ORIGINAL ARTICLE

Evaluation of antidiabetic activity of Rajata bhasma

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Abstract

Rajata bhasma (RB) has been used in the treatment of diabetes mellitus with its excellence for centuries, but their scientific evaluation has not thoroughly constituted with modern tools. The aim of the study was to evaluate the antidiabetic effect of RB in streptozotocin-induced diabetic rats. The different formulations RB1 (9 putas) and RB2 (17 putas) were prepared by the following classical methods. After the treatment with RB1 and RB2 to streptozotocin-treated rats, it significantly lowered the blood glucose level. Further, it also significantly decreased the elevated total cholesterol, triglycerides, and low-density lipoprotein level while increased the high-density lipoprotein. Glibenclamide was used as a standard drug at a dose of 0.50 mg/kg body weight. The present study shows that RB does have antidiabetic activity.

Key words: Antidiabetic activity, diabetes mellitus, Rajata bhasma

INTRODUCTION

Ayurveda, a traditional Indian system of medicine, is believed to in existence from time immemorial. There are evidences for the usage of drugs derived from minerals, vegetables, and animal products. It is believed that the teachings have been received from Lord Dhanvantari, which has taken its shape during the Vedic era and the contents mentioned in Ayurvedic classic texts, namely, *Charaka Samhita* and *Sushruta Samhita*. *Charaka Samhita* highlights the diagnosis of disease, whereas *Sushruta Samhita* deals with surgical procedures and surgical tools. Apart from treating dreadful diseases, Ayurvedic practices harmonize the well-being of an individual through Yoga exercises and meditation.[1]

Ayurveda is the science comprised Veda (information) and Ayush (life), for example, learning of life. An Ayurvedic framework receives an all-encompassing methodology toward medicinal services by adjusting the physical, mental, and profound elements of the human body. Rasa Shastra (Vedic science) is one of the pieces of Ayurveda, which deals with herbomineral/metals/non-metals preparations called Bhasmas.[2]

The bhasmas derived using several metals are known for their numerous therapeutic uses against dreadful diseases. For instance, Swarnabhasma (gold-based bhasma) has been indicated for many degenerative diseases. Tamara bhasma (copper-based bhasma) has been used to treat leukoderma, cardiac problems, and liver- and stomach-related disorders. Rajata Bhasma (RB) (silver-based bhasma) has known for effect against diabetes, fever, anemia, and psychological disorders. Vanga bhasma (tin-based bhasma) is prescribed to patients suffering from diseases such as diabetes mellitus, asthma, anemia, and gastric ulcers. Yashadha bhasma (zinc-based bhasma) has been successfully implemented to treat diabetes. Naga bhasma (lead-based bhasma) has been used to treat diabetes mellitus, and its therapeutic efficacy against liver disorders and various skin diseases is mentioned in Ayurvedic texts.[3]

Rajata is not known since Vedic era. Indians have been using silver since 10,000 years, whereas therapeutic application of silver was registered in *Charaka samhita* in

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the context of preparing enema nozzle about 3000 years ago. Rajata categorized as Suddhaloha in texts of Rasa shastra[4] being therapeutically used successfully since many centuries. It is advised to use Rajata in the form of Bhasma for internal administration. RB prepared (according to Rasa tarangini Taranga 16.6) is therapeutically used for Shosha, Dhatukshaya, Prameha, Madatyaya, Visha, Jwara, Pitta roga, Pliha roga, Budhi mandya, Garbhashaya dosha, Apasmara, Sukrameha and as Vayasthapaka, Dahahara, Smritikanti vivardhana, and Rasayana.[5] Apart from said therapeutic application, Ayurveda Prakasha highlights about Vayasthapana, Lekhana, Vatapittahara, and Kashaya amlarasa.[6] Rasaratna Samucchaya lists the benefit of RB in Rajayakshma, Udara, Pandu, Arsha, Shwasa, Kasa, Netra Roga, and all kinds of Pitta rogas.[7]

Diabetes comprises a group of common metabolic disorders that share the phenotype of hyperglycemia. With an increasing incidence worldwide, diabetes is likely to continue to be a leading cause of morbidity and mortality in the near future. RB having potential of decreasing blood sugar levels has been tested in experimental animal models and found effective. It is suggested that the Bhasma in combination with other herbs is devoid of any untoward effects.[8]

Hence, it was planned to evaluate the antidiabetic activity of two different samples acquired from RB. It is expected that the outcome of the study will substantially contribute to revalidate existing standardization with scientific evidence and to establish pharmacological properties of RB.

