THE SELECTION AND CONTENT DETERMINATION OF AN ANTI-MIGRAINE EFFECTIVE FRACTION SEPARATED FROM TIANMA GOUTENG DECOCTION BY MACROPOROUS RESIN

Zhaohuan Lou¹, Bohou Xia¹, Junyan Zhao², Yuefang Huang³, Mingjie Hu¹ and Guangji Zhang¹,*

¹Institute of Material Medica, Zhejiang Chinese Medical University, 548 Binwen Road, Hangzhou, 310053, China
²The First People Hospital of Hangzhou Xiaoshan District, 199 Shixin South Road, Xiaoshan District, Hangzhou, 311200, China
³Department of Neurology, Zhejiang Provincial Hospital of TCM, 54 Youdian Road, Hangzhou, 310006, China

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ABSTRACT

The objective of the present study was to obtain an effective part of Tianma Gouteng decoction (TMGTD) on anti-migraine and to establish a RP-HPLC method for the content determination of Jasminoidin and Baicalin in the part. For this study, TMGTD extract was prepared by decoction with water and 30%, 60% and 90% part of TMGTD were made by D-101 macroporous resin. Migraine rat model induced by subcutaneous injection of Nitroglycerine was applied to evaluate the anti-migraine effect of the samples. RP-HPLC coupled with liquid-solid extraction method was applied for the content determination of Jasminoidin and Baicalin. Results: 60% fraction was the most effective part of TMGTD on anti-migraine, with significant effect on reducing the frequency of head scratching and regulating the abnormal content of 5-HT, DA, NE and E in serum and brain tissue of migraine rats. The calibration curve of Jasminoidin and Baicalin was linear (r > 0.999) over the range of 0.0153 to 0.183 μg and 0.334 to 4.013 μg respectively, and the average recovery of each component was from 96.1% to 100.1% and 97.6% to 102.7% respectively. The content of Jasminoidin and Baicalin in 60% fraction was 1.03% and 29.6%, which was significantly higher than that in the fraction before purification process optimization. Conclusions: This was the first report on anti-migraine effective part selection and main components content determination of TMGTD. The result may provide a meaningful basis for the clinical application of TMGTD on anti migraine headache and for the new product development of TMGTD effective part.

* Corresponding author
E-mail: loutcm@gmail.com (Guangji Zhang)

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1 Introduction

Migraine is a chronic disorder characterized by recurrent moderate to severe headaches often associated with a number of autonomic nervous system symptoms. It is a common disease and incidence has increased yearly. Chinese medical treatments are popularly used in China, Chinese herbs such as Chuanxiong Rhizoma and Gastrodiae Rhizoma (Wang et al., 2013) are commonly used in migraine headache treating and TCM decoctions are maybe the best treatment in clinical TCM practice on migraine(Zhou et al., 2013). Tianma Gouteng Decoction(TMGT) originated from <Zhongyi Neike Zabing Zhengzhi Xinyi>, and it is composed of Rhizoma Gastrodiae (Tianma) 9 g, Ramulus Uncariae Cum uncis (Gouteng) 12 g, Concha Haliotidis (Shijueming) 18 g, Fructus Gardeniae (Zhizi) 9 g, Baical skullcap root (Huangqin) 9 g, Radix Cyathulae (Chuanniuxi) 12 g, Cortex Eucommiae(Duzhong) 9 g, Herba Leonuri (Yimucao) 9 g, Herba Taxilli (Sangjisheng) 9 g, Caulis polygoni multiflori (Yejiateng) 9 g and Poria (Fuling) 9 g. It has efficacy on calming liver to stop head scratching of all the rats were recorded every 30 minutes and lasted for 3.5 h. After subcutaneous injection with Nitroglycerin, frequency of head scratching of all the rats were recorded every 30 minutes and lasted for 3.5 h. After subcutaneous injection with Nitroglycerin, frequency of head scratching of all the rats were recorded every 30 minutes and lasted for 3.5 h.

2 Materials and methods

2.1 Materials and reagents

All the medical materials of TMGT were purchased from Chinese medicine factory of Zhejiang Chinese Medical University. TMGT extract was made by decoction the medical materials of TMGT with 10 BV water according to the weight of 10 prescription of the formula for twice, and the extract were collected and concentrated by rotary evaporation under vacuum (with the ratio of cut crude drug to extract volume to be 1.0 g/ml). The concentrate solution was absorbed by D-101 macroporous resin and then eluted by water, 30% (V/V), 60% and 90% ethanol solution one by one. Each ethanol elutrient was collected and concentrated under vacuum to get the separated fractions (parts): 30% fraction (part), 60% fraction (part) and 90% fraction (part) (with the ratio of cut crude drug to extract volume to be 1.0 g/ml). The reference substance of Jasminoidin and Baicalin were purchased from National Institutes for Food and Drug Control of China. Pure water was made by Milli-Q equipment.

