

Preclinical HbA1c level studies of Brihat Khadir Batika and Chandraprabha Batika after chronic administration to male Sprague-Dawley rats

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ABSTRACT

Background: Brihat Khadir Batika (BKD) and Chandraprabha Batika (CPB) are Ayurvedic preparations used as traditional medicines for different clinical indications in the rural population. BKD is used in diseases of throat and CPB is used in glandular enlargement. 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, Ayurvedic medicinal preparations BKD and CPB were administered chronically to the male Sprague-Dawley rats at a dose of 400 mg/kg. After 28 days of chronic administration of BKD and CPB the following changes were noted. In this experiment Glycated Hemoglobin A1C level was determined.

Results: The results of the study of in vitro quantitative determination of rat Glycated hemoglobin A1c concentrations in serum studies are thus: BKD caused a statistically insignificant ($p=0.066$) increase in the HbA1c level of the male rat (16.87% increase). CPB demonstrated a statistically insignificant ($p=0.079$) (17.47%) increase in the HbA1C level of the blood of the male rat.

Conclusions: Both preparation BKD and CPB found in increasing HbA1c level of the blood of the male rat.

Keywords: Ayurvedic preparations, Diabetes mellitus, HbA1c, Traditional medicines

INTRODUCTION

The term diabetes mellitus describes a metabolic disorder with heterogenous aetiologies which is characterized by chronic hyperglycaemia and disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both.¹

The long-term relatively specific effects of diabetes include development of retinopathy, nephropathy and neuropathy.² People with diabetes are also at increased risk of cardiac, peripheral arterial and cerebrovascular disease.³

As of 2015, an estimated 415 million people had diabetes worldwide, with type 2 DM making up about 90% of the cases.^{4,6} As of 2014, trends suggested the rate would continue to rise.⁶ Diabetes at least doubles a person's risk of early death.⁷ From 2012 to 2015, approximately 1.5 to 5.0 million deaths each year resulted from diabetes.⁸ The global economic cost of diabetes in 2014 was estimated to be US\$612 billion.⁹ In the United States, diabetes cost \$245 billion in 2012.¹⁰

Throughout human history, natural products have been used as remedies to cure or treat illnesses. BKD is an ayurvedic and herbal remedy used for mouth ulcer,

pharyngitis (sore throat) and other diseases of teeth, gums, tongue and throat. The formulation BKD contains Khadira Saar (Kattha) - Catechu as main ingredients.^{11,12} CPB is an ayurvedic classical medicine used for the treatment of diseases of kidneys, bladder, urinary tract, pancreas, bones, joints, and thyroid gland. It is also recommended in the management of diabetes, men's problems, women's problems and mental disorders. The formulation of CPB contains Asphaltum (Shilajit), Cimmiphora Mukul as main ingredients.^{11,12}

METHODS

Drugs, chemicals and reagents

For this study, Brihat Khadir Batika (BKD) and Chandraprabha Batika (CPB) 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 40mg/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.¹³ The concentration of HbA1c was measured immune-turbidimetrically using a microparticle agglutination inhibition method.¹⁴ The individual concentration measurements of THb and HbA1c performed automatically by the ARCHITECT Systems and the AEROSET System and can be measured in g/dL or mmol/L. The calculation of the percent HbA1c was generated using the following equation:

Statistical analysis

The group data are expressed as Mean±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

In this experiment two popular Ayurvedic medicinal preparations (BKD and CPB) were used to evaluate their HbA1c level though those drugs use in several major health conditions. BKD is an ayurvedic remedy used for mouth ulcer, pharyngitis (sore throat) and other diseases of teeth, gums, tongue and throat and CPB is an ayurvedic classical medicine used for the treatment of diseases of kidneys, bladder, urinary tract, pancreas, bones, joints, and thyroid gland. It is also recommended in the management of diabetes.

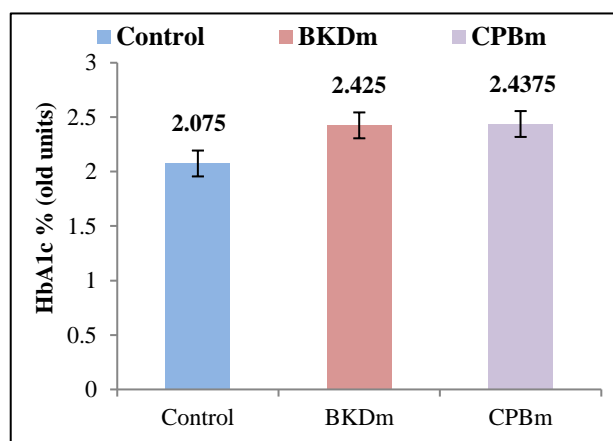


Figure 1: Effect of Brihat Khadir Batika (BKD) and Chandraprabha Batika (CPB) on HbA1c in male rats.

