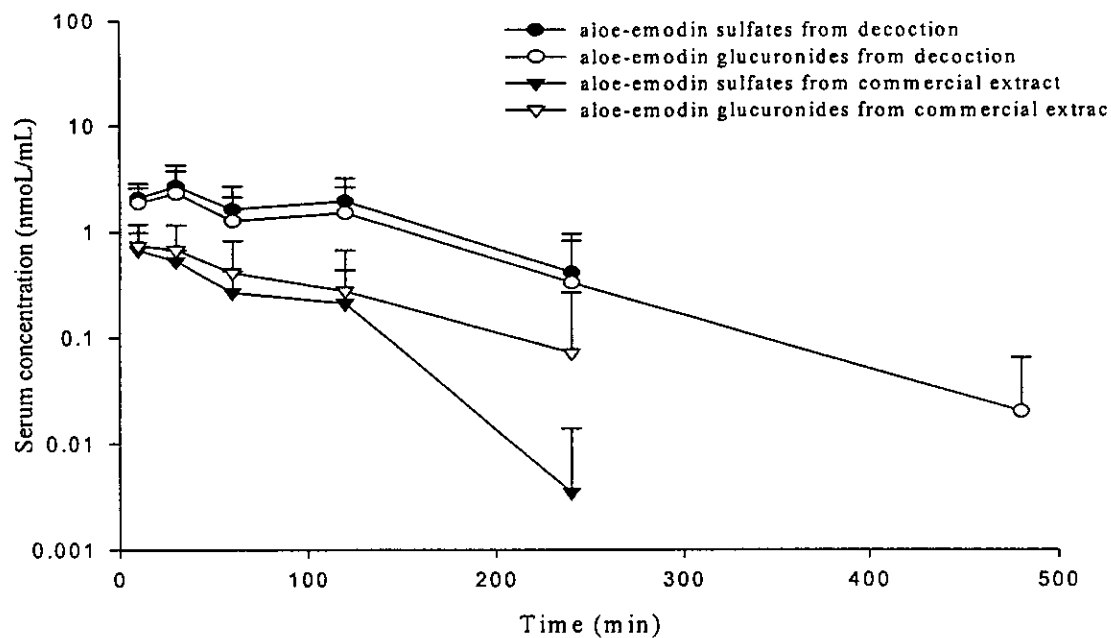
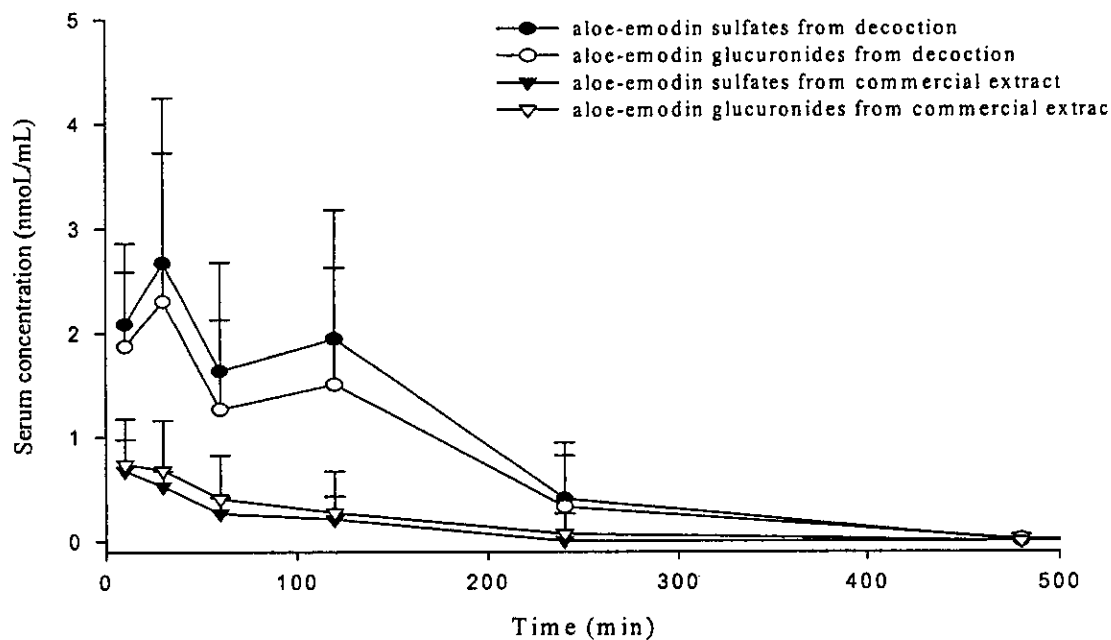


Fig. 14 (a) Comparison of mean ( $\pm$ S.D.) serum concentration-time profiles of aloe-emodin conjugates(sulfates or glucuronides) after oral administration of decoction and commercial extract of SHSST in rats (n =9) ; (b) the semi-log diagram of (a).

