ABSTRACT

Guducyadi Louha (GCD) is an ayurvedic medicine which is used in the treatment of gout. It is important to evaluate its toxicity in the animal system. Therefore, this study aimed to evaluate the toxicological effects of this ayurvedic formulation, Guducyadi Louha in male Sprague-Dawley rats. Acute toxicity tests were conducted to determine the LD$_{50}$ of the drug. In chronic study, animals were administered with GCD at a dose of and 400 mg/kg body weight for a period of 51 days. The effects of GCD on biochemical parameters (total protein content, lipid profiles, hepatic function test, kidney function test, uric acid level) were measured as indices of organ toxicity. The drug (GCD) did not affect serum total protein and globulin level; however, a significant (p<0.05) increase occurred in albumin and albumin/globulin ratio in the treated group. In lipid profile study, GCD significantly (p<0.05) increased total cholesterol (TC) and Non HDL-C level; thus leading to a statistically significant increase (p<0.05) of both Cardiac Risk Ratio (TC/HDL-C) and Atherogenic Coefficient [(TC - HDL-C)/HDL-C)]. The drug did not significantly (p<0.05) alter the levels of bilirubin, creatinine, urea, urea/creatinine ratio and uric acid. Overall, the alterations in the biochemical parameters especially lipid profile have consequential effects on the normal functioning of the organs of the animals. Therefore, the drug GCD at the dose of 400 mg/kg body weight may not be completely safe as an oral remedy and should be taken with caution if absolutely necessary.

Keywords: Guducyadi Louha, Atherogenic Coefficient, Cardiac Risk Ratio, Total Protein content, Lipid Profiles

INTRODUCTION

Medicinal plants have formed the basis of health care since the earliest days of humanity and are still widely used with considerable importance throughout the world $^{[1,2]}$. In certain African countries, however, up to 90% of the population still relies exclusively on plants as a source of medicines $^{[3]}$. Ayurveda largely uses plants as raw material for the manufacture of drugs though materials of animal and marine origin, metals and minerals are also used. Ayurvedic treatment aims to re-establish the equilibrium through various techniques, procedures, regimens, diet and medicines. Ayurvedic treatment consists of drugs, diet, exercise and general mode of life. The safety profile of this drug has not been fully investigated. It is also not clear, whether these preparations might interact with other drugs or diagnostic tests. Therefore, the continuous evaluation of these ayurvedic drugs for safety/toxicity using different animal models since the responses by these animals to drugs varies widely.

Currently, the World Health Organization (WHO) has officially recognized and recommended large-scale use of herbal (Unani and Ayurvedic) medicines, particularly in the developing countries, as an alternative system of medicine to provide health care services at the primary health care level $^{[4]}$. An estimated 1.5 billion people of the world’s population,
The use of herbal preparations with inadequate scientific studies on their safety profile has raised concerns on their toxicity. That is why; we designed our current experiment to observe the effect of chronic administration of GCD to Sprague-Dawley rats at a high dose (400 mg/kg). The objective is to have a better understanding of the potential toxicological profile of the drug. The study provides directions for further research as well.

MATERIALS AND METHODS

Drugs, Chemicals and Reagents: For the toxicological study, Guducyadi Louha (GCD) was collected from Sri Kundeswari Aushadhalaya Limited, Chittagong. Ketamine injection was purchased from ACI Pharmaceuticals Limited, Bangladesh. All other reagents, assay kits and chemicals used in this work were purchased from Human GmbH, Wiesbaden, Germany.

Experimental Animals: Six to eight-week old male Sprague-Dawley rats bred and maintained at the animal house of the Department of Pharmacy, Jahangirnagar University, were used in the toxicological experiment. These animals were apparently healthy and weighed 60-70 g. The animals were housed in a well-ventilated clean experimental animal house under constant environmental and adequate nutritional conditions throughout the period of the experiment. They were fed with rat chow prepared according to the formula developed at Bangladesh Council of Scientific and Industrial Research (BCSIR). Water was provided ad libitum and the animals maintained at 12 h day and 12 h night cycle. All experiments on rats were carried out in absolute compliance with the ethical guide for care and use of laboratory animals approved by Ethical Review Committee, Faculty of Life Sciences, Department of Pharmacy, Jahangirnagar University.

