The Study of Different Extracts from Tongkat Ali on Hyperuricemia in Rats

Liang Peng¹, Zhu Libin², Chen Ying¹, Long Xiaoyan¹, Chen Qian², Xu Zhao² and Huang Zhijun¹²*

¹College of Physiology, Wuhan University of Technology, Wuhan, China
²Jianmin Pharmaceutical Group Ltd., Wuhan, China

ABSTRACT

The aim of this research is to investigate the effects of various extracts of Tongkat Ali on uric acid excretion and renal function in rats with hyperuricemia. Sprague dawley male rats were fed with yeast extract, adenine and potassium oxonate to make the model of rats with hyperuricemia. With the animal model being made, Rats were given various extracts from Tongkat Ali for 14 d. Then, uric acid(UA), urea nitrogen(UN), creatinine(Cr) levels in serum and urine and the activity of Xanthine oxidase (XOD) in serum and liver were measured. The level of serum uric acid in the chloroform group and n-butanol group was lower than that in the model group (P<0.05). The uric acid and creatinine levels of urine in the chloroform group and n-butanol group was higher than those in the model group (P<0.05). Besides, the activity of XOD in chloroform group and n-butanol group was lower than those in the model group(P<0.05). Chloroform and n-butanol extract from Tongkat Ali can reduce the level of serum uric acid in rats with hyperuricemia which were induced by adenine, yeast extract and potassium oxonate. This function may be produced through inhibiting the activity of Xanthine oxidase.

Keywords: Tongkat ali; Hyperuricemia; Uric acid; Xanthine oxidase

INTRODUCTION

Hyperuricemia is caused by uric acid metabolic disorders which caused the level of serum uric acid increased abnormally, renal urinary deposition and gout syndrome [1-4]. An increased level of Uric acid in serum is connected with numerous cardiovascular risk factors such as arterial hypertension, hyperglycemia and diabetes. Hyperuricemia is a great damage to the human body. The incidence of hyperuricemia increased year by year. A simple fact currently is the drugs which can cure the hyperuricemia have a great side effect on humans. We need to find a new drug that has a better therapeutic effect on hyperuricemia and has small side effects. UA is a weak acid produced in the liver, muscles and intestines [5]. Purines are the precursors of UA. Xanthine oxidase (XOD) is the enzyme responsible for UA production. Studies has shown that Tongkat Ali could decrease serum uric acid levels and 24 h urinary excretion of uric acid [4] besides, The ethanol extract from Tongkat Ali can inhibit the crystallization of sodium citrate caused by gouty arthritis and relieve various symptom sites such as joint swelling, pain in the affected area and limb dysfunction [6]. However, there is nobody to research the active sites in Tongkat Ali and the mechanism that Tongkat Ali play a role on reducing serum uric acid levels in rats. In order to sloving the problem, we got different extracts from Tongkat Ali to find the extract which can reduce the level of serum uric acid and observed the change of the indexes in rats. The aim of this study was to validate the facts that Tongkat Ali can reduce the level of serum uric acid, to find the active part that could reduce the level of serum uric acid, and to explore the mechanism that Tongkat Ali play a role on reducing serum uric acid levels in rats. Last but not least,this study laid the foundation for the development and utilization of Tongkat Ali.
MATERIALS AND METHODS

Materials
Experimental animals:
Sixty healthy male sprague dawley rats(SD) of specific weight 180 ± 20 g were purchased from Hubei CDC and were individually housed in atomic stainless steel cages in constant conditions of temperature (22 ± 2)°C, relative moisture (40%-60%), and air conditioning (12 full changes of air per 1 h). The light/darkness ratio was 12 h/12 h. Rats were acclimatized for 5 days before the beginning of the study.

Reagent:
Yeast extract, adenine (purity ≥ 98.0%), potassium oxonate (purity ≥ 98.0%), allopurinol (purity ≥ 98.0%), uric acid kit, creatinine kit, urea nitrogen kit and xanthine oxidase kit were purchased from Shanghai Uprising Test Biotechnology Co., Ltd, batch number is 20161210; Tongkat Ali purchased from Chinese medicine Pieces Factory in Bozhou, batch number is 20161020.

