Preclinical Anemia Panel Studies of “Makardhvaja” after Chronic Administration to Male Sprague-Dawley Rats


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Abstract

Makardhvaja (MD) is an Ayurvedic preparation used as a traditional medicine in the treatment of sexual dysfunction in the rural population. The effect of chronic administration of Makardhvaja on the hematological parameters and serum iron profile was studied in this experiment. The acute toxicity test of MD recorded no death, even at the highest dose of 80 ml/kg body weight. During the chronic toxicity test, animals were divided into two groups. The first group was given MD preparation at a dose of 40 mg/kg body weight for 28 days while the second group that served as the control received water for the same period. After 28 days of chronic administration of the MD preparation to the male Sprague-Dawley rats, the following hematological changes were noted. Erythrocytic indices such as red blood count (RBC), hemoglobin, Hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and red cell volume distribution width (RDW) did not change significantly. In the male rats, a statistically highly significant (p = 0.003) decrease (27.35%) in the serum iron level, an increase (26.42%) in the serum ferritin level, which, though not significant, was prominent (p = 0.120), and a statistically very highly significant (p = 0.001) decrease (47.05%) in the serum total iron binding capacity (TIBC) were noted.

Keywords: Makardhvaja; Ayurvedic preparation; Hematology; Serum iron level; Ferritin; TIBC

Introduction

Anemia is a public health problem both in Bangladesh and worldwide [1]. It is defined as a "fall of hemoglobin concentration below a statistically defined threshold lying at two standard deviations below the median of a healthy population of the same age, sex, and stages of pregnancy" [2]. Although pregnant women are most frequently affected, it is also ubiquitous in nonpregnant women and other population groups, including children [3]. It has been estimated that around two billion people in the world are anemic; most of them are found in low-income countries in Asia and Africa [4]. Iron deficiency has been claimed to constitute the major part of the anemia problem. A logical intervention for its prevention and control has therefore been the provision of iron supplementation during pregnancy [5].

Drug-induced anemia is also a major problem in low-income countries [6]. There are some drugs (such as Streptomycin, Aspirin, Ceftriaxone, etc.) that can cause severe anemia [7-9]. Ayurvedic medicine also recognized as Ayurveda is one of the world’s oldest holistic (whole-body) healing systems. It is regarded as a part of complementary and alternative medicine recognized by World Health Organization (WHO), National Institutes of Health (NIH), and others [10]. They also have a good safety profile [11]. But there are reports of heavy metal contamination (such as lead) in Ayurvedic preparations resulting in intoxication [12]. The safety profile of these drugs has not been fully investigated. That is why the present study was undertaken to explore the effect of MD in the anemia profile after chronic administration of it to the male Sprague-Dawley rats.

Makardhwaj is a well-known inorganic preparation of the Ayurvedic Pharmacopoeia used in Ayurvedic antiaging and aphrodisiac treatment [13,14]. Chemically, it is red sulfide of mercury and gold in an uncombined form. It is a sublimed product made from pure mercury, sulfur, and gold. Eight parts of mercury and one part of gold leaf are mixed together to form an amalgam. To this mixture, 16 parts of sublimed sulfur are added, and the resulting mixture is ground very thoroughly in a stone mortar for 24 h or more until the whole is converted to a lusterless, fine, impalpable powder of uniform consistence. This mixture is then placed in a narrow-mouthed bottle and is gradually heated on a sand bath. On heating, the bottle is filled with reddish fumes of various hues. On cooling, Makardhwaj is found deposited in the inner surface of the neck of the bottle. MD (447 p.) is included 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).

