Experimental Study on the Effect of Shugan Jianghuo Granule on Related Indexes of Rats with Liver-fire Hypertension Syndrome

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Abstract. Objective: The study based on previous studies that have confirmed spontaneously hypertensive rats (SHR) at 14-18 wk of age belong to stable phase of liver-fire hyperactivity and chose the SHR at age of 14-18 wk as animal model, preliminarily confirmed shuganjianghuo granule’s hyperactivity effect by observed the effect of the drug on related microscopic physical and chemical indicators of rats with hypertension of liver-fire hyperactivity syndrome. Method: 24 male SHR at age of 16 wk, randomly divided into model group, the Chinese medicine group (shuganjianghuo granule group), the Western medicine group (captopril group), and normal WKY rats as control group, collected animal macroscopic appearance, systolic pressure, rotating tolerance time, irritability level, after 3 weeks treatment, detected each group rats’ serum content of angiotensin II (Ang II) and norepinephrine (NE) by enzyme-linked immunosorbent (ELISE). Result: After 4 weeks treatment, compared with model group, shuganjianghuo granule group significantly lower systolic pressure and irritability, rotating tolerance time prolonged, serum Ang II, NE content reduced. And the captopril group the indexes were improved significantly except irritability degree. Conclusion: 16-18 wk of age SHR belongs to stable phase of liver-fire hyperactivity, shuganjianghuo granule has obvious steady and long hypotensive effect and can improved the manifestation of liver-fire hyperactivity.

According to relevant literatures, spontaneous hypertensive rats (SHR) at the age of 14-18 wk showed liver-fire hyperactivity [1], and researches indicate that Ang II, NE significantly higher than other syndromes is important index [2] of hypertension of liver-fire hyperactivity syndrome. Therefore, this study chose WKY rats as the control group, as SHR aged 16 wk as the model group, to observed the influence of shuganjianghuo granule on microscopic index such as macroscopic appearance, systolic pressure, rotation tolerance time, irritability degree and Ang II, NE, preliminarily confirmed and appraised shuganjianghuo granule’s hypotensive effect.
Experimental Materials and Methods

Experimental Animals

24 male SPF spontaneously hypertensive rats (SHR) at age of 16 wk, (purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd.). 8 male WKY rats at age of 16 wk (purchased from Henan animal experiment center). All animals are raised in the laboratory animal room of Henan University of Chinese Medicine, adaptive feeding 2 wk.

Main Instruments, Reagents and Drugs

Main instruments: Shanghai precision electronic balance; Balancing rotator; Thermostatic water bath box; Japan SoftronBP-98-A noninvasive rats tail arterial pressure meter, Germany low-temperature high-speed centrifuge, the enzyme linked immunosorbent assay text kit of rat serum (Ang II, NE) from Wuhan Elabscience Biotechnology Co., Ltd.

Main drugs: Shuganjianghuo granule is national patent, the formula is: chaihu, gouteng, tianma, baishao, etc. Chinese herbal granules made from san-jiu pharmaceutical Ferrin GmbH. Captopril tablets: 25mg/ tablet *100 tablets, manufactured by chang-zhou ferrin GmbH

Animal Grouping

According to the clinical diagnostic criteria under the equivalent conversion of animal macroscopic appearance to identify the syndrome of SHR at age of 16 wk [1,2,3], then combined with systolic pressure, rotation tolerance time, irritability level, judgement of macroscopic appearance, 24 SHR all shown as liver-fire hyperactivity syndrome, randomly divided them into model group, the Chinese medicine group (shuganjianghuo granule group), the Western medicine group (captopril group); 8 normal WKY rats as control group.

Administration Method

When rats were aged 16 wk began to administration, the Chinese medicine group rats and the Western medicine group rats were given different drug lavaged, once a day, for 3 wk, the dose decided by the conversion ratio of drug dose between human and animal (rats dose dosage is about human around 6.3 times). Dose volume was 1 ml / 100g, the Chinese medicine group rats were given shuganjianghuo granule suspension 1.78 g/ 100 g/d. captopril group rats were given the dose as the captopril solution 1.0417 mg/ 100 g/d, model group rats and the control group rats were given some volume of pure water.

