

**PRECLINICAL LIPID PROFILE STUDIES OF AN AYURVEDIC PREPARATION “SARAGUNA BALIJARITA MAKARADHVAJA” USED AS ANTIPYRETIC**

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ABSTRACT

Saraguna Balijarita Makaradhvaja (SBM) is an Ayurvedic preparation used in traditional medicine as an antipyretic in the rural population. We were eager to know the effect of chronic administration of SBM on the lipid profile test. To find out the effect of chronic administration of SBM on the lipid profile, it was administered chronically to the male Sprague-Dawley rats at a dose of 400 mg/Kg. After 28 days of chronic administration of the SBM preparation, the following effects on the lipid profile were noted. There was a statistical significant increase in LDL and Non-HDL Cholesterol ($p=0.01$). A very highly significant ($p=0.001$) decrease was noticed in HDL-C level; thus leading to a statistically very highly significant ($p=0.001$) increase in Cardiac Risk Ratio (CRR) (TC/HDL-C), Castelli's Risk Index-II (CRI-II) (HDL C/LDL-C) and Atherogenic Coefficient (AC) [(TC/HDL-C)/ HDL-C]. No significant change was found in case of TG, TC, VLDL and Atherogenic Index of Plasma (AIP) (log (TG/HDL-C)).

Keywords: Saraguna Balijarita Makaradhvaja, Lipid profile, Cardiac Risk Ratio, Atherogenic Index of Plasma, Atherogenic Coefficient.

INTRODUCTION

Ayurveda which means 'Science of life' is derived from the Sanskrit words 'Ayur' meaning life and 'Veda' meaning knowledge. It takes an integrated view of the interactions of the physical, mental, spiritual and social aspects of the life of human beings. Ayurveda aims to keep the structural and physiological entities in a state of equilibrium, which signifies good health. Any imbalance due to internal or external factors may cause disease [1].

Ayurvedic medicines are somewhat inexpensive and have wide acceptability among the general populace,

particularly in rural areas. They have a good safety profile also [2]. 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 [3]. An estimated 1.5 billion people of the world's population, according to WHO, are now getting treatment with these medicines [4, 5].

Makaradhvaja is a herbo-mineral formulation mentioned in Ayurvedic formulary of India [6]. It has been used for centuries with claimed efficacy and

safety in treatment of chronic fever. It consists Solanum melongena, Oryza sativa, Trichosanthes dioica, Boerhaavia diffusa, Godugdha, Dadhi, Goghrita, Triticum aestivum, Phaseolus radiatus, Cuminum cyminum and metal including processed gold, mercury and sulfur in different ratios (1:8:16; 1:8:24 or 1:8:48) [7].

Saraguna Balijarita Makaradhvaja (SBM) is included (page 92) 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). The present study was undertaken to explore the effect of Saraguna Balijarita Makaradhvaja in the lipid profile of rat serum after chronic administration of the drug to male Sprague-Dawley rats.

MATERIALS AND METHODS

Drugs, Chemicals and Reagents: For the toxicological study, Saraguna Balijarita Makaradhvaja 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 80-100 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) [8]. Sixteen

female mice (non-pregnant, 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 (SBM) 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 SBM administration.

Chronic toxicity studies: Prior to the experiment, rats were randomly divided into 2 groups of 8 animals each. One group was treated with SBM 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 400 mg/Kg body weight [9]. 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 [10].

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 [11]. 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 [12].

Determination of Lipid Profile Parameters: Lipid profile studies involved analysis of parameters such as triglyceride (TG) level determined by GPO-PAP method [13]; total cholesterol (TC) level determined by CHOD-PAP method [14]; LDL-cholesterol level determined by CHOD-PAP method [15]; HDL cholesterol level determined by CHOD-PAP method [16]. 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 [17] 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 [18]:

Non-HDL cholesterol = Total cholesterol – HDL cholesterol.

The atherogenic indices were calculated as follows:

Cardiac Risk Ratio (CRR) = TC/HDL-C [19].

Castelli's Risk Index (CRI-II) = LDL-C/HDL-C [20].

Atherogenic Coefficient (AC) = (TC - HDL-C)/HDL-C [21].

Atherogenic Index of Plasma (AIP) = $\log(\text{TG}/\text{HDL-C})$ [22].

(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 Science) Statistics 11.5 package (SPSS Inc., Chicago Ill). All values are expressed as mean \pm 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 (SBM) 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 SBM is in the clinical use for treatment of chronic fever 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 SBM when administered at single dose is non-toxic and can be used safely in oral formulations.