Materials and Methods

Drugs, chemicals, and reagents

For the toxicological study, Makardhvaja (MD) was collected from Sri Kundeswari Aushadhalya Limited, Chittagong. Ketamine injection was purchased from ACI 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 a 12 h day and 12 h night cycle. All
The supernatant serum samples were collected using dry Pasteur sample tubes for serum generation for biochemical analysis. Serum was obtained after allowing blood to coagulate for 30 min and centrifuged at 4,000 g for 10 min using bench top centrifuge (MSE Minor, England)

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 modifications (OECD Guideline 425) [15]. Sixteen male mice (30-40 g body weight) were divided into four groups of four animals each. Different doses (50, 60, 70, and 80 ml/kg) of the experimental drug (MD) were administered by a stomach tube. The dose was divided into two fractions and given within 12 h. Then all the experimental animals were observed for mortality and clinical signs of toxicity (general behavior, respiratory pattern, cardiovascular signs, motor activities, reflexes, and changes in skin and fur texture) at 1, 2, 3, and 4 h and thereafter once a day for the next three days following MD administration.

**Chronic toxicity studies**

Prior to the experiment, rats were randomly divided into 2 groups of 8 animals each. One group was treated with MD 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 drugs were administered per oral route at a dose of 40 mg/kg body weight [16]. After acclimatization, Ayurvedic medicinal preparation was administered to the rats by intragastric syringe between the 10 to 12 am daily throughout the study period. All the experiments on the 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 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 [17].

**Blood sample collection and preparation of serum**

At the end of the 28-day treatment period, after 18 h fasting, the rats from each group were anesthetized by the administration (i.p) of ketamine (500 mg/kg body weight) [18]. Blood samples were collected from post vena cava of the rats into EDTA (Ethylene di-amine tetra acetic acid) sample tubes for hematological analysis and into plain sample tubes for serum generation for biochemical analysis. Serum was obtained after allowing blood to coagulate for 30 min and centrifuged at 4,000 g 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 h of sample collection [19].

**Determination of anemia profile studies**

Anemia profile studies involved the analysis of parameters such as red blood cells (RBC) level determined by the electrical impedance method [20], hemoglobin (HGB) level determined by the modified hemoglobin cyanide method [21], serum transferrin determined by the turbidity method [22], total iron binding capacity (TIBC), and the serum ferritin level [23,24]. MCV, MCH, and MCHC are calculated according to the formula given by Wintrobe [25] and Diem and Clenton [26]:

\[
\text{MCV} = \frac{\text{HCT} \times \text{RBC count (millions)}}{10} \\
\text{MCH} = \frac{\text{Hb (g/dL)} \times \text{RBC count (millions)}}{10} \\
\text{MCHC} = \frac{\text{Hb (g/dL)}}{\text{HCT} \times 100}
\]

**Statistical analysis**

The data were analyzed using independent sample t-test with the help of SPSS (Statistical Package for Social Science) Statistics 11.5 package (SPSS Inc., Chicago Ill). All values are expressed as mean ± SEM (standard error of the mean), and \(p < 0.05\), \(p < 0.01\), \(p < 0.001\) was taken as the level of significance.

**Results**

**Acute toxicity study**

The drug (MD) administered up to a high dose of 80 ml/kg produced no mortality. Thus, the LD50 value was found to be greater than 80 ml/kg body weight. The animals did not manifest any sign of fever; chronic skin diseases; diabetes; urinary tract disorders; sinuses; nonhealing wounds; fistula; obesity; rheumatoid arthritis; ascites; headache; gynecological disorders; and diseases of ear, nose, throat, and eyes. According to the OECD test guideline 425, when there is information in support of low toxicity or nontoxicity and immortality nature of the test material, the limit test at the highest starting dose level (80 ml/kg body weight) was conducted. There were no mortality and toxicity signs observed at 80 ml/kg body weight. Therefore, it can be concluded that MD when administered at single dose is nontoxic and can be used safely in oral formulations.