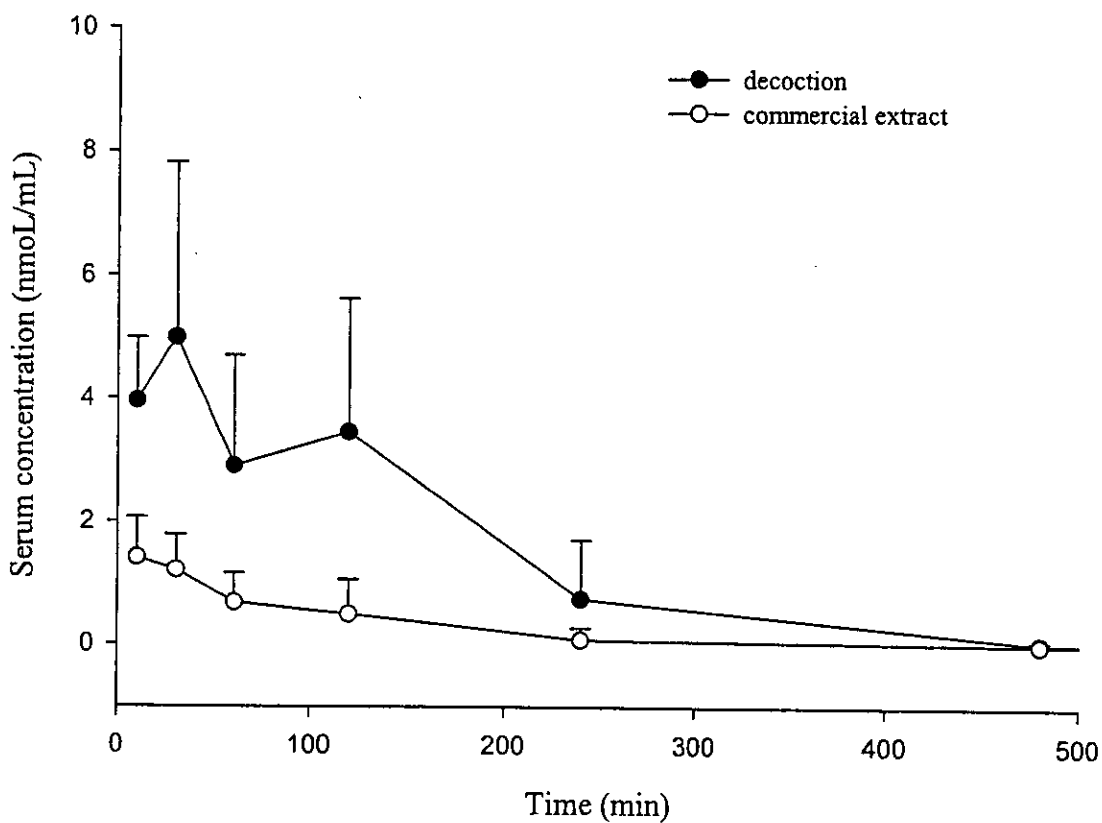


Fig. 15 Comparison of mean ( $\pm$ S.D.) serum concentration-time profiles of aloe-emodin conjugates (sulfates and glucuronides) after oral administration of decoction and commercial extract of SHSST. Conjugates represent the sum of sulfates and glucuronides. (n =9)