Experimental Design

Acute toxicity study:
The acute oral toxicity test was performed following the guidelines of Organization for Economic Cooperation and Development (OECD) for testing of chemicals with minor modifications (OECD Guideline 425) [9]. Sixteen male mice (30-40 g body weight) were divided into four groups of four animals each. Different doses (1000 mg/kg, 2000 mg/kg, 3000 mg/kg and 4000 mg/kg) of experimental drug (GCD) were administered by stomach tube. The dose was divided into two fractions and given within 12 hours. Then all the experimental animals were observed for mortality and clinical toxicity signs (general behaviour, respiratory pattern, cardiovascular signs, motor activities, reflexes and changes in skin and fur texture) at 1, 2, 3 and 4 hours and thereafter once a day for the next three days following GCD administration.

Chronic toxicity studies:
Prior to the experiment, rats were randomly divided into 2 groups of 8 animals each. One group was treated with GCD and another was used as a control. The control animals were administered with distilled water only as per the same volume as the drug treated group for 51 days. For all the pharmacological studies the drugs were administered per oral route at a dose of 400 mg/kg body weight [10]. After acclimatization, Ayurvedic medicinal preparation was administered to the rats by intra-gastric syringe between the 10 am to 12 am daily throughout the study period. All experiments on rats were carried out in absolute compliance with the ethical guide for care and use of laboratory animals. The experimental animals were marked carefully on the tail which helped to identify a particular animal. By using identification mark, responses were noted separately for a particular period prior to and after the administration [11].

Blood Samples Collection and Preparation of Serum:
At the end of the 51 days treatment period, after 18 hour fasting, rats from each group were anaesthetized by administration (i.p) of ketamine (500 mg/kg body
weight) \(^{(12)}\). Blood samples were collected from post vena cava of rats into plain sample tubes for serum generation for biochemical analysis. Serum was obtained after allowing blood to coagulate for 30 minutes and centrifuged at 4000 rpm for 10 min using bench top centrifuge (MSE Minor, England). The supernatant serum samples were collected using dry Pasteur pipette and stored in the refrigerator for further analysis. All analyses were completed within 12 hours of sample collection \(^{(13)}\).

**Determination of Biochemical Parameters:**

Biochemical analysis was carried out on serum to assess the state of the liver \(^{(14)}\) and kidney \(^{(15)}\). Biochemical studies involved analysis of parameters such as Total Protein \(^{(16)}\), Albumin by Bromacresol green method \(^{(17)}\), Creatinine \(^{(18)}\), Blood Urea Nitrogen (BUN) \(^{(19)}\) and Uric Acid \(^{(20)}\). Total cholesterol (TC) level \(^{(21)}\) and HDL-cholesterol level \(^{(22)}\) determined by CHOD-PAP method and total bilirubin was determined by Jendrassik and Grof method \(^{(23)}\).

While the serum non-HDL cholesterol concentration was determined as reported by Brunzell \(^{(24)}\). Non-HDL cholesterol = Total cholesterol − HDL cholesterol.

The atherogenic indices were calculated as follows:

Cardiac Risk Ratio (CRR) = TC/HDL-C \(^{(25)}\).

Atherogenic Coefficient (AC) = (TC - HDL-C)/HDL-C \(^{(26)}\).

The absorbances of all the tests were determined using spectrophotometer (UV-Visible Spectrophotometer Model No. UV-1601 PC).

**Statistical Analysis:**

The data were analyzed using independent sample t-test with the help of SPSS (Statistical Package for Social Sciences) Statistics 11.5 package (SPSS Inc., Chicago Ill). All values are expressed as mean ± SEM (Standard error of the mean) and \(p<0.05\) was taken as the level of significance.

**RESULTS AND DISCUSSION**

**Acute Toxicity Study:**

The drug (GCD) administered up to a high dose of 4000 mg/kg produced no mortality of the experimental animals. Thus the LD\(_{50}\) value was found to be greater than 4000 mg/kg body weight. The animals did not manifest any sign of fever, diarrhea, dysentery, bleeding per rectum, mal-absorption syndrome. Since GCD is in the clinical use for fever, diarrhea, dysentery, bleeding per rectum, mal-absorption syndrome treatment for many years, a limit test was performed in acute oral toxicity study.

According to the OECD test guideline 425 when there is information in support of low or non-toxicity and immortality nature of the test material, then the limit test at the highest starting dose level (4000 mg/kg body weight) was conducted. There were no mortality and toxicity signs observed at 4000 mg/kg body weight. Therefore, it can be concluded that GCD when administered at single dose is non-toxic and can be used safely in oral formulations.

