

Preclinical HbA1c level studies of makaradhwaja and siddha makaradhwaja after chronic administration to male Sprague-Dawley rats

Arjyabrata Sarker¹, Kushal Biswas^{2*}, Manjurul I. Chowdhury³, Farzana Khan², Taposhi Sultana², Farjana Afrin⁴, Md. Afaz Uddin¹, Md. Al Foyjul Islam¹, M. S. K. Choudhuri¹

¹Department of Pharmacy, Jahangirnagar University, Savar, Dhaka 1342, Bangladesh

²Department of Pharmacy, East West University, Dhaka 1212, Bangladesh

³Global Centre for Environmental Remediation, The University of Newcastle, Callaghan, NSW 2308, Australia

⁴Department of Pharmacy, Noakhali Science and Technology University, Noakhali 3814, Bangladesh

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***Correspondence to:**

Kushal Biswas,

Email: kushal71@outlook.com

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ABSTRACT

Background: Makaradhwaja (MD) and Siddha Makaradhwaja (SMD) are Ayurvedic preparations used as traditional medicines for different clinical indications in the rural population. Principle purpose of using MD is controlling hypotension and while SMD is useful in peripheral circulatory failure treatment. In this study we evaluate the influence of these preparations on HbA1c (%) level.

Methods: To find out the average plasma glucose concentration over prolonged period of time, MD and SMD were administered chronically to the male Sprague-Dawley rats at a dose of 40 mg/kg. After 28 days of chronic administration of MD and SMD the following changes were noted and Glycated Hemoglobin (HbA1c) level was determined.

Results: The results of the study of in vitro quantitative determination of rat HbA1c concentrations in serum studies, MD demonstrated a negligible (0.61%) decrease in the HbA1c level of the blood of the male rat ($p=0.902$). Whereas SMD demonstrated a negligible (1.83%) increase in the HbA1c level of the blood of the male rat ($p=0.782$).

Conclusions: Between these preparation MD slightly decreases HbA1c level of the blood of the male rat, whereas SMD found in increasing HbA1c level of the blood of the male rat.

Keywords: Diabetes mellitus, HbA1c, Makaradhwaja, Siddha Makaradhwaja

INTRODUCTION

The countries of Indian subcontinent are the mines of traditional medicines and medicinal plants. Among many of the lifesaving or disease curing preparations Makardhwaja (MD) and Siddha Makardhwaja (SMD) are

the two names which are used in enormous purposes. These are mineral containing Ayurvedic traditional formulation.^{1,2} MD is mainly prepared with purified mercury (Suddha Parad), purified sulphur (Suddha Gandhak) and gold (Swarna) which processed in Aloe Vera juice and other herbs in almost same proportion.

Only difference between the formulation of MD and SMD is in the proportion of gold, where SMD lacks of it. These preparations are well known for their efficacy and safety in the treatment of neurological disorders, rheumatoid arthritis, tuberculosis, bradycardia, pneumonia and longevity of life.^{3,4} MD has been used for so many years for strengthening heart, as an anti-aging and aphrodisiac treatment, to relieve fever, dyspepsia and to improve immunity.⁵ However, SMD possesses exception for rejuvenation and convalescent therapy.⁶

Diabetes mellitus is a group of metabolic diseases that results from the defects of insulin secretion from β - cells of Islets of Langerhans of pancreas, insulin action or may be both and characterized by constant hyperglycemia.⁷ Blood sugar levels can vary from one day to the next depending on food intake and activity levels. Hemoglobin A1c is a form of hemoglobin that is bound by glucose and is often referred to as glycated hemoglobin.^{8,9} Measurement of hemoglobin A1c (HbA1c) level is a combined measure of circulating glucose levels in individuals over time period (around 120 days). The higher glucose level in the plasma indicates that glucose level inside the red blood cell is high. That is more hemoglobin A1c is bound by the glucose.¹⁰⁻¹⁴ Therefore measuring the glycated hemoglobin level in the blood, the state of hyperglycemia as well as diabetes mellitus can be examined. That's been made the HbA1c as an ideal character for the determination. Beside these, HbA1c is an important tool for measuring some other possibilities, such as, detecting diseases of cardiovascular system, immunity and dyslipidemia.¹⁵⁻¹⁷

As MD and SMD are well known herbo-mineral preparation for several sign and symptoms, this research has been conducted to study the HbA1c level in male Sprague Dawley rats after chronic administration of this two Ayurvedic drugs. The aim of this study is to find out the relation between intake of those preparations (Makaradhwaja and Siddha Makaradhwaja) and its impact on HbA1c level.

METHODS

Drugs, chemicals and reagents

For this study, Makaradhwaja (MD) and Siddha Makaradhwaja (SMD) were collected from Sri Kundeswari Aushadhalaya Limited, Chittagong. 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, which were used in this toxicological experiment. These animals were apparently healthy and weighed 50-70g. 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 by Bangladesh Council of Scientific and Industrial Research (BCSIR). Water was provided *ad libitum* and the animals were 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.

Specimen collection

For the experiment, the liquid was administered at an oral dose of 40 mg/kg of the body weight by oral route, without affecting the total fluid volume in animal. Ketamine was administered intra-peritoneally (500mg/kg i.p.) as anesthetic agent.

