PRECLINICAL LIPID PROFILE STUDIES OF A CLASSICAL AYURVEDIC PREPARATION MAKARADHWAJ RAS AFTER CHRONIC ADMINISTRATION TO MALE SPRAGUE-DAWLEY RATS

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ABSTRACT

Makaradhwaj Ras (MRS) is an Ayurvedic preparation used in traditional medicine as a cure for debility in chronic fatigue syndrome in the rural population. The present study is conducted to evaluate the effect of conventionally prepared MRS on different lipid profile parameters in experimental animals. Acute toxicity tests were conducted to determine the LD$_{50}$ of the drug. To find out the effect of chronic administration of MRS on serum lipid profile it was administered chronically to the male Sprague-Dawley rats at a dose of 40 mg/kg for 28 days. The drug (MRS) did not affect triglyceride (TG), total cholesterol (TC), LDL-C, VLDL-C, HDL-C and Non HDL-C level significantly; thus leading to an insignificant change in atherogenic indices like Cardiac Risk Ratio (TC/HDL-C), Atherogenic Coefficient [(TC - HDL-C)/HDL-C], Castelli’s Risk Index-II (CRI-II) (HDL-C/LDL-C) and Atherogenic Index of Plasma (AIP) (log (TG/HDL-C)). This experimental data will help the clinician for the logical use of MRS in different disease conditions with findings like no significant change in the lipid profile parameters.

Keywords: Ayurvedic preparation, lipid profile, Cardiac Risk Ratio, Atherogenic Index of Plasma, Atherogenic Coefficient.

INTRODUCTION

Ayurveda or Ayurveda medicine, is a system of medicine with historical roots in the Indian subcontinent [1]. Globalized and modernized practices derived from Ayurveda traditions are a type of complementary or alternative medicine [2-3]. In the Western world, Ayurveda therapies and practices have been integrated in general wellness applications and as well in some cases in medical use [4]. Ayurveda originated in India more than 3,000 years ago and remains as one of the country’s traditional health care systems. Its concepts about health and disease promote the use of herbal compounds, special diets, and other unique health practices. Ayurvedic medicines are regarded as a part of complementary and alternative medicine recognized by World Health Organization (WHO), National Institutes of Health (NIH) and others [5].

Makaradhwaj Ras (MRS) is an Ayurvedic preparation used in traditional medicine as a cure for debility in chronic fatigue syndrome in the rural population [6-11]. Makardhwaj Ras is included (page 289) in the Bangladesh National Formulary of Ayurvedic Medicine 1992 (Approved by the Government of Bangladesh vide Ministry of Health and Family Welfare Memo No. Health-1/Unani-2/89/(Part-1) 116 dated 3-6-1991) [6]. Bangladesh National Formulary of Ayurvedic Medicine is compiled by the National Unani and Ayurvedic Formulary Committee and published by the...
Bangladesh Board of Unani and Ayurvedic Systems of Medicine, 38, Bangabandhu Avenue, Dhaka-1000 under the authority vested in the Board vide section 13(j) of the Bangladesh Unani and Ayurvedic practitioners Ordinance, 1983 in collaboration with the World Health Organization. Permission to manufacture at industrial scale is printed in page no. 534 (column 1: Product code 12.81). Directorate of Drug Administration has issued Notification DA/Admin/1-10/96/6212 dated 19th October 1996 has issued license under Drug Act, 1940 and Rules there under and Drug (Control) Ordinance 1982 for local manufacture and sale in Bangladesh. (Published Bangladesh Gazette #24 Part VI dated Thursday, June 11th 1998.)

At present a good number of ayurvedic manufactures are manufacturing and marketing the classical ayurvedic medicinal preparation. Ayurvedic medicine uses a variety of products and practices. The safety profile of these drugs has not been fully investigated. It is also not clear, whether these preparations might interact with other drugs or diagnostic tests. The present study was undertaken to explore the effect of the drug in the lipid profile of rat serum after chronic administration of the drug.