Draw Material and Prepared Sample

Draw material were performed at the end of the third week, Rats were fasted for one night. The next day, along the Mid distance cut open quickly after anesthesia by the injection of 10% chloral hydrate. All sample were put into vacuum Blood Collection Tube with the sodium citrate, stood for one hour, then centrifuged five minutes used low-temperature high-speed centrifuge (3000 R/M), took out the serum, saved to - 80 °C refrigerator for index detection.
Detection of experimental Indicators

**Blood Pressure Measurement**

Blood pressure measured by pressured rat tail artery. Specific operation steps as the follows: First, put and fixed rats in a cage under the rats awake. Second, put the cage into a constant temperature box, preheating of about 10 minutes caused the tail vessel to dilate. Last, exposed the tail fully, clamped the tail by the blood pressure clamp then measuring the systolic pressure, repeated 3 times, and took the average measured value as the result of blood pressure, Measured 1 times 1 week.

**Rotation Tolerance Time**

Set the speed of Balancing Rotator (JD-SH) at 60 R/M, then the rat was placed in the center of the rotating platform. Recorded the time of start the power unit to the rats fell off the rotating Platform.

**The Reference Rating of Irritability Degree, According to Relevant Literature [3], See Table 1 for Its Reference Rating Standards.**

Table 1. Reference rating for irritability degree.

<table>
<thead>
<tr>
<th>sequence</th>
<th>level</th>
<th>Evaluation criteria and score</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Class I</td>
<td>There was no obvious reaction while holding the rat’s neck (score 1)</td>
</tr>
<tr>
<td>2</td>
<td>Class II</td>
<td>Screaming and jumping while holding the rat’s neck (score 2)</td>
</tr>
<tr>
<td>3</td>
<td>Class III</td>
<td>Biting while holding the rat’s neck or frequently fighting with other rats (score 3)</td>
</tr>
</tbody>
</table>

**Statistics**

Dates were analyzed using SPSS 18.0. Difference of measurement date was compared with analysis of variance, represented as ( \( \bar{x} \pm S \) ), enumeration date use the rank sum text. \( P<0.05 \) was considered significant.

**Conclusion**

**Comparison on Macroscopic Appearance of Different Groups**

Under the equivalent transformation of clinical syndrome standard, 32 SHRS at age of 15 wk all showed liver-fire hyperactivity syndrome, specific manifestations as follow: excitement, like fighting, revolt violently, and tongue was thin and dry, the blood vessel of ear and claw was engorged, the urine was less and yellow and the feces was dry and hard. After 4 wk of treatment, the symptoms were significantly improved in
each dose group of shuganjianghuo granule: no revolt violently, tongue was relatively moderate, and the urine and feces was relatively normal.

Comparison on Blood Pressure of Different Groups

The table 2 shown the systolic pressure’s difference between each group when the rats at age of 18 wk after the drug intervention 3 wk. The systolic pressure of Chinese medicine group and Western medicine group were significantly lower than the model group, the difference was significant ($P<0.05$).

Table 2. comparison on blood pressure of different groups

\[ (\bar{x} \pm S) \ (n=8) \ [\text{mmHg}] \]

<table>
<thead>
<tr>
<th>Group</th>
<th>W16</th>
<th>W17</th>
<th>W18</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>125.42±7.59</td>
<td>125.08±5.25</td>
<td>125.86±9.46</td>
</tr>
<tr>
<td>Model group</td>
<td>214.93±12.02</td>
<td>220.40±13.6</td>
<td>212.75±8.57*</td>
</tr>
<tr>
<td>TCM group</td>
<td>188.65±7.19</td>
<td>184.48±7.69*</td>
<td>183.98±7.55*</td>
</tr>
<tr>
<td>Western medicine group</td>
<td>187.63±9.81</td>
<td>185.63±8.63*</td>
<td>186.38±6.92*</td>
</tr>
</tbody>
</table>

Note: compare with the control group *$P<0.05$; compare with the model group, ●$P<0.05$;

Comparison on Irritability Score of Different Groups

The table 3 shown the irritability score of the Chinese medicine group were significantly lower than the model group after 3 treatment, the difference was significant ($P<0.05$), while that of the western medicine group was not significant.

Table 3. comparison on irritability score of different groups (n=8).