Experimental equipment:
Ultrasonic cell grinder, TY96-II; Microplate reader, Multiskan Go 1510; Waterproof incubator, GNP-9080; Automatic biochemical analyzer, Beckman AU680 type; Positive microscope and other laboratory other devices.

Methods
The preparation of different extracts in Tongkat Ali:
10 kg dried root of Tongkat Ali were taken and crushed. 10 times 70% ethanol was added in the flask to extract the roots for 3 times, 2 hours every time. Then the ethanol extract were Combined and dried in a vacuum dryers, The alcohol extract could be achieved after drying. Add some water to the alcohol extracts, and then add chloroform, ethyl acetate and n-butanol in turn to get the extracts of chloroform, ethyl acetate and n-butanol.

Experimental design:
Sixty rats were randomly divided into six groups: blank group (A), model group (B), positive control group (C), chloroform group (D), ethyl acetate group (E) and n-butanol group (F). Except for group A, the rats in the other group were treated with 7.5 g/(kg/d) yeast extracts, 100 mg/(kg/d) adenine and 15 mg/(kg/d) potassium oxonate (It is called as the molding agent) [1-3]. The rats were given modeling agent for two weeks; the dose is 1.5 ml/100 g per day. Took the blood from jugulars in rats after two weeks, and measured the level of serum uric acid in rats. The level of serum uric acid in rats which were given modeling agent has no significant difference, and the level of serum uric acid in rats which were given modeling agent was significantly higher than that in the control group (P<0.05), It is indicated that the model was established successfully. After the establishment of the model, Groups other than group A were given the molding agent for two weeks. After giving molding agent for 1h, the molding agent groups other than group B were given the chloroform extract, ethyl acetate extract and n-butanol extract of Tongkat Ali respectively for two weeks, the dosage is 1.08 g/(kg/d). Allopurinol (71 mg/(kg/d)) was given to the rats in group C for two weeks.

Determination of urine index:
Rats were fed in a single cage for 24 hours after the last administration with the condition that no food but water. Collected urine, recorded urine volume of 24 hours and measured 10 ml urine. The urine was centrifuged for 10min at a centrifuge at 4°C with the speed of 3500 r/min and measured the levels of UA, Cr, and UN of serum in rats. The excretion of 24 h UA in rats was calculated according to the urine volume and the concentration of UA. The UA excretion fraction (FEUA) of each group was calculated according to the concentration of UA and Cr in serum and urine.

\[
\text{FEUA} (%) = \left( \frac{\text{blood Cr} \times \text{urine UA}}{\text{blood UA} \times \text{urine Cr}} \right) \times 100
\]

Determination of serum indicators:
0.2% sodium pentobarbital sodium was utilized to anesthetize the rats after collecting the urine. 6 ml of blood were collected from the aorta of abdomens in rats. The blood was put in room temperature for 1 hour, centrifuged for 10 min at a centrifuge at 4°C with the speed of 3500 r/min and measured the levels of UA, XOD (xanthine oxidase), Cr, UN of serum in rats.

Determination of liver index:
The liver of rats was taken quickly after collecting the blood, cleaned at physiological saline and mashed with scissors. Took 0.2 g of liver tissue, added 1.8 ml of physiological saline at 4°C to the liver tissue, mashed it in ice baths for 2 min and centrifuged for 10 min at a centrifuge at 4°C with the speed of 3500 r/min measured the XOD activity of the liver supernatant at last.
Kidney pathology:
The kidney of rats was taken quickly after taking the liver, weighed with a balance and fixed with 10% formaldehyde for 48 hours. The kidneys tissue was taken, dehydrated, embedded with the paraffin, dyed, and observed with the microscope.