MATERIALS AND METHODS

Drugs, Chemicals and Reagents: For the toxicological study, MRS 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: 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 50-70 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 hours day and 12 hours 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 Co-operation and Development (OECD) for testing of chemicals with minor modification (OECD Guideline 425) [12]. Sixteen male mice (35-40 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 (MRS) 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 behavior, 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 MRS administration.

Chronic toxicity studies: Prior to the experiment, rats were randomly divided into 2 groups of 8 animals each. One group was treated with MRS 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 28 days. For all the pharmacological studies the drug was administered per oral route at a dose of 40 mg/Kg body weight [13]. 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 experiment animals were marked carefully on the ear 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 [14].

Blood Samples Collection and Preparation of Serum: At the end of 28 days treatment, after 18 hours fasting, blood samples were collected from post vena cava of the rats anaesthetizing with Ketamine (500 mg/Kg body, intra peritoneal) and transferred into plain sample tubes immediately for serum generation [15]. Blood was then centrifuged at 4,000 g for 10 minutes using bench top centrifuge (MSE Minor, England). The supernatant plasma samples were collected using dry Pasteur pipette and stored in the refrigerator for further analyses. All analyses were completed within 12 hours of sample collection [16].

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Determination of Lipid Profile Parameters: Lipid profile studies involved analysis of parameters such as triglyceride (TG) level determined by GPO-PAP method [17]; total cholesterol (TC) level determined by CHOD-PAP method [18]; LDL-cholesterol level determined by CHOD-PAP method [19]; HDL cholesterol level determined by CHOD-PAP method [20]. The absorbance of all the tests was determined using Humalyzer, Model No-3500 (Human GmbH, Wiesbaden, Germany).

Serum VLDL and LDL cholesterol concentrations were calculated using the Friedewald equation [21] as follows:

i. LDL cholesterol (mg/dl) = Total Cholesterol − (HDL cholesterol −Triglyceride / 5)

ii. VLDL cholesterol (mg/dl) = Triglyceride / 5.

While the serum non-HDL cholesterol concentration was determined as reported by Brunzell [22], Non-HDL cholesterol = Total cholesterol − HDL cholesterol.

The atherogenic indices were calculated as follows:

Cardiac Risk Ratio (CRR) = TC/HDL-C [23],

Castelli’s Risk Index (CRI-II) = LDL-C/HDL-C [24],

Atherogenic Coefficient (AC) = (TC − HDL-C)/ HDL-C [25],

Atherogenic Index of Plasma (AIP) = log (TG/HDL-C) [26].

(Note: for calculation of atherogenic indices mg/dl values of TC, HDL-C, LDL-C and TG were converted into mmol/l).

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, p<0.01, p<0.001 were taken as the level of significance.

RESULTS

Acute toxicity study: The drug (MRS) administered up to a high dose of 4000 mg/Kg produced no mortality of the experimental animals. Thus the LD50 (Median Lethal Dose) value was found to be greater than 4000 mg/Kg body weight. The animals did not manifest any sign of restlessness, respiratory distress, general irritation or convulsion. Since MRS is in the clinical use for treatment of cardiovascular diseases 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 nontoxicity 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 ml/Kg body weight. Therefore, it can be concluded that MRS when administered at single dose is non-toxic and can be used safely in oral formulations.

Chronic lipid profile studies

Effect of MRS on lipid profile of male rats: There is a (p=0.098, 18.09%) decrease in the triglyceride content of the serum of the male rat, the decrease though not significant yet it was noticeable. There is a (p=0.213, 6.41%) increase in the total cholesterol content, the decrease though not significant yet it was prominent. There is a negligible (p= 0.744, 4.33%) increase in the LDL level, which was statistically not at all significant. There is a (p=0.098, 18.09%) decrease in the VLDL level, the decrease though not significant yet it was noticeable. There was no change noticed in the non-HDL level of the serum of the male rat.