MATERIALS AND METHODS

Preparation of RB

The RB was prepared in two different forms, RB1 prepared by 9 puta while RB2 prepared by 17 puta. The detailed procedure is given below.[9]

- Rajat foil cut into small pieces and amalgam was formed with parade in mortar
- Purified gandhaka was added to amalgam and triturated till formation of proper Kajjali
- Followed by impregnation with kumara swarasa to the preparation of Chakrikas (Pellets)
- Dried chakrikas were placed in shravana and laghuputa was given
- After first puta, Rajata was in completely powder form
- In subsequent two putas, half amount of kajjali was added, triturated with kumara swarasa and puta was given
- From 4–9 putas, half part of gandhaka was added in place of kajjali (considered as a RB1)
- Remaining puta were followed without addition of Kajjali or Gandhaka
- 17 putas were given to obtain Rajat Bhasma that passing all classical parameter (considered as a RB2).

Adiabatic Activity of RB1 and RB2

Oral glucose tolerance test (OGTT) of RB1 and RB2

The OGTT was performed in overnight fasted (18 h) normal rats. The rats were divided into five groups (n = 6). Group I served as normal control rats, administered drinking water daily; Group II had glucose control rats; Group III rats were administered standard drug glibenclamide (0.5 mg/kg); Group IV rats were administered RB1 (50 mg/kg body weight) orally; Group V rats were administered RB1 (100 mg/kg body weight) orally; Group VI rats were administered RB2 (50 mg/kg body weight) orally; and Group VII rats were administered RB2 (100 mg/kg body weight) orally. Glucose (2 g/kg) was fed to rats of Groups II–VII, 30 min before the administration of the extracts and standard drug. Blood was withdrawn from the retro-orbital sinus after 0, 30, and 90 min of extract and standard drug administration, and the plasma obtained after centrifugation at 3000 rpm was estimated for fasting plasma glucose levels using a glucose oxidase-peroxidase glucose estimation kit.[10-13]

Induction of non-insulin-dependent diabetes mellitus (NIDDM)

NIDDM was induced in overnight fasted adult rats weighing 170–220 g by a single intraperitoneal injection of 60 mg/kg streptozotocin, 15 min after i. p. administration of 120 mg/kg of nicotinamide. Streptozotocin was dissolved in a citrate buffer (pH 4.5) and nicotinamide was dissolved in normal saline. Hyperglycemia was confirmed by the elevated glucose levels in plasma, determined at 72 h and then on day 7, after injection. The threshold value of fasting plasma glucose to diagnose diabetes was taken as >125 mg/dl. Only those rats that were found to have permanent NIDDM were used for the study.

Experimental Design

Evaluation of antidiabetic activity of RB1 and RB2

The animals were segregated into five groups of six rats each. The extract was administered for 28 days. Group I served as normal control rats, administered drinking water daily for 28 days; Group II had diabetic control rats, administered drinking water daily for 28 days; Group III diabetic rats were administered standard drug glibenclamide (0.5 mg/kg); Group IV rats were administered RB1 (50 mg/kg body weight) orally; Group V rats were administered RB1 (100 mg/kg body weight) orally; Group VI rats were administered RB2 (50 mg/kg body weight) orally; and Group VII rats were administered RB2 (100 mg/kg body weight) orally for 28 days. The fasting glucose levels were determined on days 0, 7th, 14th, and 28th of RB1 and RB2 administration.