<table>
<thead>
<tr>
<th>Group</th>
<th>W16</th>
<th>W17</th>
<th>W18</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>1.25±0.71</td>
<td>1.13±0.35</td>
<td>1.13±0.35</td>
</tr>
<tr>
<td>Model group</td>
<td>2.88±0.35</td>
<td>3±0</td>
<td>3±0*</td>
</tr>
<tr>
<td>TCM group</td>
<td>2±0.76</td>
<td>1.38±0.52*</td>
<td>1.38±0.52*</td>
</tr>
<tr>
<td>Western medicine group</td>
<td>2.63±0.74</td>
<td>2.53±0.74</td>
<td>2.5±0.52▲</td>
</tr>
</tbody>
</table>

Note: compared with the control group *$P<0.05$; compared with the model group, ●$P<0.05$; Compared with the TCM group, ▲$P<0.05$;
Comparison on Rotatory Tolerance Time of Different Groups

The table 4 shown the irritability score’s difference between each group when the rats at age of 18 wk after the drug intervention 3 wk. The irritability score of Chinese medicine group and Western medicine group were significantly prolonged than model group, the difference was significant (P<0.05).

Table 4. comparison on rotatory tolerance time of different groups (x ± S) (n=8).

<table>
<thead>
<tr>
<th>Group</th>
<th>W16</th>
<th>W17</th>
<th>W18</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>78.84±18.48</td>
<td>80.65±20.26</td>
<td>82.63±17.29</td>
</tr>
<tr>
<td>Model group</td>
<td>19.75±6.32</td>
<td>16±4.93</td>
<td>13.13±4.39</td>
</tr>
<tr>
<td>TCM group</td>
<td>25.45±5.98</td>
<td>32.26±9.62</td>
<td>37.89±8.86</td>
</tr>
<tr>
<td>Western medicine group</td>
<td>25.38±7.87</td>
<td>28.28±7.09</td>
<td>30.38±7.96</td>
</tr>
</tbody>
</table>

Note: compared with control group *P<0.05; compare with model group, ●P<0.05;

Comparison on the Serum Ang II and NE Level of Different Groups

The table 5 shown the serum Ang II and NE level of Chinese medicine group and Western medicine group were significantly lower than model group when rats was aged 18 wk (P<0.05).

Table 5. comparison on the serum Ang II and NE level of different groups (x ± S) (n=8) [mmol/l].

<table>
<thead>
<tr>
<th>Group</th>
<th>NE</th>
<th>Ang II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>1.39±0.14</td>
<td>77.98±4.90</td>
</tr>
<tr>
<td>Model group</td>
<td>3.66±0.29*</td>
<td>147.65±5.83*</td>
</tr>
<tr>
<td>TCM group</td>
<td>2.23±0.52*</td>
<td>102.89±6.23*</td>
</tr>
<tr>
<td>Western medicine group</td>
<td>2.52±0.22*</td>
<td>105.82±6.91*</td>
</tr>
</tbody>
</table>

Note: compared with the control group *P<0.05; compare with the model group ●P<0.05;

Discussion

Hypertension is a term of western medicine; Chinese medicine literature did not record the name of disease. Based on the theory of traditional Chinese medicine and according to the clinical symptoms of hypertension, hypertension belong to “vertigo”,

429
“headache”, “wind dazzled”, etc. The differentiation of hypertension syndrome can be sorted into four categories [4]: liver-fire hyperactivity, yin deficiency yang hyperactivity, yin-yang deficiency, phlegm-dampness, according to the epidemiological survey study shows that liver-fire hyperactivity is a common syndrome among young and middle-aged hypertension patient [5,6,7]. With the change of person’s life, bad habits of eating, especially in the high nervous state of spirit and nerves for a long time, the incidence trend of this category of hypertension increases year by year and tend to be younger.

The TCM hold that long-term affect-mind dissatisfaction or in a state of fear stress and mood swings lead to “excessive anger damages the liver”, excessive anger most easily affect the physiological function of liver governs free flow of qi, the dysfunction of liver governs free flow of qi cause disorder of qi, qi stagnation cause qi depression, gave birth to internal fire and wind, cause hypertension of liver-fire hyperactivity syndrome.

Professor Li-Hua Zhou based on the view of state medicine [8,9], treat clearing away the fire from the liver as the therapeutic principle, use shuganjianghuo granule treat the hypertension of liver-fire hyperactivity syndrome, Shuganjianghuo granule has a more satisfactory curative effect in clinical. The study took the SHR as research objects, by analyzing the microscopic index change, confirm that shuganjianghuo granule had accuracy curative effect to treat hypertension and can improved the manifestation of liver-fire hyperactivity. Shuganjianghuo granule can decrease the serum Ang II and NE level significantly. However, it’s not completely clear about the mechanism of action of the Chinese medicine compound, because of the Chinese medicine compound’s ingredient is diverse and complex, which needs further research and exploration.

References