Effect of MRS on atherogenic indices of male rats: There is an (p=0.350, 11.59%) increase in the Cardiac Risk Ratio, the increase though not significant yet it was prominent. There is an (p=0.286, 25.40 %) increase in the Castelli’s Risk Index II, the increase though not significant yet it was prominent. There is an (p=0.350, 19.97%) increase in the Atherogenic Coefficient (AC), the increase though not significant yet it was prominent. There is a negligible (p=0.860, 8.58%) decrease in the Atherogenic Index of Plasma (AIP), which was statistically not at all significant.

DISCUSSION

A lipid profile is a measurement of various lipids that are found in the blood. This kind of blood test is often used to assess risk of heart disease. A lipid profile contains information about several different kinds of lipid that normally circulate in the blood. Values are numerical, but in order to simplify explanation, ranges of numerical values are often placed into categories such as ‘low risk,’ or ‘high risk.’ For example, a total cholesterol level over 240 mg/dl is said to be ‘high risk’, but that doesn’t mean a reading of 238 is fine. With total cholesterol and LDL cholesterol the higher the number, the higher the risk. Conversely, the lower the LDL cholesterol,
the lower the risk. However, a low number is not a guarantee against heart disease. The population with low cholesterol is at lower risk of heart disease, but heart disease is not absent in this population.

**Effect of MRS on lipid profile of male rats:**
Dyslipidemia is one of the main contributors to cardiovascular risk. High level of cholesterol, low-density lipoprotein cholesterol (LDL-C) and triglycerides (TG), and low levels of high-density lipoprotein cholesterol (HDL-C) are relevant risk factors for adverse cardiovascular events. Classically, management of dyslipidemia has been focused on LDL-C. However, other deleterious lipid profiles and their association with cardiovascular clinical events have gained attention during last years.

A high plasma triglyceride level is both an independent and synergistic risk factor for cardiovascular diseases and is often related with hypertension, obesity and diabetes mellitus [27-29]. In this study, a lower serum triglyceride level was observed in the animals treated with MRS. Lower serum triglyceride may have positive impact on body.

While higher HDL levels are correlated with cardiovascular health, no medication used to increase HDL has been proven to improve health [30]. In other words, while high HDL levels might correlate with better cardiovascular health, specifically increasing one's HDL might not increase cardiovascular health. The remaining possibilities are that either good cardiovascular health causes high HDL levels, there is some third factor which causes both, or this is a coincidence with no causal link. Reduced serum HDL cholesterol is a risk factor for cardiovascular disease [31] and is often found in hypertension [28, 32]. So, in the present study, the lower serum HDL cholesterol level, recorded for the treated groups is suggestive of the cardio-toxic effect of the drug.

High levels of VLDL cholesterol have been associated with the development of plaque deposits on artery walls, which narrow the passage and restrict blood flow. In this study, lower serum VLDL level was observed in the animals treated with MRS.

**Effect of MRS on atherogenic indices of male rats:**
In this study, MRS augmented almost all the atherogenic indices except AIP but all are not significant. Atherogenic indices are strong indicators of the risk of heart disease: the higher the value, the higher the risk of developing cardiovascular problems and vice versa [23-26].

**CONCLUSION**
From the above experiment it can be concluded that MRS should not be administered chronically at a higher dose as it increase LDL-C and almost all atherogenic indices except AIP and decrease HDL-C level. 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.