HbA1c assay

HbA1c Assay consists of two separate concentration measurements, the glycated hemoglobin (HbA1c) and the total hemoglobin (THb). The two concentrations were used to determine the percent HbA1c or hemoglobin fraction. The individual concentration values of HbA1c and THb generated by the assay were used for calculating the percent HbA1c. The whole blood specimen was first pre-treated with the MULTIGENT Hemoglobin Denaturant. The erythrocytes were lysed and the hemoglobin was degraded by the proteolytic enzyme, pepsin, to form a hemolysate. Both the THb and the HbA1c concentrations were determined from the same hemolysate. The concentration of total hemoglobin was determined calorimetrically and was based on the method described by Zander et al.¹⁸ The concentration of HbA1c was measured immune-turbidimetrically using a microparticle agglutination inhibition method.¹⁹ The individual concentration measurements of THb and HbA1c performed automatically by the ARCHITECT Systems and the AEROSSET System and can be measured in g/dL or mmol/L. The calculation of the percent HbA1c was generated using the following equation:

$$\left(\frac{\text{HbA1c} \left(\frac{\text{g}}{\text{dL}} \right)}{\text{THb} \left(\frac{\text{g}}{\text{dL}} \right)} \times 100 \right) - 3 + \left(0.2 \times \text{THb} \left(\frac{\text{g}}{\text{dL}} \right) \right) = \% \text{HbA1c}$$

Statistical analysis

The group data are expressed as Mean \pm SEM (Standard Error of the Mean). Independent sample 't' tests were done for statistical significance analysis. SPSS (Statistical Package for Social Science) for WINDOWS (ver. 16.0) was applied for the analysis of data. Differences between

groups were considered significant at $p < 0.05^*$, 0.01^{**} and 0.001^{***} .

RESULTS

The HbA1c assay is used for the quantitative in vitro measurement of percent HbA1c (HbA1c fraction) in whole blood. To find out the average plasma glucose concentration over prolonged period of time, Ayurvedic medicinal preparations MD and SMD were administered

chronically to the male Sprague-Dawley rats at a dose of 40 mg/kg. After 28 days of chronic administration of MD and SMD the following changes were noted. In this experiment HbA1c level was determined. MD demonstrated a negligible (0.61%) decrease in the HbA1c level of the blood of the male rat, which was statistically not at all significant ($p=0.902$). Whereas SMD demonstrated a negligible (1.83%) increase in the HbA1c level of the blood of the male rat, which was statistically not at all significant ($p=0.782$).

Table 1: HbA1c (%) value of control, MD and SMD.

Parameters	Control (Mean±SEM)	MD (Mean±SEM)	SMD (Mean±SEM)
HbA1c (%)		2.03750±0.06797	2.0875±0.11090
t/p	2.05000±0.07315	0.125 / 0.920	- 0.282 / 0.782
Overall output		↓ Decrease 0.609756%	↑ Increase 1.82927%

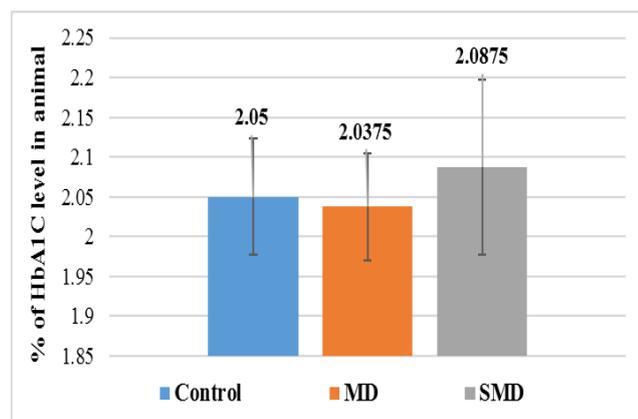


Figure 1: Effect of MD and SMD on HbA1c in male rats.

DISCUSSION

Makaradhwaja (MD) and Siddha Makaradhwaja (SMD) are Ayurvedic preparations used as traditional medicines for different clinical indications in the rural population.²⁰⁻²² Makaradhwaja is used in hypotension and Siddha Makaradhwaja is used in peripheral circulatory failure. The HbA1c assay is intended to aid in the monitoring of long-term blood glucose control of the marketed Ayurvedic medicinal preparation. In this experiment, we found different HbA1c level after treating the lab animals with those preparations. MD demonstrated a negligible (0.61%) decrease in the HbA1c level of the blood of the male rat. Decreases in HbA1c can occur in the several nondiabetic conditions, like hemolytic anemia, chronic blood loss or chronic renal failure of patient.²³⁻²⁶ There are some drug also involved in decreasing HbA1c level, like- acarbose, deferoxamine, diltiazem, enalapril, glipizide, glyburide etc.²⁷ Whereas SMD demonstrated a negligible (1.83%) increase in the

HbA1c level of the blood of the male rat, which was statistically not at all significant ($p=0.782$). From this study we can say that, non-diabetic person can easily take both of the preparation but patient with high HbA1c (%) level should be careful in taking SMD as it can elevate the HbA1c (%) level a little bit. But MD is somehow beneficial for diabetic patient in some way as it decreases the glycated haemoglobin level in blood. Further study is needed for better understanding of the nature and features of those preparations.

CONCLUSION

Two herbo-mineral preparation (MD and SMD) were examined to find out the relation of chronic administration of those preparations and their effects on HbA1c (%) level in rats. Between these preparation, MD demonstrated a negligible (0.61%) decrease in the HbA1c level of the blood of the male rat, whereas SMD demonstrated an increased (1.83%) HbA1c level of the blood of the male rat.

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Conflict of interest: None declared

Ethical approval: The study was approved by the Ethical Review Committee, Faculty of Life Sciences, Department of Pharmacy, Jahangirnagar University

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