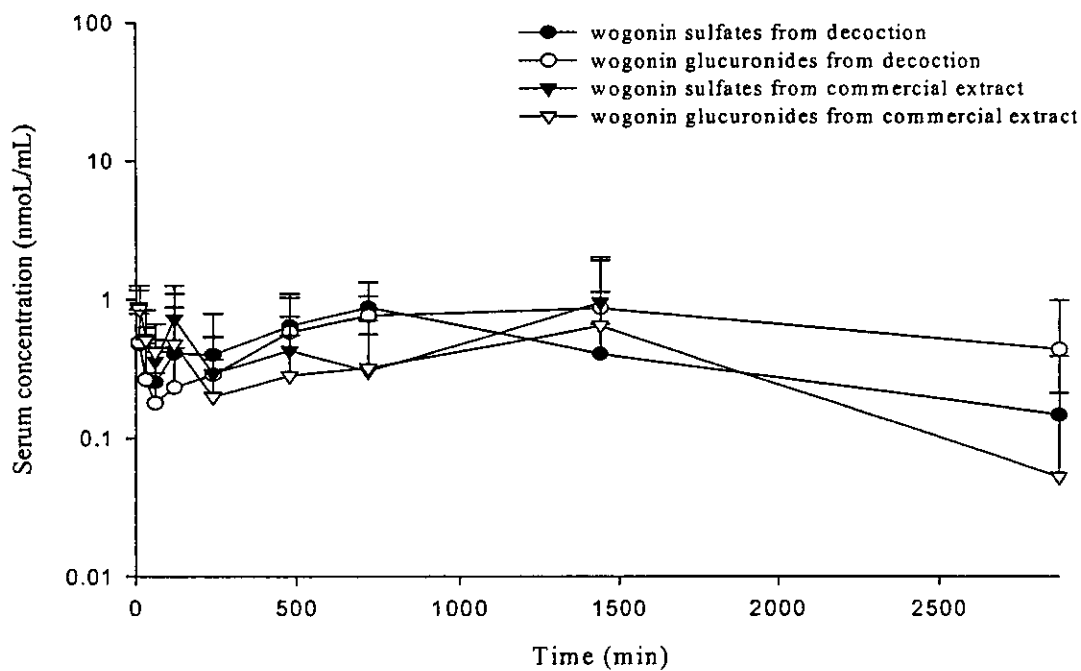
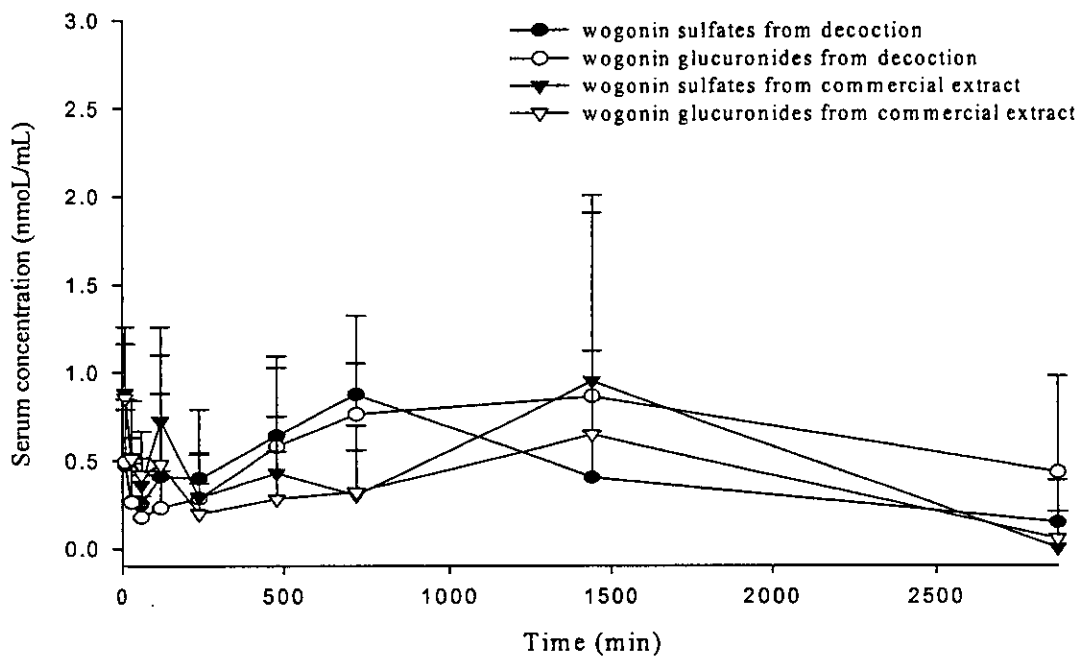


Fig. 16 (a) Comparison of mean ( $\pm$ S.D.) serum concentration-time profiles of wogonin conjugates (sulfates or glucuronides) after oral administration of decoction and commercial extract of SHSST in rats ( $n = 9$ ) ; (b) the semi-log diagram of (a).