Statistical Data
Results were expressed as mean ± SD in all cases. The comparison between the groups was analyzed by one-way ANOVA, and the LSD procedure was used to compare the two groups. Differences were considered to be statistically significant when p was less than 0.05. Data were analysed using a statistical software package (SPSS for Windows 10.0.1, 1999, SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION
Effects of Different Extracts from Tongkat Ali on UA, Cr and UN Levels in Rats Serum and Urine
Serum UA level in groups which were given modeling agent had no statistically significant, serum UA levels in group B were significantly higher than those in group A (P<0.05) after the rats were given modeling agent for two weeks. It showed that the model was established successfully. The results were shown in Table 1, the level of serum UA in group B was significantly higher than that in group A (P<0.001) and the level of urine UA in group B was significantly lower than that in group A (P<0.01) after the rats were given modeling agent for four weeks. The results showed that the level of serum Uric acid in group C, D and F were significantly lower than those in group B (P<0.05) after the rats were given the extracts of Tongkat Ali. It indicated that the extracts of chloroform and n-butanol from Tongkat Ali had significant effect of reducing uric acid. Compared with group B, urine UA level in group C, D and F were significantly increased (P<0.05) after the rats were given the extracts of Tongkat Ali, indicating that the extracts of chloroform and n-butanol from Tongkat Ali could promote UA excretion in rats.

As is shown in Table1, the serum creatinine level in group B was significantly higher than that in group A (P<0.01), and the level of urine creatinine in group B was significantly lower than that in group A (P<0.05). Compared with group B, serum Cr levels in group C and F were significantly decreased (P<0.01), and serum Cr level in group D was significantly decreased (P<0.05). It indicated that both chloroform and n-butanol extracts have the ability to reducing the level of serum Cr in rats, and the effect of n-butanol extract is better than that of chloroform extract. The levels of urine Cr in group C and F were significantly higher than that in group B (P<0.01), and the level of urine Cr in group D was significantly higher than that in group B (P<0.05), it indicated that the extracts of chloroform and n-butanol could promote the excretion of creatinine in rats, and the effect of n-butanol extracts are stronger. As is shown in Table1, the level of serum UN in group B was significantly higher than that in group A (P<0.05), and the level of urine UN in group B was significantly lower than that in group A (P<0.05). The levels of serum UN in C, D, E and F group were lower than that in group B, but there was no statistically significant (P>0.05). The levels of urine UN in group C, D, E and F were higher than that in group B, but there was no statistically significant (P>0.05)

Table 1: Effects of different extracts from Tongkat Ali on uric acid, creatinine and urea nitrogen levels in rats serum and urine (n=10)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Serum UA(μmol/L)</th>
<th>Serum Cr(μmol/L)</th>
<th>Serum UN(mmol/L)</th>
<th>Urine UA(μmol/L)</th>
<th>Urine Cr(μmol/L)</th>
<th>Urine UN(mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank group(A)</td>
<td>187.05 ± 27.24</td>
<td>41.33 ± 3.75</td>
<td>3.87 ± 0.55</td>
<td>296.74 ± 29.55</td>
<td>194.26 ± 33.77</td>
<td>11.94 ± 2.45</td>
</tr>
<tr>
<td>Model group(B)</td>
<td>253.57 ± 38.08</td>
<td>60.29 ± 10.17</td>
<td>10.04 ± 3.39</td>
<td>338.64 ± 12.48</td>
<td>136.43 ± 12.31</td>
<td>4.83 ± 0.98</td>
</tr>
<tr>
<td>Positive control group(C)</td>
<td>207.22 ± 23.03</td>
<td>44.42 ± 7.94</td>
<td>7.28 ± 2.90</td>
<td>308.54 ± 34.16</td>
<td>214.17 ± 27.97</td>
<td>7.11 ± 1.78</td>
</tr>
<tr>
<td>Chloroform group(D)</td>
<td>219.56 ± 33.05</td>
<td>47.40 ± 5.17</td>
<td>8.59 ± 1.65</td>
<td>313.44 ± 31.66</td>
<td>224.34 ± 39.90</td>
<td>6.84 ± 1.06</td>
</tr>
<tr>
<td>Ethyl acetate group(E)</td>
<td>236.38 ± 34.32</td>
<td>56.06 ± 5.68</td>
<td>9.68 ± 3.93</td>
<td>298.07 ± 32.48</td>
<td>198.26 ± 35.24</td>
<td>5.30 ± 1.19</td>
</tr>
<tr>
<td>N-butanol group(F)</td>
<td>217.49 ± 22.08</td>
<td>45.22 ± 6.40</td>
<td>7.42 ± 2.68</td>
<td>322.02 ± 30.10</td>
<td>230.22 ± 31.29</td>
<td>6.40 ± 1.91</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SD; a denotes statistical significance between group A and the other groups (p<0.001), b denotes statistical significance group A and the other groups (p<0.01), c denotes statistical significance group A and the other groups (p<0.05), d denotes statistical significance group B and the other groups (p<0.01). e denotes statistical significance group B and the other groups (p<0.01). f denotes statistical significance group B and the other groups (p<0.05).