<p>| Table 1: Name of the ingredients/herbs used in the preparation of Makardhwaj Ras |
|-------------------------------------------------|------------------|------------------|------------------|------------------|------------------|</p>
<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Plant part</th>
<th>Botanical name</th>
<th>Family</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Rasa Sindura</td>
<td>Calcined</td>
<td>Hydragentum</td>
<td>Mineral</td>
<td>10 g.</td>
</tr>
<tr>
<td>2. Svarna bhasma</td>
<td>Calcined</td>
<td>Processed Gold</td>
<td>Mineral</td>
<td>10 g.</td>
</tr>
<tr>
<td>3. Lauha bhasma</td>
<td>Calcined</td>
<td>Purified Iron oxide</td>
<td>Mineral</td>
<td>10 g.</td>
</tr>
<tr>
<td>4. Lavanga</td>
<td>Flower</td>
<td>Syzygium aromaticum</td>
<td>Myrtaceae</td>
<td>10 g.</td>
</tr>
<tr>
<td>5. Camphor</td>
<td>Whole plant</td>
<td>Cinnamomum camphora</td>
<td>Lauraceae</td>
<td>10 g.</td>
</tr>
<tr>
<td>6. Jatiphala</td>
<td>Seed</td>
<td>Myristica fragrans</td>
<td>Myristicaceae</td>
<td>10 g.</td>
</tr>
<tr>
<td>7. Mrgamada (kasturi)</td>
<td>Musk</td>
<td>Moschus moschiferus</td>
<td>Moschidae</td>
<td>10 g.</td>
</tr>
<tr>
<td>8. Pan</td>
<td>Leaf</td>
<td>Piper betle</td>
<td>Piperaceae</td>
<td>Q.S.</td>
</tr>
</tbody>
</table>

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Table 2: Effect of Makardhwaj Ras on lipid profile of rat serum

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>MRS</th>
<th>p values</th>
<th>% Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triglycerides</td>
<td>38.00±3.18</td>
<td>31.13±2.22</td>
<td>0.098</td>
<td>18.09% decrease</td>
</tr>
<tr>
<td>Total Cholesterol</td>
<td>64.38±2.50</td>
<td>60.25±1.93</td>
<td>0.213</td>
<td>6.41% decrease</td>
</tr>
<tr>
<td>VLDL-C</td>
<td>7.60±0.64</td>
<td>6.23±0.44</td>
<td>0.098</td>
<td>18.09% decrease</td>
</tr>
<tr>
<td>LDL-C</td>
<td>28.88±2.52</td>
<td>30.13±2.79</td>
<td>0.744</td>
<td>4.33% decrease</td>
</tr>
<tr>
<td>Non HDL-C</td>
<td>36.75±2.58</td>
<td>36.75±2.99</td>
<td>1.00</td>
<td>0.00%</td>
</tr>
<tr>
<td>HDL-C</td>
<td>27.63±1.59</td>
<td>23.50±1.48</td>
<td>0.078</td>
<td>14.93% decrease</td>
</tr>
</tbody>
</table>

Independent sample t-test was performed to analyze this data set. All values are expressed as mean ± SEM and p<0.05, p<0.01, p<0.001 were taken as the level of significant

Table 3: Effect of Makardhwaj Ras on atherogenic indices of rat serum.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>MRS</th>
<th>p values</th>
<th>% Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRR</td>
<td>2.38±0.16</td>
<td>2.66±0.24</td>
<td>0.35</td>
<td>11.593% increase</td>
</tr>
<tr>
<td>CRI-II</td>
<td>1.09±0.14</td>
<td>1.37±0.21</td>
<td>0.286</td>
<td>25.4032% increase</td>
</tr>
<tr>
<td>AIP</td>
<td>0.1328±0.05</td>
<td>0.1214±0.04</td>
<td>0.86</td>
<td>8.58437% decrease</td>
</tr>
<tr>
<td>AC</td>
<td>1.38±0.16</td>
<td>1.66±0.24</td>
<td>0.35</td>
<td>19.9653% increase</td>
</tr>
</tbody>
</table>

Independent sample t-test was performed to analyze this data set. All values are expressed as mean ± SEM and p<0.05, p<0.01, p<0.001 were taken as the level of significant

REFERENCES

5. Valiathan MS. Current Science (Indian Academy of Sciences), 2006; 90 (1); 5–6.

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