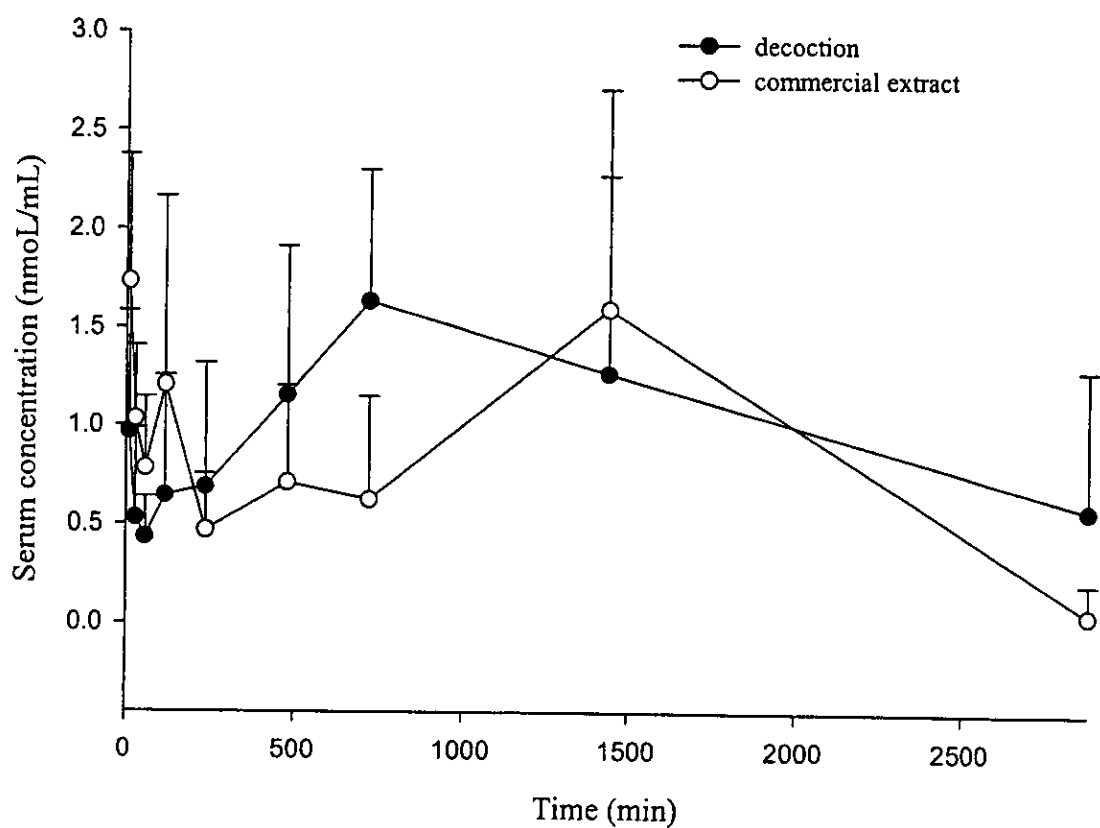


Fig. 17 Comparison of mean ( $\pm$ S.D.) serum concentration-time profiles of wogonin conjugates (sulfates and glucuronides) after oral administration of decoction and commercial extract of SHSST. Conjugates represent the sum of sulfates and glucuronides. (n =9)

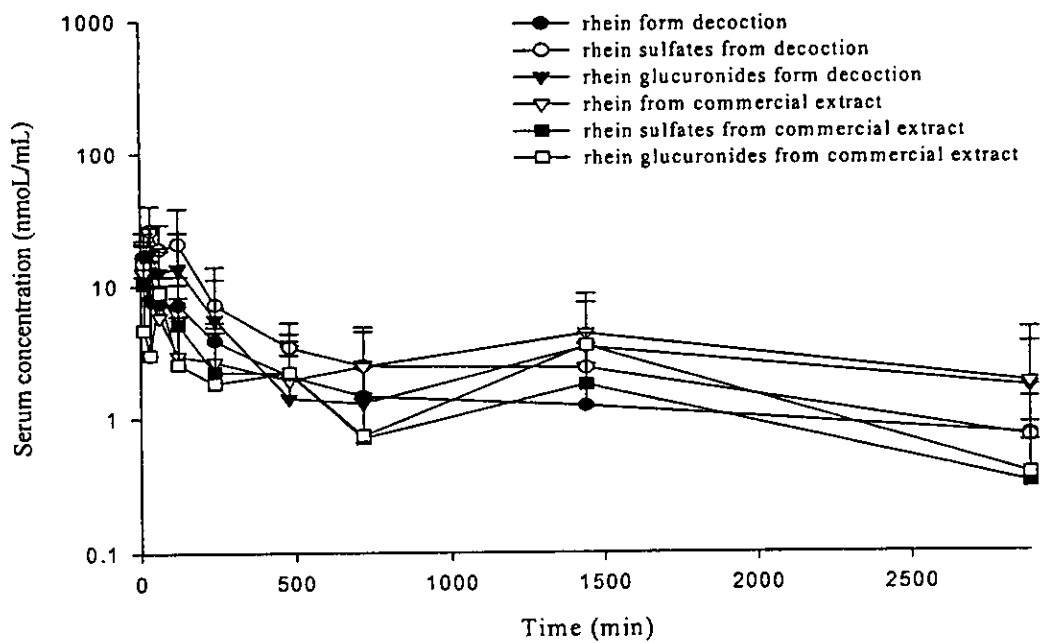
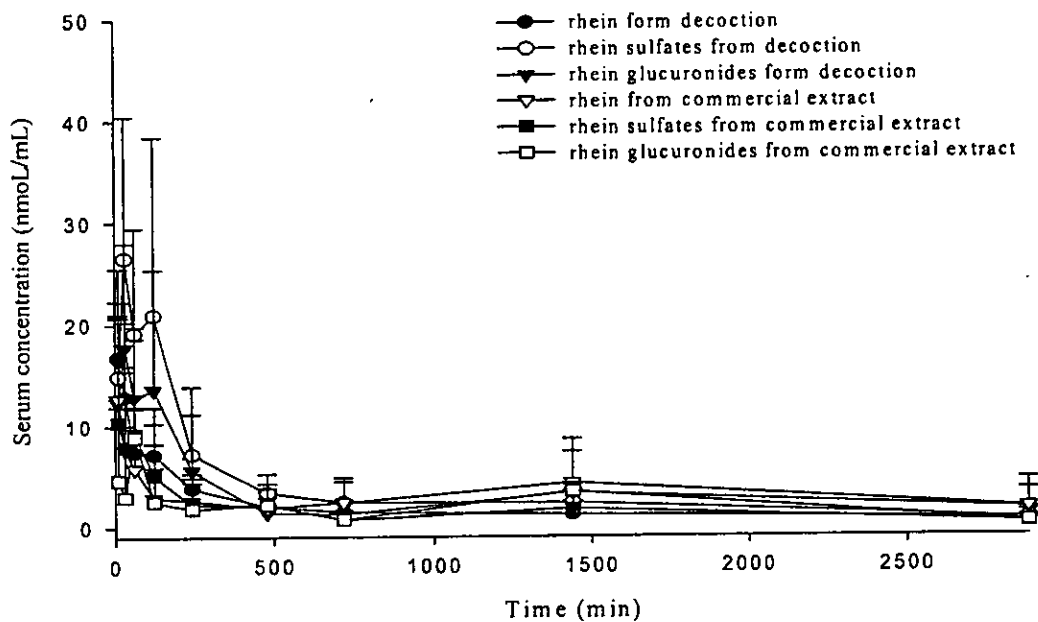