Effects of Different Extracts of Tongkat Ali on Serum and Liver XOD Activity
The effects of extracts from Tongkat Ali on the XOD activity of serum and liver were shown in Table 2. The activity of XOD in serum and liver of rats in group B was significantly higher than that in group A (P<0.05). The activity of XOD in serum and liver in group C was significantly lower than that in group B (P<0.05). The serum XOD activity in group D was significantly lower than that in group B (P<0.01). The serum XOD activity in group F was significantly lower than that in group B (P<0.001). It indicated that chloroform extract and n-butanol extract have a certain inhibitory effect on the activity of serum XOD.
Results are expressed as mean ± SD denotes statistical significance between group A and the other groups (p<0.001). b denotes statistical significance group A and the other groups (p<0.01). e denotes statistical significance group A and the other groups (p<0.05). d denotes statistical significance group B and the other groups (p<0.001). e denotes statistical significance group B and the other groups (p<0.01). f denotes statistical significance group B and the other groups (p<0.05).

Effects of Different Extracts of Tongkat Ali on FEUA and the Weight of Kidney

The results were shown in Table 3. The FEUA of group C, D, E and F were higher than that in group B, but there was no statistically significant (P>0.05). The weight of kidney in group B, D, E and F were significantly higher than that in group A (P<0.001), the weight of kidney in group C was significantly higher than that in group A (P<0.01), It indicated that the modeling agent could affect the weight of kidney in rats and lead to kidney lesions.

Results are expressed as mean±SD; a denotes statistical significance between group A and the other groups (p<0.001), b denotes statistical significance group A and the other groups (p<0.01), c denotes statistical significance group A and the other groups (p<0.05). d denotes statistical significance group B and the other groups (p<0.001). e denotes statistical significance group B and the other groups (p<0.01). f denotes statistical significance group B and the other groups (p<0.05).

Table 2: Effects of different extracts of Tongkat Ali on serum and liver XOD activity (n=10)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Activity of XOD in serum (ng/L)</th>
<th>Activity of XOD in liver(ng/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank group(A)</td>
<td>40.94 ± 7.14</td>
<td>36.44 ± 5.32</td>
</tr>
<tr>
<td>Model group(B)</td>
<td>52.11 ± 4.61</td>
<td>40.22 ± 3.25</td>
</tr>
<tr>
<td>Positive control group(C)</td>
<td>41.44 ± 7.37</td>
<td>35.91 ± 3.38</td>
</tr>
<tr>
<td>Chloroform group(D)</td>
<td>40.25 ± 6.26</td>
<td>36.71 ± 2.62</td>
</tr>
<tr>
<td>Ethyl acetate group(E)</td>
<td>45.09 ± 6.10</td>
<td>39.38 ± 4.44</td>
</tr>
<tr>
<td>N-butanol group(F)</td>
<td>38.99 ± 4.51</td>
<td>39.00 ± 5.46</td>
</tr>
</tbody>
</table>