Chronic Lipid Profile Studies

Effect of SBM on lipid profile of male rats: After chronic administration of SBM, there were a very highly significant decrease in the HDL-C (27.60%; $p = 0.001$) level and a highly significant increase in the LDL-C (31.17%; $p = 0.01$) level in the serum of male rats. Also a statistically significant increase was noticed in case of Non-HDL (24.49%; $p = 0.02$) level. No significant change was noticed in case of TG, TC and VLDL level. All the results are presented in Table 2.

Effect of SBM on Atherogenic indices of male rats:

In this study, SBM augmented almost all the Atherogenic indices except Atherogenic Index of Plasma (AIP). The increase in Cardiac Risk Ratio (CRR) (38.69 % increase), Castelli's Risk Index-II (CRI-II) (75.64 % increase) and Atherogenic Coefficient (AC) (66.63 % increase) was statistically very highly significant ($p = 0.001$). A statistically insignificant ($p = 0.38$) increase in case of Atherogenic Index of Plasma (AIP) (42.92 %) was noticed. All the results are presented in Table 3.

DISCUSSION

Ayurvedic medicines have achieved greater importance as an alternative to conventional therapy. To enhance the safe use of a plant-based medicine, one should take into account their historical applications on humans and animals as well as toxicity evaluation of the medicinal herbs and their active components [23]. Although significant advances have been made in the development and application of *in vitro* toxicity assays, *in vivo* safety evaluation remains the most useful tool for detecting target organ toxicity [24]. The rat has been the species of choice for the majority of preclinical toxicology studies. Recent findings revealed that rat and mouse is a suitable model for early safety assessment since earlier identification of preclinical toxicities are generally predictive of human toxicity and could save time, money, and effort spent [25].

Effect of SBM on lipid profile of male rats: High level of plasma LDL cholesterol is a risk factor for cardiovascular disease and often accompanies hypertension and obesity [26-29]. In this study, significantly higher plasma LDL cholesterol level was observed in the animals treated with SBM. Reduced serum HDL cholesterol is a risk factor for cardio-vascular disease [30] and is often found in hypertension [28, 31]. So, in the present study, the low serum HDL cholesterol level, recorded for the treated groups is suggestive of the cardio-toxic effect

of the drug. Measurement of non-HDL cholesterol (non-HDL-C) is one of the principal components of cardiovascular risk assessment and a critical target for lipid-lowering therapy as it reflects the cholesterol in all lipoprotein particles that are considered to be atherogenic. [32]. Non-HDL-C has been strongly associated with predicting coronary artery disease [33]. Additionally, in a consensus report by the American Diabetes Association and American College of Cardiology Foundation, they identified that non-HDL-C was a better marker than low-density lipoprotein (LDL) cholesterol for identifying high-risk patients at cardiovascular risk [18].

Effect of SBM on Atherogenic indices of male rats:

In this study, SBM augmented all the Atherogenic indices. The increase in Cardiac Risk Ratio (CRR), Castelli's Risk Index-II (CRI-II) and Atherogenic Coefficient (AC) was statistically highly significant. The increase in Atherogenic Index of Plasma (AIP) was not statistically 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 [20-21].

CONCLUSION

From the above experiment it can be concluded that SBM should not be administered chronically at a higher dose as it increases total cholesterol (TC), LDL, Non-HDL, all Atherogenic indices and decrease HDL level. Further studies should be done by reducing the administered dose.

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Table 1: Effect of Saraguna Balijarita Makaradhvaja (SBM) on Lipid profile in Male Rats

| Parameters | CON (Mean±SEM) | SBM (Mean±SEM) | p-Value | % Change |
|------------|-------------------|-------------------|---------|-----------|
| TG | 38.00±3.18 | 31.13±1.87 | 0.08 | ↓ 18.09 % |
| TC | 64.38±2.50 | 65.75±2.77 | 0.72 | ↑ 2.14 % |
| LDL | 28.88±2.52 | 37.88±1.83 | 0.01 | ↑31.17 % |
| VLDL | 7.60±0.64 | 6.23±0.37 | 0.08 | ↓ 18.09 % |
| HDL | 27.63±1.59 | 20.00±0.94 | 0.001 | ↓ 27.60 % |
| Non-HDL | 36.75±2.58 | 45.75±2.18 | 0.02 | ↑ 24.49 % |

Table 2: Effect of Saraguna Balijarita Makaradhvaja (SBM) on Atherogenic Indices

| Parameters | CON (Mean±SEM) | SBM (Mean±SEM) | p-Value | % Change |
|------------|-------------------|-------------------|---------|-----------|
| CRR | 2.38±0.16 | 3.31±0.12 | 0.001 | ↑ 38.69 % |
| CRI- II | 1.09±0.14 | 1.92±0.12 | 0.001 | ↑ 75.64 % |
| AC | 1.38±0.16 | 2.31±0.12 | 0.001 | ↑ 66.63 % |
| AIP | 0.13±0.05 | 0.19±0.04 | 0.38 | ↑ 42.92% |

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