Fig. 18 (a) Comparison of mean ( $\pm$ S.D.) serum concentration-time profiles of rhein free form and conjugates (sulfates or glucuronides) after oral administration of decoction and commercial extract of SHSST in rats ( $n = 6$ ) ; (b) the semi-log diagram of (a).



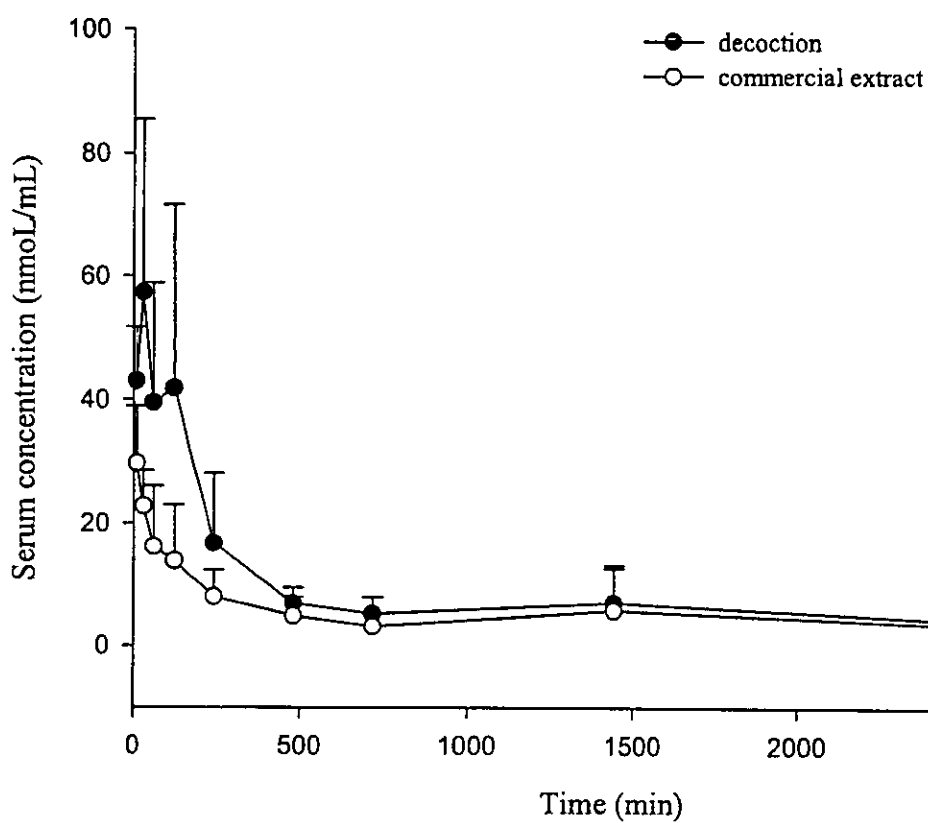


Fig. 19 Comparison of mean ( $\pm$ S.D.) serum concentration-time profiles of rhein free form and conjugates (sulfates and glucuronides) after oral administration of decoction and commercial extract of SHSST. Conjugates represent the sum of sulfates and glucuronides. (n =6)