Renal Pathology

The results were shown in Figure 1, the kidney of rats in group A had no obvious abnormalities which has a complete kidney capsule and has a clear structure of glomerular, proximal tubule and distal convoluted tubule. The kidney of rats in group B was damaged seriously. The phenomenon of renal tubular dilatation, renal tubular epithelial cells degeneration, necrosis and loss, a large number of interstitial fibers hyperplasia, inflammatory cell infiltration, a large number of brown crystals in the lumen and a small amount of macrophages could be observed obviously in rats of group B. The phenomenon of partial tubular dilatation, renal tubular epithelial cell degeneration and a little brown crystal in the lumen could be found in the kidney of rats in group C. The phenomenon of renal tubular epithelial cell degeneration, some interstitial cell proliferation and a little urate crystallization could be observed in the kidney of rats in group D, and the mount of urate crystallization in kidney of rats in group D is less than that in rats of group B. The phenomenon of renal tubular cells degeneration, part of the renal tubular epithelial cell shedding, a small amount of macrophages and a little brown crystal in the lumen could be observed in the kidney of rats in group E. The only change of the kidney in rats of group F was the degeneration of the renal tubular epithelial cell, this change was mild. The renal lesion in rats of group D and F was lighter than that in rats of group B, the renal lesion in rats of group D and F was also lighter than that in rats of group C. It indicated that the extracts of chloroform and n-butanol from Tongkat Ali had a certain protective effect on the kidney of rats.

In this research, we established the model of hyperuricemia by giving rats yeast extract, adenine and potassium oxonate [7-12], observed the functions of different extracts from Tongkat Ali and verified the role of Tongkat Ali in reducing uric acid. This study gave us the chance to find the active sites which have the effect of reducing uric acid in different extracts from Tongkat Ali laid the foundation for the subsequent separation of monomeric compounds with the activity of reducing uric acid.

Table 3: Effects of different extracts of Tongkat Ali on FEUA and the weight of kidney (n=10)

<table>
<thead>
<tr>
<th>Groups</th>
<th>FEUA (%)</th>
<th>Average value of kidney weight(g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank group(A)</td>
<td>35.86 ± 7.85</td>
<td>3.04 ± 0.20</td>
</tr>
<tr>
<td>Model group(B)</td>
<td>31.49 ± 5.52</td>
<td>4.83 ± 0.53</td>
</tr>
<tr>
<td>Positive control group(C)</td>
<td>38.08 ± 8.06</td>
<td>4.33 ± 0.60</td>
</tr>
<tr>
<td>Chloroform group(D)</td>
<td>39.46 ± 8.57</td>
<td>4.68 ± 0.59</td>
</tr>
<tr>
<td>Ethyl acetate group(E)</td>
<td>37.88 ± 7.86</td>
<td>4.55 ± 0.53</td>
</tr>
<tr>
<td>N-butanol group(F)</td>
<td>34.02 ± 6.34</td>
<td>4.47 ± 0.44</td>
</tr>
</tbody>
</table>
Figure 1: Representative optic micrographs of the slices of kidney in rats of different groups represents the blank group (B) represents the model group (C) represents the positive control group (D) represents the chloroform group (E) represents the ethyl acetate group (F) represents the n-butanol group

CONCLUSION

Research showed that the extract of chloroform and n-butanol could reduce serum uric acid levels and the effect of chloroform and n-butanol extract was as similar as that of allopurinol. Chloroform and n-butanol extract could decrease the level of serum creatinine and the effect of n-butanol extract is stronger than that of chloroform extract. The kidney is responsible for elimination of 70% of the daily UA production. It was obvious that the molding agent has bad influence on excretion of kidney from the results of renal pathology. The kidney of rats which were given the molding agent was damaged severely. Compared with the rats which were given the molding agent, the kidney of rats in chloroform group and n-butanol group was damaged slightly. It showed that the extracts of chloroform and n-butanol from Tongkat Ali had a certain protective effect on the kidney. In the process of purine metabolism, XOD is the only enzyme which can inhibit the production of uric acid. The facts that the activity of XOD decreased indicated that the speed of producing uric acid in the body was slowing down [13]. The extracts of chloroform and n-butanol could inhibit the activity of serum XOD and the effect of n-butanol extract is stronger than that in chloroform extract. The mechanisms of reducing the level of uric acid of the extracts of chloroform and n-butanol from Tongkat Ali may be related to inhibiting the activity of XOD. The fact that the extracts of Tongkat Ali had the effect of lowering uric acid has been confirmed, but there are some issues need to be further studied, such as the specific mechanism of reducing uric acid, the active monomer compounds, other pharmacological effects and so on.

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REFERENCES