2.2 Animals

Sprague Dawley (SD) rats weighing 180 to 220 g were obtained from Experimental Animal Center of Zhejiang Academy of Medical Sciences (Certificate No.: SCXK (Zhe) 20080033). They were housed in cages at 25 ± 2 °C and exposed to a 12:12 h light–dark cycle, free access to food and water. Animals were fasted but free access to water for 12 h before experiment and 2 h after drug administration. All procedures were in strict accordance with the China legislation on the use and care of laboratory animals and with the guidelines established by the Institutional Animal Ethics Committee and Committee for the Purpose of Control and Supervision of Experiments on Animals in China.

2.3 Selection of effective-migraine fractions of TMGT

2.3.1 Animal treatments

70 SD rats were equally partitioned into 7 groups (Male and female in each group were the same in the number). Rats in treatment groups were administrated i.g. once each day for 7 days as follows: normal group (normal) and negative control (Flunarizine) were treated with 1.7 mg/kg Flunarizine (Xian-Janssen Pharmaceutical LTD. Beijing China) solution; and the other four groups were treated with 10 g/kg TMGT extract, 30% ethanol elution, 60% ethanol elution and 90% ethanol elution respectively. 0.5 h after the last administration, all groups except the normal one were treated with 10 mg/kg subcutaneous injection of 5 mg/ml Nitroglycerine (Guangzhou Baiyun Shan Ming Xing Pharmaceutical Co. Ltd. Guangzhou China ) as migraine models.

Dosage of administration: The dosage of TMGT complex prescription for a person is 114 g (cut crud drug) per day, and the equivalent dose for rat’s test is about 10 g/kg body weight.

2.3.2 Index of ethology

After subcutaneous injection with Nitroglycerin, frequency of head scratching of all the rats were recorded every 30 minutes and lasted for 3.5 h.

2.3.3 Assaying of monoamine neurotransmitter in blood and brain tissue

4 h after subcutaneous injection, the blood were collected from eye-orbit venous sinus of each rat, then centrifuged at 3000 r/min for 10 min at 4 °C. The plasma was collected and stored at −80 °C until analysis. Then, all rats were sacrificed with ether anesthesia and their brains were taken out immediately and washed with cold sodium chloride. 10% brain tissue homogenate were made with sodium chloride.
Hydroxytryptamine (5-HT), epinephrine (E), dopamine (DA) and norepinephrine (NE) in serum and homogenate were determined according to the kits directions (5-HT, E, DA, NE kits were purchased from Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

2.3.4 Statistical analysis

All data were processed by SPSS 16.0. Measurement data were shown as mean ± SD, and the comparison between two groups was performed with t-test. Differences were considered statistically significant if the P value < 0.05.

2.4 Assaying of Jasminoidin and baicalin in TMGTD effective part

2.4.1 Chromatographic condition

Chromatographic separation was achieved on a XDB-C_{18} column (4.6 mm ×250 nm i.d., 5 μm) at temperature of 25°C. The mobile phase consisted of methanol and 0.05% (v/v) phosphoric acid aqueous solution with gradient elution (methanol: during 0-20 min, from 30% to 45%; during 20-50 min, from 45% to 60%). The flow rate was 1.0 ml/min. The gradient detection wavelength was set at 238 nm during 0-17 min and at 280 nm during 17-55 min. The λ_{max} of Jasminoidin is 238 nm (Wu et al., 2010; YH, 2013) and of Baicalin is 280 nm (Wang et al., 2011a).

2.4.2 Preparation of the standard solution

The stock solution of Jasminoidin and Baicalin was respectively prepared by dissolving the reference substance in methanol to a concentration of 0.305 mg/ml and 0.352 mg/ml. Then 0.4 ml Jasminoidin standard solution, 7.60 ml Baicalin standard solution and 2 ml methanol was mixed as a commixture standard solution. 1, 2, 5, 8, 10 and 12 μL commixture standard solution was injected into HPLC apparatus and the peak areas were collected.

2.4.3 Preparation of sample solution

0.1 g TMGTD effective part was dissolved in 25 ml 60% (v/v) ethyl-alcohol and extracted with hypersonic for 30 min, and before determination the solution was filtered by 0.45 μm micropore film.

