Research Article

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SCREENING OF ANTIDIABETIC EFFECT OF NAGA BHASMA IN ALLOXAN INDUCED HYPERGLYCEMIC RATS

Deshmukh Smita M[1]*, Bhingare Chandrashekhar L[2], Kshirsagar Sanjay J[1]
[1]Department Of Quality Assurance, AISSMS College Of Pharmacy, Pune, Maharashtra, India
[2]Department of Quality Assurance, MM College of Pharmacy, Thergaon (Kalewadi), Pune, Maharashtra, India

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***Corresponding author**
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ABSTRACT
Naga bhasma is traditionally used in the treatment of diabetes; literature does not document its influence on experimentally induced hyperglycemia in animal models. Therefore, it was proposed to investigate the influence of Naga bhasma in alloxan-induced hyperglycemic rats. In the present study, Naga bhasma was subjected to evaluate the anti diabetic activity in alloxan-induced diabetic rats. Graded doses of Naga bhasma (100 and 200mg/kg) were administered orally with suspension of milk using oral feeding needle to control and experimental diabetic rats. Weight variation study and glucose tolerance test was conducted in rats while Antidiabetic effect was evaluated in alloxan-induced hyperglycemic rats. Glibenclamide was used as reference standard. Treatment with Naga bhasma showed no change in blood glucose level in normal rats but normalized the impaired glucose tolerance and alloxan-induced hyperglycemia on long-term treatment. In conclusion, Naga bhasma, on prolong administration exhibits anti hyperglycemic effect.
Keywords: Alloxan, Diabetes, Glibenclamide, Naga bhasma

INTRODUCTION

Diabetes mellitus is one of the major risk factor for metabolic syndrome, characterized by hyperglycemia, which results from defects in insulin secretion, insulin resistance or both. This hyperglycemia in turn damages many of the body's systems, in particular the blood vessels and nerves. The increasing worldwide incidence of diabetes mellitus in adults constitutes a global public health burden. It was predicted that by 2030, India, China and USA will have the largest number of people with diabetes[1]. Despite appreciable progress made in the management of diabetes mellitus using conventional Antidiabetic management strategies, the search for products of natural origin for control of diabetes mellitus continues. The World Health Organization (WHO) has also long back recommended that this practice should be encouraged, especially in countries where access to conventional treatment of diabetes mellitus is not adequate[2]. Many herbal formulations are used in treatment of diabetes[3,4] and their results are also promising. In Ayurvedic system of medicine, materials from different sources viz., plants, animals, metals and minerals were used to prepare the formulations. In metal-based preparations, the metal was not used as it is but used after subjecting to various purification methods along with herbs. The output of such purification and calcinations process is a fine powder, called Bhasma, which is used either as such or in combination with other herbs. Naga bhasma is among such bhasma preparations that is purified and calcinated form of Naga i.e. lead with additional herbs. In the traditional literature, Naga bhasma was used in urinary diseases (prameha), pyorrhea, loss of appetite, inflammatory conditions, gastric troubles, respiratory disorders like asthma, polyurea, skin diseases, snakebite, helminthiasis, cancer, chronic fever, neurological disorders, sexually transmitted diseases, etc. Besides these, it was also used in nervine tonic, rejuvenators, aphrodisiac, leucorrhea, tuberculosis, and lipid disorders[5]. Recently, it is demonstrated that Naga bhasma is an ideal drug for treatment of madhumeha (diabetes mellitus)[6]. Diabetes mellitus is one of the most challenging public health problems of the 21st century and the prevalence of diabetes mellitus is increasing rapidly[7]. Diabetes mellitus is a complex syndrome characterized primarily by the imbalance in blood glucose homeostasis leading to hyperglycemia (high glucose blood sugar) and a series of secondary complications caused by an absolute or relative lack of insulin[8]. In Ayurveda, diabetes mellitus is described as madhumeha kshaudrameha, which literally means "excessive urine with sweet taste like honey," or dhatupak janya vikriti, which means a disease caused by a defective metabolism leading to derangement in body tissue (seven dhatus) transformation process[9]. The prevalence of diabetes mellitus increases with age and it peaks at 60-69 years of age in all populations[10]. Worldwide 140 million people suffer from diabetes and an estimated 31.7 million people in India suffers from the same disorder. The number of affected individuals with diabetes is expected to double by 2025 [11].

MATERIALS AND METHODS

Til Oil (sesame oil), Buttermilk (Takra), Gomutra (Cow's Urine), Kanji (Starch Paste), Kulith Kwatha, Ashwattha (*Ficus religiosa*), Amlika (*Tamrindus indica*), Turmeric powder and Aloe vera juice from S.G.Phytopharma Kolhapur were procured. Obtained authentic glibenclamide as gift sample from Sun Pharma, Baroda. Alloxan monohydrate (SD- Fine Chemicals, India) were purchased from supplier. All other reagents were of analytical grade manufactured by Glaxo-Qualigens,

Mumbai, India.