**Chronic anemia profile studies**

**Effect of MD on hematological profile of male rats**

The results of the anemia panel studies are thus: There is a statistically insignificant decrease (\(p = 0.681\)) [1.69%] in the total number of red blood cells in the male rat. There is a statistically insignificant decrease (\(p = 0.641\)) [1.91%] in the hemoglobin content in the blood of the male rat. There is a negligible decrease [0.44%] in the hematocrit level of the blood of the male rat, which was statistically not at all significant (\(p = 0.904\)). There is a statistically insignificant increase (\(p = 0.616\)) [0.53%] in the mean corpuscular volume, a red cell index of the male rat. There is a negligible decrease [0.14%] in the mean corpuscular hemoglobin, a red cell index of the male rat, which was statistically not at all significant (\(p = 0.898\)). There is a decrease [0.58%] in the mean corpuscular hemoglobin concentration, a red cell index of the male rat; the decrease, though not significant, was prominent (\(p = 0.447\)). There is an increase [1.79%] in the red cell volume distribution width, a red cell index of the male rat; the increase, though not significant, was prominent (\(p = 0.381\)).

**Effect of MD on serum iron profile of male rats**

In the male rats, a statistically highly significant (\(p = 0.003\)) decrease (27.35%) in the serum iron level, an increase (26.42%) in...
The measurement of hemoglobin, the oxygen-carrying protein, is a more sensitive and direct test for anemia. Anemia is generally defined as hemoglobin values below the fifth percentile in a healthy reference population. It is most commonly used to screen iron deficiency. Anemia occurs when the numbers of red blood cells decreases as iron stores are depleted. However, serum iron may remain normal or even lower value may occur in iron deficiency, but the normal or even lower value may occur in iron deficiency anemia and is known to decrease when iron deficiency anemia is present [32]. MCV is useful for categorizing anemia as microcytic, normocytic, and macrocytic. MCV and MCH (the mean corpuscular hemoglobin) values are reduced usually in anemia patients, and the mean corpuscular hemoglobin concentration (MCHC) is reduced in severe diseases [30]. The mean corpuscular volume (MCV) is an indicator of iron deficiency anemia and is known to decrease when iron deficiency anemia is present [32]. The measurement of hemoglobin, the oxygen-carrying protein, is a more sensitive and direct test for anemia. Anemia is generally defined as hemoglobin values below the fifth percentile in a healthy reference population. It is most commonly used to screen iron deficiency. Anemia occurs when the numbers of red blood cells decreases as iron stores are depleted. However, serum iron may remain normal or even lower value may occur in iron deficiency anemia and is known to decrease when iron deficiency anemia is present [32]. MCV is useful for categorizing anemia as microcytic, normocytic, and macrocytic. MCV and MCH (the mean corpuscular hemoglobin) values are reduced usually in anemia patients, and the mean corpuscular hemoglobin concentration (MCHC) is reduced in severe diseases [30].

### Table 3: Effect of MD on hematological profile of rat

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>MD</th>
<th>p value</th>
<th>(% Decrease/increase)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBC</td>
<td>6.3075 ± 0.20577</td>
<td>6.2012 ± 0.14710</td>
<td>0.681</td>
<td>Decr 1.69%</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>11.0225 ± 0.38284</td>
<td>10.812 ± 0.21748</td>
<td>0.641</td>
<td>Decr 1.91%</td>
</tr>
<tr>
<td>Hematocrit</td>
<td>33.7750 ± 0.12378</td>
<td>33.6250 ± 0.66272</td>
<td>0.904</td>
<td>Decr 0.44%</td>
</tr>
<tr>
<td>MCV</td>
<td>53.9625 ± 0.35654</td>
<td>54.2500 ± 0.43221</td>
<td>0.616</td>
<td>Incr 0.53%</td>
</tr>
<tr>
<td>MCH</td>
<td>17.4750 ± 0.16448</td>
<td>17.4500 ± 0.09820</td>
<td>0.898</td>
<td>Decr 0.14%</td>
</tr>
<tr>
<td>MCHC</td>
<td>32.3875 ± 0.19127</td>
<td>32.2000 ± 0.14392</td>
<td>0.447</td>
<td>Decr 0.58%</td>
</tr>
<tr>
<td>RDW</td>
<td>12.5625 ± 0.17107</td>
<td>12.7875 ± 0.18071</td>
<td>0.381</td>
<td>Incr 1.79%</td>
</tr>
</tbody>
</table>

rho ≤ 0.05, ρ** ≤ 0.01, ρ*** ≤ 0.001.

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References