2.4.4 Tests of precision, stability and reproducibility

The commixture standard sample was consecutively injected into HPLC for 6 times in one day to investigate the precision of the apparatus. The commixture standard sample were stored at room temperature (about 25°C) for 24 h before sample processing and sample peak area were obtained at the storage time of 0, 2, 4, 6, 10, 14, 20, and 24 h. The precision and stability of the established method were evaluated as the relative standard deviation (RSD) of the concentration. 7 pieces of test sample were prepared and the concentration of Jasminoidin and Baicalin in each sample was determined, and then the reproducibility of the method was evaluated as the RSD of the percentage content.

2.4.5 Test of recovery

TMGTD effective part (containing 0.76% Jasminoidin and 20.5% Baicalin) was added with corresponding standard solution which equivalently to 80%, 100%, 120% of Jasminoidin and Baicalin in samples respectively, three replicates of each concentration were used. The recoveries were evaluated as the ratio between the determined and the added quality of the standards.

3 Results

3.1 Effects on frequency of scratching head

The action of scratching head with forward limb of rats is similar with the behavior of human beings with migraine. It is a symptom of migraine headache and its frequency embody the severity of ache to some extent. After subcutaneous injection of nitroglycerin, the rats were monitored for head scratching every 30 min. During the observation times (30-210 min after nitroglycerin injection), there were only a few head scratching actions in normal rats, while in control group it increased noticeably (P<0.01). Compared with the control group, 60% fraction could obviously reduce the frequency of head scratching all the observation times (P<0.01, P<0.05), the effect of 30% fraction was observed in 120-150 min time span (P<0.05) and the effect of 90% fraction was in 120-150 min and 150-180 min after nitroglycerin injection (P<0.05) (See Figure 1). Therefore, 60% fraction has a good effect in relieving headache symptom of migraine.

3.2 Effects of TMGTD fractions on monoamine neurotransmitter levels in serum

After subcutaneous injection with Nitroglycerin for 4 h, the concentration of 5-HT in serum of the model rats decreased significantly (P<0.01), while the adnephrin increased (P<0.01). TMGTD and 30% , 60% and 90% fraction can increase the content of 5-HT(P<0.01) and decrease the adnephrin (P<0.05 or P<0.01) obviously in serum. The efficacy of 60% fraction in increasing 5-HT level and decreasing E level in serum was superior to TMGTD and 30% fraction respectively. There is no statistical difference between effects of 60% fraction and 90% fraction on 5-HT and E in between, but the serum level of 5-HT and E in 60% group is get closer to the normal group than 90% fraction which indicated that the 60% fraction might be the effective part of TMGTD on anti-migraine. Coupled with the result of head scratching and considering that 60% ethanol elute has lower cost and more suitable for industry, we select the 60% fraction as the effective part of TMGTD (See Figure 2).
Figure 1 The frequency of head scratching with forward limb of rats in different time span after subcutaneous injection of nitroglycerin. The head scratching frequency was recorded every 30 minutes with the values expressed as means ± S.D (n=10). △P<0.05 and △△P<0.01 vs. normal group; *P<0.05 and **P<0.01 vs. model group.

Figure 2 The contents of 5-HT (A) and E (B) in serum of rats after subcutaneous injection of nitroglycerin. The values were expressed as means ± S.D (n=10). △P<0.05 and △△P<0.01 vs. normal group; *P<0.05 and **P<0.01 vs. model group.
3. Effects of 60% part on monoamine neurotransmitter levels in serum and in brain of model rats

As mentioned above, we selected the 60% fraction as the effective part of TMGTD on anti-migraine. After that, we took a validation test to ensure the effect of 60% part on anti-migraine. As shown in Figure 3, after subcutaneous injection with Nitroglycerin for 4 h, the serum content of 5-HT and DA of model rats significantly decreased and the content of NE and E increased obviously ($P<0.05$). But in brain, all the contents of 5-HT, DA, NE and E increased ($P<0.05$). Compared with model rats, each dosage of 60% part can increase the serum content of 5-HT and DA ($P<0.05$, $P<0.01$), and decrease the content of NE and E in serum and 5-HT, DA, NE and E in brain tissue ($P<0.01$). These results showing that the 60% part of TMGTD has a good effect on improve the abnormal level of monoamine neurotransmitter in serum and in brain tissue of migraine rats.
3.4 Assaying of Jasminoidin and Baicalin in TMGTD effective part

3.4.1 Specificity

The result of system adaptability experiments show that under the condition described above, Jasminoidin and Baicalin can be separated from other components in the sample, and there were no interfering peaks within the elution times for either reference standard and tested samples. The retention time (t_R) of Jasminoidin and Baicalin was 9.8 min and 31.0 min as shown in Figure 4.