Preparation of Naga bhasma

Process of the Naga bhasma done according to the "Satiput Naga bhasma" process described in the "Bharat Bhaishashya Ratnakar"[12].

Lead metal was melted in iron ladle and poured into a vessel containing quenching of molten lead into different herbal juices. Quenching with Til Oil (sesame oil), Buttermilk (Takra), Gomutra (Cow's Urine), Kanji (Starch Paste), Kulittha Kwatha purifies Lead and *Aloe vera* juice removes toxicity to generate specific organo-metallic complex. The process was repeated seven times with fresh herbal juice taken each time. In the first puta (step), Melted lead was stirred constantly with bark Ashwattha (*Ficus religiosa*) and Amlika (*Tamrindus indica*) stick till it becomes dried powder. After powdering followed by heating with Turmeric powder (*Curcuma longa* Linn.) for eight hours. After cooling, powder is sieve through mesh (80#) then triturated with the juice of Aloe Vera juice for 24 hours then remove mixture and put it into the oven at 105^0 C for eight hours then powder is sieve through mesh (100#) This was the first puta (step) of Naga bhasma sample.

The process was repeated six times to get the Naga bhasma finished product . The powdered material was packed in airtight containers.

Acute oral toxicity study

The acute oral toxicity study of different crude drugs and selected material was carried out as per the guidelines set by Organization for Economic Cooperation and Development (OECD) guideline 423. Acute oral toxicity studies were carried out as per fixed dose procedure of OECD 423[13]

Study design for acute oral toxicity

For acute toxicity studies, following group configuration was followed with three mice each group. (Group 1: Control, Group 2: 5 mg/kg, and Group 3: 50 mg/kg, Group 4: 300 mg/kg, Group 5: 2000 mg/kg)

Observation was made post administration during first 30 min, 4 h, 6 h and 24 h carefully thereafter for 14 days. Parameters were observed for 24 h. i.e. cage side observations, body weight, mortality (30 min, 24 h, 3, 7 and 14 day).

Antidiabetic study

Evaluation of animals for antidiabetic study

Wistar albino rats weight of 200-250gm were taken. Animals were housed in uniform environment of temperature 18-21^{o}C humidity and light under 12h day and night cycles throughout the experiment.

Standard and tests administrated orally to three separate groups consisting of six rats each. Glucose in Blood sample was estimated by glucometer. 120 mg/kg alloxan induced animals after 3 day increased in sugar level were then given dose of Naga bhasma with milk suspension considered as test:1; 100mg/kg and test: 2 were given dose of 200mg/kg. Glibenclamide 2 mg/kg as standard drug was given. Doses were prepared as suspension in milk and daily fresh suspension was prepared for oral administration using oral feeding needle. Administration was carried out at fixed time of day. Weight recordings of individual rat were done on electronic balance on 0, 3, 7, 14, 21 day and doses were calculated according to recent weight record. Institutional Animal Ethics Committee approved the study protocol (Protocol no is AISSMS/IAEC/11-12/01-13).

RESULT

Acute oral toxicity (AOT)

The acute oral toxicity study was carried out as per OECD guidelines. The study was performed at fixed doses of 5, 50, 300, 2000 mg/kg using three animals per group. Animals were observed individually after dosing at least once during the first 30 min, periodically during the first 24h with special attention given during the first 4 h and daily thereafter, for a total of 14 days.

Effect on general behavior and mortality

A summary of the results of the mortality and gross symptoms of toxicity observed in the animals treated with Naga bhasma:

Table 1: Results of the mortality and Gross Symptoms of toxicity

Group mg/kg/ml	Mice	Sex	D*/T	Symptoms of toxicity*
Vehicle	3	Male	0/3	None
5	3	Male	0/3	None
50	3	Male	0/3	None
300	3	Male	0/3	None
2000	3	Male	0/3	Decreased locomotors activity and nasal secretion increase
Total	15	Male	0/15	-

Mortality and gross symptoms of toxicity observed after per oral administration of test materials to rats. D: Deaths. T: Total animal in-group. * All animals were monitored for any deviations in normal behavior, movements and mortality on a daily basis during the 14 days period of study.

There was noticeable deviation in the behavior of the animals treated with Naga bhasma (2000 mg/kg) dose i.e. decreased locomotor activity up to 24h Compared to that of the control (Vehicle) group. However, no incidence of mortality was observed during 14 days.

Effect of treatment on body weight

Decrease in food and water consumption is an important sign of deterioration of health or an indicator of poor health, and generally results in loss of body weight. Changes in the body-weight have also been used as an indicator of adverse effects of drugs and chemicals. The dose at which the body weight is decrease by 10 % or

more is considered a toxic effect whether or not it is accompanied by any other changes. In this study, weight was recorded at 0, 6, 24, 48h. Post administration and then finally at 7 and 14 day post administration of test materials in respective groups. There was slight changes in the behavior of the animals and body-weight in the treated groups of Naga bhasma (2000 mg/kg) but there is no significant changes in behavior and body weight in the treated group of Naga bhasma (50 and 300 mg/kg) compared to that of the control group.