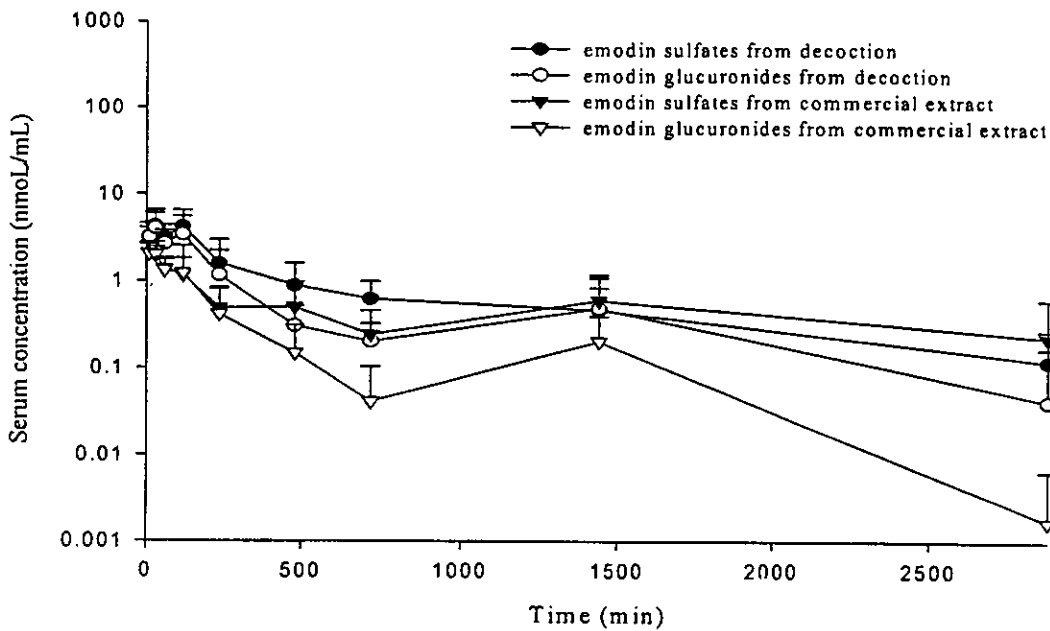
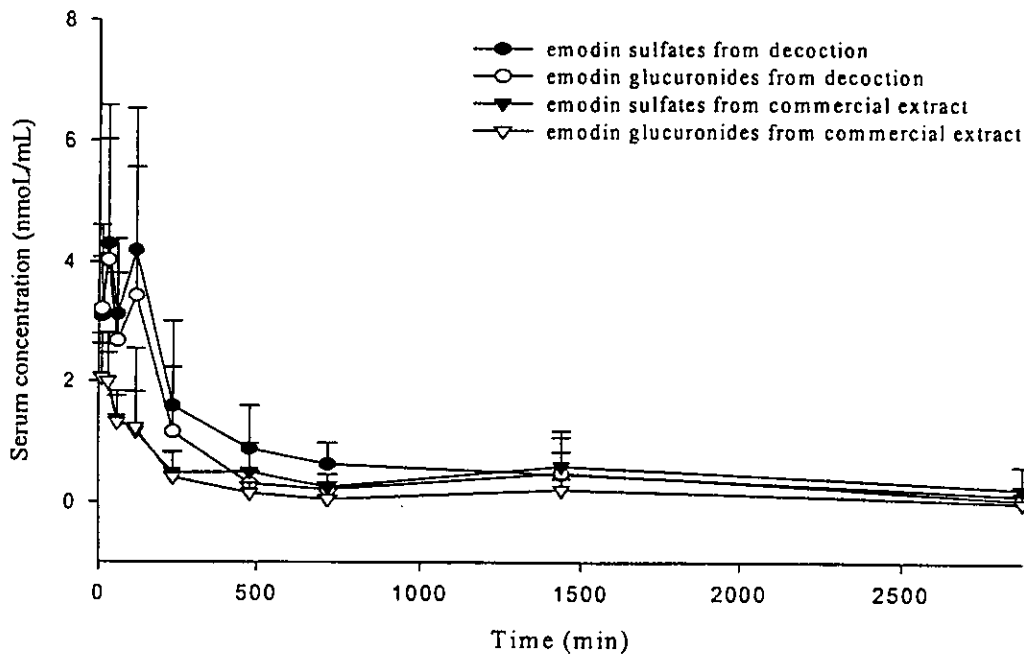


Fig. 20 (a) Comparison of mean ( $\pm$ S.D.) serum concentration-time profiles of emodin conjugates (sulfates or glucuronides) after oral administration of decoction and commercial extract of SHSST in rats ( $n=9$ ); (b) the semi-log diagram of (a).