The HbA1c assay is used for the quantitative in vitro measurement of percent HbA1c (HbA1c fraction) in whole blood which is significant factor for diabetes. To find out the average plasma glucose concentration over prolonged

period of time, Ayurvedic medicinal preparations BKD and CPB were administered chronically to the male Sprague-Dawley rats at a dose of 400mg/kg. After 28 days of chronic administration of BKD and CPB the following changes were noted. In this experiment HbA_{1c} level was determined. The results of the study of the quantitative determination of rat Glycated hemoglobin A_{1c} concentrations in serum studies are thus.

Though we did not find any significant changes, there were some noticeable points in the studies. BKD caused an (16.87%) increase in the HbA_{1c} level of the male rat, the

increase though not significant yet it was noticeable (p=0.066) (Table 1). CPB demonstrated an (17.47%) increase in the HbA_{1c} level of the male rat, the increase though not significant yet it was noticeable (p=0.079) (Table 1). It is clearly visible from the graph that the level of HbA_{1c} rose for both drugs than control which is probable evidence of increasing HbA_{1c} level after administration of BKD and CPB though the increasing pattern was remain almost steady for both drugs each other (Figure 1).

Table 1: HbA_{1c} (%) value of control, BKD and CPB.

Parameters	Control (Mean±SEM)	BKD	CPB
HbA _{1c} (%)		2.4250±0.09774	2.4375±0.12383
p	2.0750±0.14608	0.066	0.079
Overall output		Incr 16.87%	Incr 17.47%

p*≤0.05, p**≤0.01, p***≤0.001

DISCUSSION

Blood sugar levels can vary from one day to the next depending on food intake and activity levels. Hemoglobin A_{1c} is a form of hemoglobin that is bound by glucose and is often referred to as glycated hemoglobin.^{15,16} Measurement of hemoglobin A_{1c} (HbA_{1c}) 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 A_{1c} is bound by the glucose.¹⁷⁻²¹ 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 HbA_{1c} as an ideal character for the determination. Beside these, HbA_{1c} is an important tool for measuring some other possibilities, such as, detecting diseases of cardiovascular system, immunity and dyslipidemia.²²⁻²⁴

Brihat Khadir Batika (BKD) and Chandraprabha Batika (CPB) are Ayurvedic preparations used as traditional medicines for different clinical indications in the rural population. The HbA_{1c} assay is intended to aid in the monitoring of long-term blood glucose control of the marketed Ayurvedic medicinal preparation. The term *HbA_{1c}* refers to glycated haemoglobin. It develops when haemoglobin, a protein within red blood cells that carries oxygen throughout your body, joins with glucose in the blood, becoming 'glycated'. By measuring glycated haemoglobin (HbA_{1c}). By measuring glycated haemoglobin (HbA_{1c}), clinicians are able to get an overall picture of what our average blood sugar levels have been over a period of weeks/months.²⁵ Now we can say, for

people with diabetes this is important as the higher the HbA_{1c}, the greater the risk of developing diabetes-related complications. In this experiment, we found different HbA_{1c} level after treating the lab animals with those preparations. BKD and CPB increased in the HbA_{1c} level of the blood of the male rat where BKD increased 16.87% and CPB increased 17% though this increase was not significant it was noticeable (Table 1, Figure 1). From this study authors can say that, non-daibetic person can easily take both of the preparation but patient with high HbA_{1c} (%) level should be careful in taking these medicines as it can elevate the HbA_{1c} (%) level a little bit which is indicator of diabetes mellitus and in diabetes mellitus, higher amounts of glycated hemoglobin, indicating poorer control of blood glucose levels, have been associated with cardiovascular disease, nephropathy, neuropathy, and retinopathy. Further study is needed for better understanding of the nature and features of those preparations.

CONCLUSION

From the above experiment it can be concluded that Brihat Khadir Batika (BKD) and Chandraprabha Batika (CPB) should not be administered chronically at a higher dose to the diabetic person as it increases HbA_{1c} level. Further studies should be done by reducing the administered dose.

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