Antidiabetic study

Table 2: Group design of animals for Antidiabetic study

Groups	Dose mg/kg/ml	Observation
Control	-	0,3,7,14 th day
Naga Bhasma Test	100mg/kg	Wt and glucose level check.
Naga Bhasma Test	200mg/kg	Wt and glucose level check.
Glibenclamide (2mg/kg)	2mg/kg	Wt and glucose level check.

Table 3: Result of effect of treatment of body weight

Treatments mg/kg/ml	Wt of animal Before treatment (gm)	Wt of animal After treatment(gm)
Control	256 ± 2.54	257± 3.57
Naga Bhasma (100mg/kg)	255.87 ± 3.65	221 ± 3.72
Naga Bhasma (200mg/kg)	267.81 ± 3.98	209 ± 3.42
Glibenclamide (2mg/kg)	265.54 ± 2.43	226 ± 2.46

Table 4: Result of Glucose level of animal

Treatment mg/kg	Glucose level Before treatment(mg/dl)	Glucose level After treatment(mg/dl)
Control	87 mg/dl	89 mg/dl
Naga Bhasma (100mg/kg)	256 mg/dl	98 mg/dl
Naga Bhasma (200mg/kg)	254 mg/dl	96 mg/dl
Glibenclamide (2mg/kg)	263 mg/dl	91 mg/dl

Both the doses have shown decrease in blood glucose level of hyperglycemic rats after 14 days treatment.

DISCUSSION

Metallic herbal preparations offer advantages over plant drugs by virtue of their stability over a period, lower dosage, easy storability, sustained availability and contain minerals and metals as integral part of the formulations[14]. They are being used with an intention to give therapeutic efficacy to the designated illness. The metals and minerals are mixed with herbs because they are considered non-living and by treating them with herbs they are converted to a viable state thereby becoming bio-compatible. The same metal processed with different herbs acts on different organs in the human body[15]. The present study was conducted to evaluate the Antidiabetic activity of Naga bhasma in alloxan-induced hyperglycemic rats to scientifically validate its traditional use in diabetes. The results of the investigation revealed that treatment with a daily single dose of Naga bhasma for fourteen days did not show any significant decrease in the basal glucose level in normal rats. The blood glucose levels remained unchanged after 24 h, 3rd, 7th & 14th day, during the period of administration of Naga bhasma in normal rats. Similarly, glibenclamide have shown no change in basal serum glucose levels. This is in accordance with the reports, which demonstrated that glibenclamide does not produce hypoglycemia in non-diabetic state[16]. This investigation suggest that Naga bhasma has no hypoglycemic effect in non-diabetic state. This indicates the efficacy of Naga bhasma to suppress the elevated blood glucose levels. Excessive hepatic glycogenolysis and gluconeogenesis associated with decreased utilization of glucose by tissue is the fundamental mechanism underlying hyperglycemia in the diabetic state. It is well known fact that alloxan destroys the beta cells of the pancreas and causes hyperglycemia in rats[17]. In alloxan induced hyperglycemic rats, both the doses i.e. 100 and 200 mg/kg of Naga bhasma showed dose-dependent decrease in glucose levels and brought it to normal on 14th day of treatment. The effect of the drug was found to be time dependant. The effect of Naga bhasma was comparable to glibenclamide. It is possible that Naga bhasma like glibenclamide will be improving insulin action at the cellular level or enhancing the action of insulin or by increasing the glucose metabolism or glucose homeostasis in diabetic animals[18-20]. Recently, treatment with Naga bhasma has shown remarkable effect in madhumeha (diabetes mellitus)[6] patients and further strengthen the result of present investigation in animals. The mechanism of the hypoglycemic effect of Naga bhasma is not elucidated in the present study. It is noteworthy to mention here that the Naga bhasma used in the present study was prepared according to method described in "**Bharat Bhaishashya Ratnakar. B.B.R.-111 SL NO: 3629, 3621**." In this method, it is mention that potency of Naga bhasma was augmented (bhavna) with addition of herbs like Turmeric powder and Aloe vera juice. Hence, it is possible that observed Antidiabetic effect is might be due to these herbs along with lead metal. Literature has also mentioned the Antidiabetic effect of these herbs in animal experiments [21, 22]. However, the detailed and long-term administration studies are required to comment on the efficacy and exact mechanism of antihyperglycemic effect of Naga bhasma. To conclude, it can be stated that, Naga bhasma possesses significant antihyperglycemic effect against alloxan induced hyperglycemic rats only after long-term administration.

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