Estimation of biochemical parameters

The biochemical parameters were determined on day 28 after the animals were sacrificed by cervical dislocation. Total cholesterol, triglycerides (TGL), high-density lipoprotein (HDL), and low-density lipoprotein (LDL) were determined by the glucose oxidase method, using an auto-analyzer.[14-17]

Statistical Analysis

The results are expressed as mean ± SD of six independent experiments. Statistical significance between the groups was evaluated by one-way analysis of variance followed by Dunnett's test. $P < 0.05$ was considered as statistically significant.

RESULTS AND DISCUSSION

Conventional modern medicine is not always successful to control diabetes mellitus in all cases. Insulin is not always indicated due to the development of insulin resistance and generation of insulin antagonists in the body, whereas the oral hypoglycemic agent is found to be limited use in many cases due to the major side effects. Therefore, search for better remedies from ayurvedic resources continues. Ayurvedic drugs not only have hypoglycemic effect but also correct metabolic derangements, help in maintaining the Agni and Ojas status, i.e., metabolic stability and immune strength in diabetic patients, and retard the complications of diabetes mellitus and having less side effects.

Oral Glucose Tolerance Effects of RB1 and RB2

The effects of RB1 and RB2 the plasma glucose level are demonstrated in Table 1. The rise in glucose level was observed in glucose control, RB1 and RB2 treated, and standard group animals after the administration of glucose. The animals treated with RB1 and RB2, there was a significant reduction in plasma glucose level which was observed compared to control group animals. In addition, significant decrease in plasma glucose level was noted in glibenclamide-treated group.

Effect on NIDDM Mellitus of RB1 and RB2

Induction of diabetes in experimental rats was confirmed by the presence of a high fasting plasma glucose level. The effect of RB1 and RB2 on serum glucose levels of normal and streptozotocin-induced rats is shown in Table 2.

The animals treated with streptozotocin, namely, diabetic control group, a significant increase in serum glucose level

Table 1: Effect of RB1 and RB2 on oral glucose tolerance test

Group	Plasma glucose concentration (mg/dl)		
	0 min	30 min	90 min
Normal control	78.21±6.84	79.14±2.43	75.36±4.86
Glucose control	82.13±2.35	192.43±8.32[a]	150.71±8.53[a]
Glucose+glibenclamide (0.5 mg/kg)	76.29±6.21	105.47±6.37*	82.73±6.12*
RB1 (50 mg/kg)	79.32±4.11	132.43±7.53*	98.24±8.35*
RB1 (100 mg/kg)	77.64±6.54	102.73±8.45*	83.25±7.13*
RB2 (50 mg/kg)	76.42±7.23	125.17±9.52*	92.32±8.32*
RB2 (100 mg/kg)	80.94±5.24	109.82±9.74*	78.36±6.32*

Values are expressed as mean±SD (number of animals, n=6); significantly different at [a]P<0.05 when compared with normal control group, *P<0.05 when compared with diabetic control group. RB: Rajata bhasma

Table 2: Effect of RB1 and RB2 on fasting plasma glucose level in rats

Group	Fasting plasma glucose concentration (mg/dl)			
	Day 0	Day 7th	Day 14th	Day 28th
Normal control	83.45±2.75	81.72±5.21	86.14±2.52	79.58±3.54
Diabetic control (streptozotocin)	135.17±8.23[a]	203.71±10.53[a]	242.35±8.12[a]	283.49±3.42[a]
Diabetic+standard glibenclamide (0.50 mg/kg)	142.64±7.58	112.45±4.49*	90.74±5.23*	75.32±6.56*
RB1 (50 mg/kg)	147.64±7.61	160.72±8.34*	123.29±6.32*	92.47±7.14*
RB1 (100 mg/kg)	137.52±6.27	128.64±6.11*	109.57±6.02*	86.14±7.52*
RB2 (50 mg/kg)	135.71±7.56	156.24±7.83*	115.17±5.34*	89.24±3.56*
RB2 (100 mg/kg)	131.48±9.56	121.85±7.63*	102.47±7.25*	82.65±8.67*