3.4.2 Linear, precision, stability and reproducibility

The linear regression of Jasminoidin and Baicalin were obtained between the peak area (Y) and the amount of standard injected (X). And the calibration curve of Jasminoidin and Baicalin displayed good linearity among 0.0153–0.183 μg and 0.334–4.013 μg respectively (See Table 1). The precision and reproducibility were assessed by performing replicate analysis (n=6 and n=7 respectively) of standards and samples, and their RSDs were less than 3%, indicating an acceptable precision and accuracy of the present method. The results of stability test showing that the Jasminoidin and Baicalin were stable for at least 24 h at room temperature, since no obvious degradation of them in solution occurred within the time period under the storage condition. See in Table 2.

Table 1. The linear regression equations for calibration curves of Jasminoidin and Baicalin in TMGTD effective part

<table>
<thead>
<tr>
<th>Components</th>
<th>Regression equations</th>
<th>Correlation coefficients (r)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jasminoidin</td>
<td>Y=1280.9X+2.257</td>
<td>0.9998</td>
</tr>
<tr>
<td>Baicalin</td>
<td>Y=2646.5X+1.3341</td>
<td>1.0000</td>
</tr>
</tbody>
</table>

Table 2 The precision stability and reproducibility of Jasminoidin and Baicalin in sample

<table>
<thead>
<tr>
<th>Components</th>
<th>precision</th>
<th>reproducibility</th>
<th>Stability</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Measured (µg/ml)</td>
<td>RSD (%)</td>
<td>Measured (%)</td>
</tr>
<tr>
<td>Jasminoidin</td>
<td>15.6±0.20</td>
<td>1.31</td>
<td>1.02±0.01</td>
</tr>
<tr>
<td>Baicalin</td>
<td>339.4±3.56</td>
<td>1.05</td>
<td>29.5±0.26</td>
</tr>
</tbody>
</table>

Table 3 Recoveries of Jasminoidin and Baicalin

<table>
<thead>
<tr>
<th>Percentage of add</th>
<th>Added (µg)</th>
<th>Measured (µg)</th>
<th>Recoveries (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Jasminoidin</td>
<td>Baicalin</td>
<td>Jasminoidin</td>
</tr>
<tr>
<td>80%</td>
<td>15.0</td>
<td>415.0</td>
<td>14.4±0.3</td>
</tr>
<tr>
<td>100%</td>
<td>18.5</td>
<td>498.2</td>
<td>18.9±0.3</td>
</tr>
<tr>
<td>120%</td>
<td>22.6</td>
<td>595.3</td>
<td>22.4±0.8</td>
</tr>
</tbody>
</table>

Figure 5 HPLC chromatograms of TMGTD (1. Gastrodin; 2. Jasminoidin; 3. Baicalin; 4. Baicalein; 5. Wogonin).
The selection and content determination of an anti-migraine effective fraction separated from TianMa GouTeng Decoction by macroporous resin.  

Table 4 Content of Jasminoid and Baicalin in TMGTD samples

<table>
<thead>
<tr>
<th>Samples</th>
<th>Contents (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Jasminoidin</td>
</tr>
<tr>
<td>TMGTD</td>
<td>0.45</td>
</tr>
<tr>
<td>30% part</td>
<td>0.59</td>
</tr>
<tr>
<td>60% part</td>
<td>0.61</td>
</tr>
<tr>
<td>90% part</td>
<td>0.80</td>
</tr>
<tr>
<td>60% part*</td>
<td>1.03</td>
</tr>
</tbody>
</table>

* This 60% part was obtain after purification process optimization.

3.4.3 Recovery

The recoveries of Jasminoidin and Baicalin were listed in Table 3. These were obtained from three replicate analyses of samples added with 80%, 100% and 120% standards respectively. The RSDs of Jasminoidin’s recoveries were range from 1.8% to 2.9% and of baicalin were from 1.9% to 3.1%. These results represented acceptable recoveries of Jasminoidin and Baicalin in samples of TMGTD effective part, indicating satisfactory reliability and validity of the present method.

3.4.4 Jasminoidin and Baicalin content assay

The sample solution of TMGTD and other separated parts were made according to the method mentioned in “2.2.3” and the Jasminoidin and Baicalin were determined by HPLC. As showed in Table 4, 60% part containing the most content of Baicalin and 90% part has the most Jasminoidin, and 60% part is the sample containing the most total amount of Jasminoidin and Baicalin. And after the purification process optimization, the contents of two components have proved significantly.