**Chronic toxicity study**

**Effect of GCD on plasma protein of male rat:**

Proteins are important parts of all cells and tissues. The total protein test measures the total amount of two classes of proteins found in the fluid portion of blood: albumin and globulin. Albumin helps prevent fluid from leaking out of blood vessels and globulins are an important part of immune system \(^{(27, 28)}\).

In the male rats there were increase in the Total protein (5.56 %), the Albumin (27.86 %), Albumin / Globulin (53.24 %) and decrease in the Globulin (20.70 %) content in the plasma. A statistically significant increase (\(p<0.05\)) in the Albumin and Albumin / Globulin content in plasma were noted. The Total protein and Globulin content though not significantly different from their corresponding control values, yet they were noticeable.

**Effect of GCD on lipid profiles in Male Rats**

In the male rats after chronic administration of GCD the total cholesterol level was 269.63 % (\(p=0.001\)) increased in male rats group which was statistically very highly significant and only 4.901% increase of HDL level was noticed which was not statistically significant (\(p=0.657\)). The increase in Non-HDL-C, Cardiac Risk Ratio and Atherogenic Coefficient were statistically significant (\(p<0.05\)).

Elevated serum total cholesterol level is a familiar and well-known risk factor for developing atherosclerosis and other cardiovascular diseases \(^{(29)}\). In this study, GCD augmented almost all the atherogenic indices. Atherogenic indices are strong indicators of the risk of heart diseases: the higher the value; the higher the risk of developing cardiovascular problems and vice versa \(^{(26, 30)}\).

Therefore GCD may have been responsible for the hypercholesterolemic effect, observed in this study.

**Effect of GCD on liver function test in Male Rats**

The liver function test was performed to assess the state of the liver by the determination of plasma bilirubin level in the rats. After chronic administration of Guducyadi Lauha (GCD) to the male rats a negligible decrease of bilirubin level (2.27 %) in the plasma was noted in comparison to their
control group which obviously was not statistically significant (p=0.969).

Effect of GCD on Kidney function test in Male Rats

Kidney function test performed to measure the creatinine and urea content in the plasma. These two contents provides information about the effectiveness of the kidney function. Besides Urea/Creatinine ratio will also reveal the underlying cause of kidney dysfunction. There was a negligible decrease in the plasma creatinine (5.36%) and Urea (4.99%) and Urea/Creatinine (5.84%) level in the GCD treated male rats, and this decrease was not significant. Also a statistically insignificant increase in the Uric acid (29.86%) content in plasma was noted.

CONCLUSION

From the above experiment it can be concluded that GCD should not be administered chronically at a higher dose as it increase Albumin, Total Cholesterol (TC), Non HDL-C and almost all atherogenic indices. Further studies should be done by reducing the administered dose.

ACKNOWLEDGMENT

The authors are thankful to Focused Research on Ayurvedic Medicine and Education (F.R.A.M.E) Laboratory, Department of Pharmacy and all faculty members and the technical staffs of the Department of Pharmacy, Jahangirnagar University for their kind co-operation. We would express our special thanks to Mr. Shafiqul Islam for ensuring a constant supply of animals followed by proper maintenance and care of these animals during all throughout the experimental period.

Table 1: Name of the ingredients/herbs used in the preparation of “Guducyadi Lauha” (GCD)

<table>
<thead>
<tr>
<th>Sanskrit Name</th>
<th>Scientific Name</th>
<th>Quantity Used</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Guduci sara</td>
<td>Tinospora cordifolia</td>
<td>1 part</td>
</tr>
<tr>
<td>2. Sunthi</td>
<td>Zingiber officinale</td>
<td>1 part</td>
</tr>
<tr>
<td>3. Marica</td>
<td>Piper nigrum</td>
<td>1 part</td>
</tr>
<tr>
<td>4. Pippali</td>
<td>Piper longum</td>
<td>1 part</td>
</tr>
<tr>
<td>5. Haritaki</td>
<td>Terminalia chebula</td>
<td>1 part</td>
</tr>
<tr>
<td>6. Bibhitaka</td>
<td>Terminalia bellerica</td>
<td>1 part</td>
</tr>
<tr>
<td>7. Amalaki</td>
<td>Emblica officinalis</td>
<td>1 part</td>
</tr>
<tr>
<td>8. Vidanga</td>
<td>Embelia ribes</td>
<td>1 part</td>
</tr>
<tr>
<td>9. Citraka</td>
<td>Plumbago zeylanica</td>
<td>1 part</td>
</tr>
<tr>
<td>10. Musta</td>
<td>Cyperus rotundus</td>
<td>1 part</td>
</tr>
<tr>
<td>11. Lauha</td>
<td>Iron</td>
<td>10 part</td>
</tr>
</tbody>
</table>