Values are expressed as mean±SD (Number of animals, n=6); significantly different at [a]P<0.05 when compared with normal control group, *P<0.05 when compared with diabetic control group. RB: Rajata bhasma

Table 3: Determination of biochemical parameters after treatment with RB1 and RB2

Group	Lipid profile (mg/dl)			
	Triglyceride	Total cholesterol	HDL	LDL
Normal control	79.58±4.30	72.49±6.12	66.27±7.12	35.62±6.13
Diabetic control (Streptozotocin)	173.43±5.81[a]	195.52±7.84[a]	22.41±5.32[a]	132.39±7.19[a]
Diabetic+Standard Glibenclamide (0.50 mg/kg)	75.32±3.21*	79.43±6.15*	63.71±4.52*	40.12±7.43*
RB1 (50 mg/kg)	98.35±6.17*	105.26±8.41*	45.82±5.17*	90.14±6.14*
RB1 (100 mg/kg)	79.24±8.20*	78.59±6.34*	62.54±6.71*	45.12±5.43*
RB2 (50 mg/kg)	90.42±6.13*	98.12±7.34*	50.17±2.72*	85.43±5.77*
RB2 (100 mg/kg)	76.85±7.53*	73.17±8.12*	65.98±3.73*	42.68±8.15*

Values are expressed as mean±SD (Number of animals, n=6); significantly different at [a]P<0.05 when compared with normal control group, *P<0.05 when compared with diabetic control group. HDL: High-density lipoprotein, LDL: Low-density lipoprotein. RB: Rajata bhasma

was observed on 0, 7th, 14th, and 28th day compared with normal control group rats. The animals treated with glibenclamide (0.5 mg/kg p. o.) demonstrated a significant decrease in serum glucose level compared to diabetic control rats. After the oral administration RB1 and RB2 to diabetic animals, a significant reduction in blood glucose level was observed.

Antihyperlipidemic Activity

The outcomes of lipid profiles in control and experimental rats are exhibited in Table 3. The animals of diabetic control showed significant increase in serum TGL, total cholesterol, and LDL while increase in HDL when compared with normal animals. The animals treated with glibenclamide also reduced TGL, total cholesterol, LDL, and increased HDL compared to diabetic control group. The RB1 and RB2 treated animals showed a significant decrease in total cholesterol, LDL, TGL, and a significant increase in HDL. All these effects were observed on day 28.

The observed improved glucose clearance in OGTT can be attributed to two major known mechanisms. First, silver ash could inhibit intestinal alpha-glucosidase enzyme and thereby reduce glucose absorption for an organo-silver complex. Second, increased glucose-stimulated insulin secretion by silver could result in better glucose disposal. Silver accumulates in pancreatic beta-cells and results in increased glucose-stimulated insulin secretion. Similar mechanisms may be operative in the case of zinc ash. Further, improved glucose clearance in OGTT after multiple dosing and the fact that these results were comparable to glibenclamide treatment suggests insulin sensitization as a possible mechanism. In the present study, a reduction in serum insulin levels was also seen after silver ash treatment, similar to glibenclamide. These results also suggest that zinc ash possibly works as an insulin sensitizer. Reduction in non-fasted as well as fasted glucose levels was seen in silver ash-treated type 1 (insulin deficient) and type 2 (insulin resistant) diabetic rats, suggesting that multiple mechanisms may be involved. However, further experimentation is necessary to identify the targets of action.

CONCLUSION

Ayurvedic medicine RB comprises submicronic particles. Antidiabetic activity of RB has been pharmacologically validated using diabetes rat models. The findings indicate that the administration of RB (RB1 and RB2) exhibited significant antidiabetic effect by controlling the blood glucose level. In addition, the RB decreased total cholesterol, TGL, and LDL with increase in HDL at the end of the treatment. Taking encouragement from the fact that Bhasma contains submicronic that enhances bioavailability, metal-based nanomedicines can give a scientific background. This would help in utilizing the age-old wisdom of Ayurveda for the development of newer drugs in modern medicine.

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