4 Discussions

Experimental animal model with analogue representation of mankind disease is a necessary condition for the drug action and safety evaluation. Migraine animal model induced by nitroglycerine is a commonly used animal model for the evaluation of anti-migraine effect. The praxiology appearance, such as ears turn red and scratch head with forward limb etc., and pathological biochemistry changing of this model is similar to the appearance of person in migraine(Tassorelli et al., 2003), this is corresponded to the principles, such as standardization, similarity, reproducibility, applicability and economy, which need to abide by during experimental animal model design. Behavior symptomatology evaluation is an important index of the experimental migraine animal models induced by nitroglycerine, and the segmentation counting in persistence time is a modus operandi for behavior symptomatology evaluation quantization. Observation of the frequency of head scratching in an unit time span would objectively reflect the start time of drug effect, the process and degree of the drug action, and it is a reasonable index for the evaluation of the drug on anti migraine (Fu et al., 2005; Sun et al., 2012). In present study, it was reported that at the first time span after subcutaneous injection with Nitroglycerin, the frequency of head scratching of the model rat has increased significantly, and it researche a max at the second time span, then the frequency reduced gradually, indicating that the model was made successfully. The 60% part could effectively reduce the head scratching frequency all the observation time, showing it has a good effect on headache relieve.

Migraine is a common disabling headache disorder that is conventionally classified according to the presence or absence of aura. The pathogenesis of this disorder entails a complex interplay of neurovascular factors that trigger reduction of cerebral blood flow followed by reactive vasodilatation(Lippi et al., 2014). And with the changing of exterior and interior environmental agents, the catecholamines such as E and NE would be released abnormal which induced the migraine attack (Wang et al., 2009). When in migraine, the blood vessel dilatation and priming volume of cerebral blood flow decreased, and then the externalization of neurone on 5-HT, DA, NE, E would be influenced, and if the composition balance of these substance be broken, pathological changes would be happened(Liu et al., 2008). Some research results indicate that there are some direct and indirect relationship between migraine and the content of 5-HT, NE, E and supersensitivity of DA in brain tissue(Yin et al., 2008; Zeng et al., 2008). 5-HT is a modulator of central nervous activity, and during migraine period of onset, its’ content degraded, while in intermission increased. These validated that the abnormal content of 5-HT is one of the bases of migraine(Tan & Fang, 2012).

Genetics data show there are some variations of dopaminergic receptor in classical migraineurs, and this maybe the important reason for the morbidity of some migraine(SJ, 1997).So the contents of 5-hydroxytryptamine (5-HT), norepinephrine (NE) and dopamine (DA) in plasma and brain can be used as indexes to evaluate the effect of drugs to treat on migraine(Wang et al., 2011b; Wu et al., 2014). In this research, it was found that compared with the normal rats the contents of 5-HT and DA in migraine model rat serum were significantly decreased but in brain all of them have increased, this showing that there are some disorders of externalization of 5-HT and DA. TMGTD and its’ 60% part can regulate the content of 5-HT, DA, NE and E to approach the normal level and to reduce the head scratching frequency, indicating that they have effects to treat migraine. The studies on dose-effect relationship of TMGTD 60% part validate its’ anti-migraine effect further.
According to previous knowledge, the clinical therapeutic effect of traditional Chinese medicine is determined by the content and composition ratio of active components and helper constituents containing in it (Fu et al., 2006). From the HPLC analysis result of TMGTD samples, it was observed that in TMGTD, there are Gastrodin, Jasminoidin, Baicalin, Baicalein and Wogonin in it, all the three part of TMGTD have containing the Jasminoidin and Baicalin, but there is difference on the content of these two components among the samples, and that maybe the cause of the difference effect of the samples on anti-migraine. Baicalin is the active component of Scutellaria baicalensis on anti-migraine headache (Liang et al., 2012), and it has a most amount in 60% part, so we supposed that it may be one of the main active components in 60% part, and then we selected it as the index for the optimization of macroporous resin purification process and the quality control of TMGTD 60% part. From the results listed above, it was found that the D-101 macroporous resin can effectively enriching the content of Jasminoidin and Baicalin in TMGTD 60% part. And the HPLC content determination method established in this research was convenient and accurate, and can be applied to the quality control of TMGTD 60% part.

Present study was devoted to find the effective part of TMGTD on anti-migraine and to analysis the main components in it, the pharmacodynamical material basis and their mechanism need further studies.

Acknowledgements

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Conflict of interest

The authors have declared that there is no conflict of interest.

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