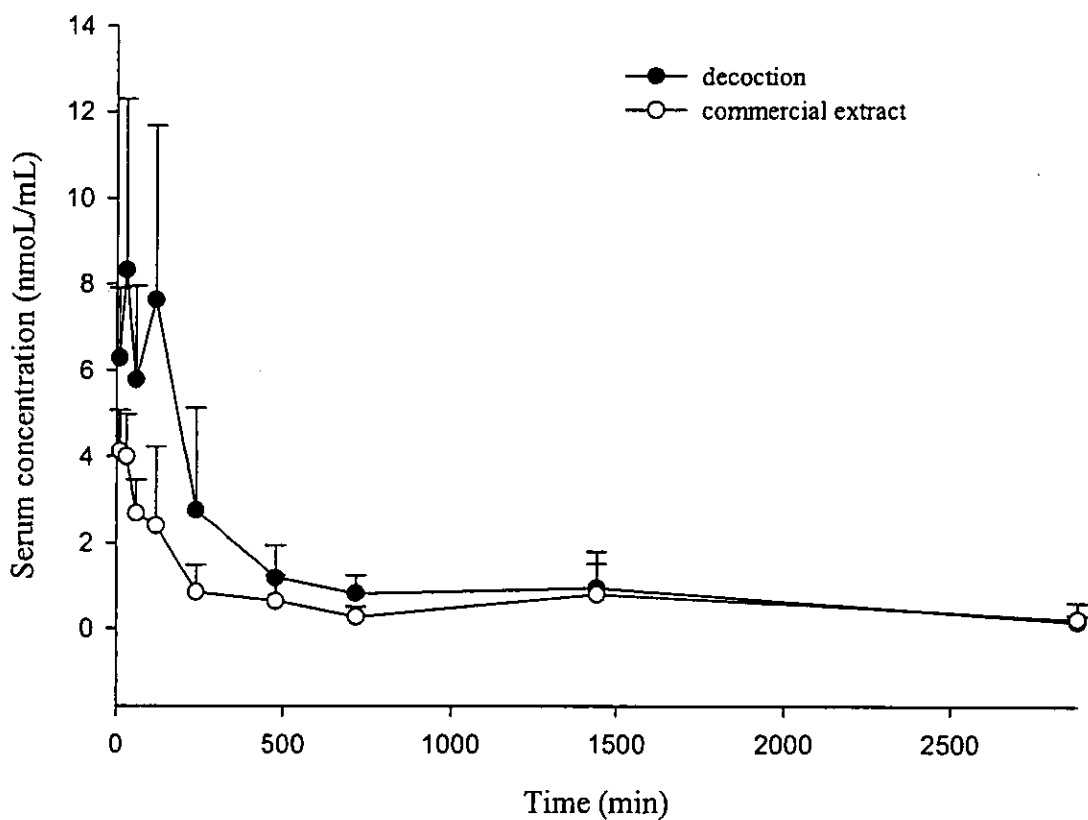


Fig. 21 Comparison of mean ( $\pm$ S.D.) serum concentration-time profiles of emodin conjugates (sulfates and glucuronides) after oral administration of decoction and commercial extract of SHSST. Conjugates represent the sum of sulfates and glucuronides. (n =9)

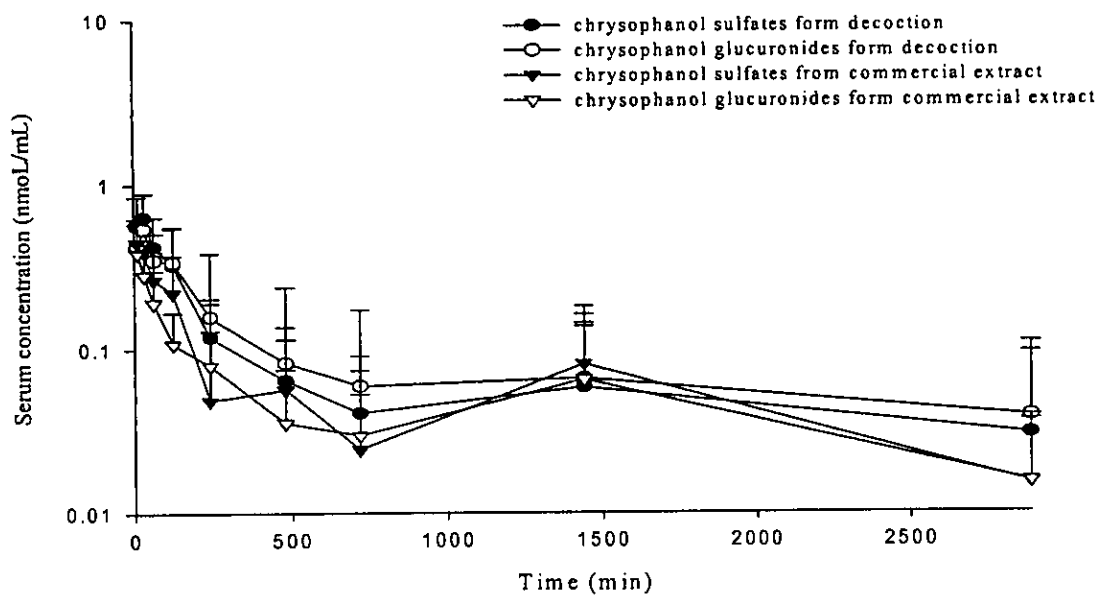
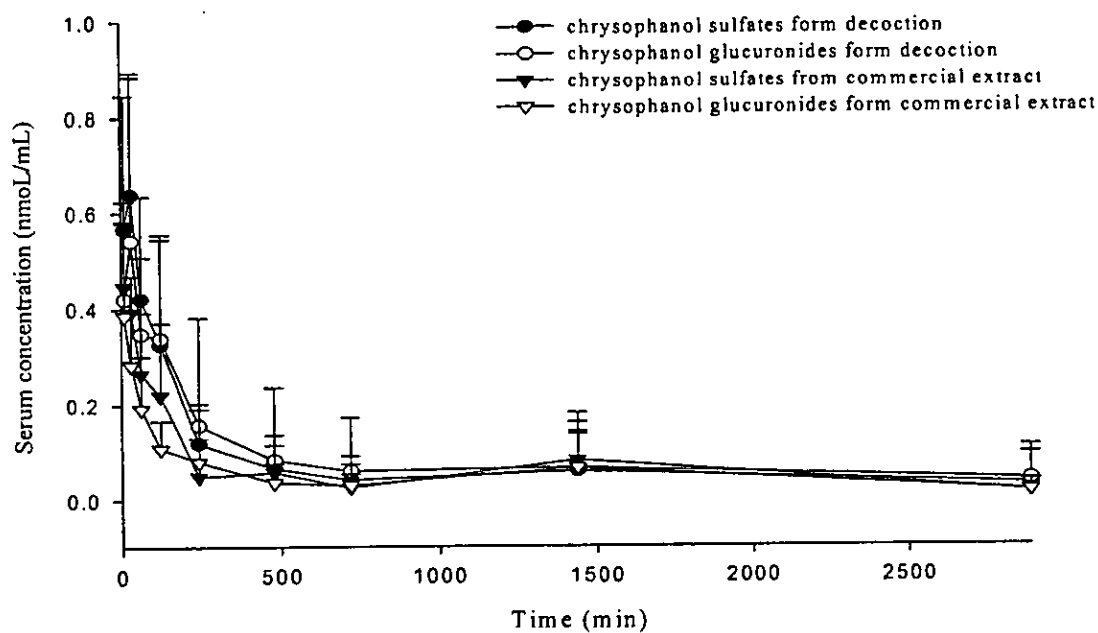