Table 2: Effect of GCD on Serum Protein, Albumin, Globulin content and A/G ratio in Male Rats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>GCD</th>
<th>p value</th>
<th>% increase/decrease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Protein</td>
<td>5.79±0.15</td>
<td>6.11±0.26</td>
<td>0.340</td>
<td>↑5.56%</td>
</tr>
<tr>
<td>Albumin</td>
<td>2.38±0.18</td>
<td>3.04±0.10</td>
<td>0.007</td>
<td>↑27.86%</td>
</tr>
<tr>
<td>Globulin</td>
<td>3.87±0.34</td>
<td>3.07±0.24</td>
<td>0.071</td>
<td>↓20.70%</td>
</tr>
<tr>
<td>A/G</td>
<td>0.68±0.10</td>
<td>1.05±0.09</td>
<td>0.017</td>
<td>↑53.24%</td>
</tr>
</tbody>
</table>

Values are presented as mean ± SEM (n=8). Independent sample t-test was performed to analyze this dataset. p<0.05 was considered statistically significant when compared against control. ↑: increase, ↓: decrease.

Table 3: Effect of GCD on lipid profiles in Male Rats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>GCD</th>
<th>p value</th>
<th>% increase/decrease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Cholesterol</td>
<td>12.30±3.51</td>
<td>45.45±6.08</td>
<td>0.001</td>
<td>↑269.63%</td>
</tr>
<tr>
<td>HDL-C</td>
<td>12.22±0.81</td>
<td>12.82±0.95</td>
<td>0.657</td>
<td>↑4.90%</td>
</tr>
<tr>
<td>Non HDL-C</td>
<td>0.08±0.01</td>
<td>32.87±0.88</td>
<td>0.001</td>
<td>↑40.987%</td>
</tr>
<tr>
<td>AC</td>
<td>0.007±0.01</td>
<td>2.58±0.09</td>
<td>0.001</td>
<td>↑36.757%</td>
</tr>
<tr>
<td>CRR</td>
<td>0.53±0.14</td>
<td>2.60±0.48</td>
<td>0.019</td>
<td>↑392.86%</td>
</tr>
</tbody>
</table>

Values are presented as mean ± SEM (n=8). Independent sample t-test was performed to analyze this dataset. p<0.05 was considered statistically significant when compared against control. ↑: increase, ↓: decrease.
Table 4: Effect of GCD on Bilirubin in male rats.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>GCD</th>
<th>p value</th>
<th>% increase/decrease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bilirubin</td>
<td>0.24±0.08</td>
<td>0.23±0.10</td>
<td>0.969</td>
<td>↓2.27%</td>
</tr>
</tbody>
</table>

Values are presented as mean ± SEM (n=8). Independent sample t-test was performed to analyze this dataset. p<0.05 was considered statistically significant when compared against control. ↑: increase, ↓: decrease.

Table 5: Effect of GCD on Kidney function test in Male Rats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>GCD</th>
<th>p value</th>
<th>% increase/decrease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Creatinine</td>
<td>2.0645±0.15891</td>
<td>1.9539±0.21933</td>
<td>0.70</td>
<td>↓5.36%</td>
</tr>
<tr>
<td>Urea</td>
<td>51.4888±6.06257</td>
<td>48.9213±4.23947</td>
<td>0.73</td>
<td>↓4.99%</td>
</tr>
<tr>
<td>Urea/Creatinine</td>
<td>26.7413±5.08590</td>
<td>25.1791±4.02209</td>
<td>0.81</td>
<td>↓5.84%</td>
</tr>
<tr>
<td>Uric acid</td>
<td>2.0898±0.60176</td>
<td>2.7138±0.88262</td>
<td>0.57</td>
<td>↑29.86%</td>
</tr>
</tbody>
</table>

Values are presented as mean ± SEM (n=8). Independent sample t-test was performed to analyze this dataset. p<0.05 was considered statistically significant when compared against control. ↑: increase, ↓: decrease.

REFERENCE