Fig. 22 (a) Comparison of mean ( $\pm$ S.D.) serum concentration-time profiles of chrysophanol conjugates (sulfates or glucuronides) after oral administration of decoction and commercial extract of SHSST in rats (n=9) ; (b) the semi-log diagram of (a).

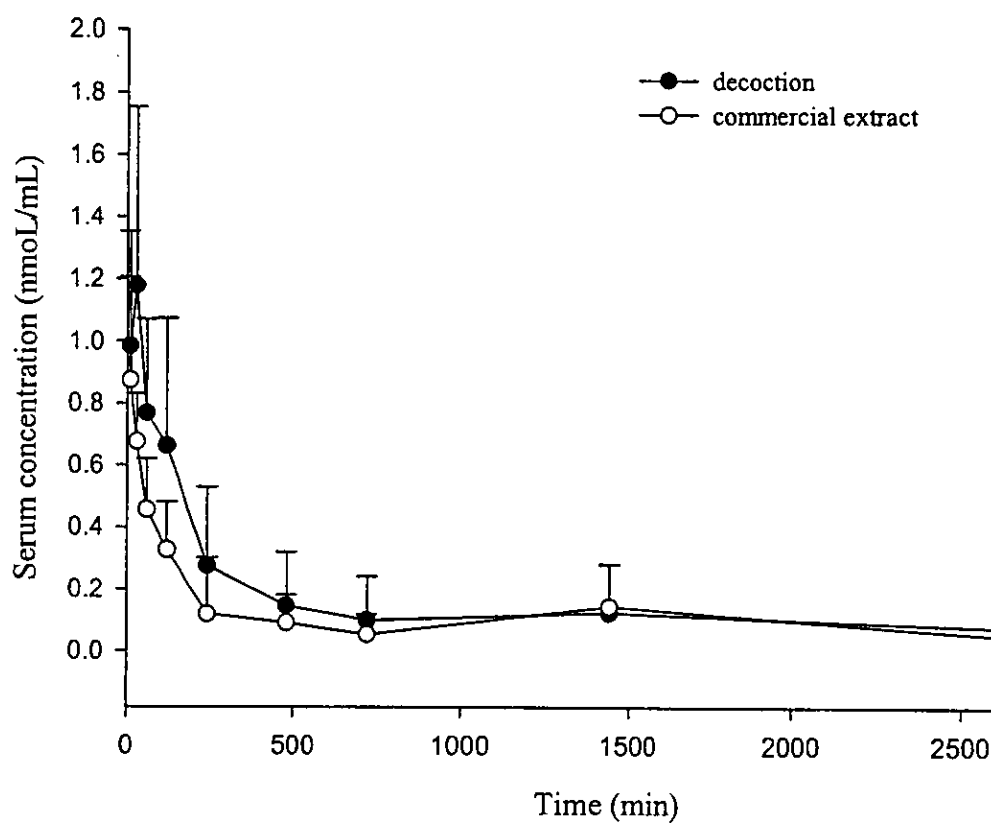


Fig. 23 Comparison of mean ( $\pm$ S.D.) serum concentration-time profiles of chrysophanol conjugates (sulfates and glucuronides) after oral administration of decoction and commercial extract of SHSST. Conjugates represent the sum of sulfates and glucuronides. (n=9)