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SCREENING OF ANTIDIABETIC EFFECT OF VANGA BHASMA (TIN ASH) IN ALLOXAN-INDUCED HYPERGLYCEMIC RATS

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ABSTRACT
Bhasmas are unique Ayurvedic metallic preparations used for medicinal purposes since ancient times. Vanga bhasma, an Ayurvedic preparation of tin is used traditionally for treatment of diabetes. In the present study, vanga bhasma was subjected to evaluate the antidiabetic activity in alloxan-induced diabetic rats. Graded doses of vanga bhasma (25 and 50 mg/kg) were administered intragastrically to normal and experimental diabetic rats. Normoglycemic study and oral glucose tolerance test were conducted in normal rats while antidiabetic effect was evaluated in alloxan-induced hyperglycemic rats. Metformin was used as reference standard. Vanga bhasma treatment did not influence the blood glucose in normal rats but normalized the impaired glucose tolerance and alloxan-induced hyperglycemia on long term treatment. In conclusion, vanga bhasma, on prolong administration exhibits antihyperglycemic effect.
KEY WORDS: Alloxan, Diabetes, Metformin, Vanga bhasma

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INTRODUCTION

Diabetes mellitus is categorized as a metabolic disease, characterized by hyperglycemia which results from defects in insulin secretion, insulin action 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 is 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 of medicines viz., plants, animals, metals and minerals are used to prepare the formulations. In metal-based preparations, the metal is 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. Vanga bhasma is among such bhasma preparations that is purified and calcinated form of vanga i.e tin with additional herbs.

In the traditional literature, vanga bhasma is used in urinary diseases (prameha), pyorrhea, loss of appetite, inflammatory conditions, gastric troubles, respiratory disorders like asthma, polyurea, skin diseases, snake bite, helminthiasis, cancer, chronic fever, neurological disorders, sexually transmitted diseases, etc. Besides these, it is also used in diabetes mellitus, nervine tonic, rejunuvators, aphrodisiac, leucorrhea, tuberculosis, and lipid disorders[5]. Recently, it is demonstrated that vanga bhasma is an ideal drug for treatment of madhumeha (diabetes mellitus)[6].

Although vanga 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 vanga bhasma on blood glucose in alloxan-induced hyperglycemic rats.

MATERIALS AND METHODS

Drugs and chemicals

Vanga bhasma was procured from local market (Shri Baidhyanath Ayurved Bhavan, Jhansi, India). Alloxan monohydrate was procured from CDH Chemicals, India while Metformin Hydrochloride was gift sample from ZIM Laboratories Ltd., Nagpur, India. Glucose Oxidase Peroxidase glucose estimation kit was procured from Span Diagnostic Ltd., Surat, India. All other reagents were of analytical grade and procured from Glaxo-Qualigens, Mumbai, India.

Experimental animals

Healthy adult albino rats of Wistar strain weighing about 200–250 g of either sex between 2–3 months of age were used in the present study. Animals were bred and maintained at Central Animal Facility of the Institute under standard housing conditions of temperature 25 ± 2°C, relative humidity 55–65% and light and dark cycles of 12 h, respectively. Animals were provided with standard pellet diet (Ashirwad Brand, Chandigarh) and water *ad libitum*. The studies were approved by Institutional Animal Ethics Committee constituted for the purpose of supervision on conducting the experiments on animal (proposal no. CRI-GWL/IAEC/2009/03).

Authentication of vanga bhasma

Vanga bhasma was authenticated using "Phased spot test"[7] in which a Whatman paper No. 1 was impregnated with 10% potassium iodide and dried carefully by keeping on a clean sheet of glass. About 0.25 g of vanga bhasma was taken in a micro tube and 0.5 ml of 5N HNO_3 was added. The solution was slightly heated and allowed to settle for 24 h shaking vigorously. A drop of this supernatant solution was carefully put on Whatman paper (chemical reacting paper) with the help of a dropper. Immediately after application of spot on Whatman paper, an instantaneous characteristic spot began to appear and changing rapidly and continuously for some time. The characteristic colour changes and reactions were observed during the three phases. The characteristic colour changes were compared with the standard colour changes pattern mentioned in the literature.

Induction of hyperglycemia

Hyperglycemia was induced by single dose of alloxan monohydrate in overnight fasted rats. Alloxan was prepared freshly in normal saline and administered intravenously through tail vein at a dose of 65 mg/kg[8]. Glucose solution (1% w/v) was immediately administered intragastrically (i.g.) to alloxan treated rats in order to prevent transient hypoglycemia. In overnight fasted rats, 48 h after the administration of alloxan, blood was collected from retro-orbital plexus under light ether anesthesia and clear serum was obtained after centrifugation at 3000 rpm[9]. Fasting serum glucose levels were estimated using a Glucose Oxidase- Peroxidase glucose estimation kit[10]. The rats exhibiting serum glucose more than 250 mg/dl were considered hyperglycemic and included in the study.

Grouping and treatments

Rats were divided into four different groups (n = 5). All normoglycemic and hyperglycemic rats received vehicle (4% gum acacia in normal saline) or vanga bhasma (25 and 50 mg/kg, i.g.) or metformin (500 mg/kg, i.g.) as a reference standard[11]. The doses of vanga bhasma were determined from approximate human equivalent dose calculated on the basis of surface area ratio[12].

Effect of vanga bhasma on serum glucose in normoglycemic rats

The rats were divided into four different groups (n = 5). Rats were fasted overnight (18 h) and optimum care was exercised to avoid coprophagia. Vehicle treated group received 4% gum acacia in normal saline while test group received vanga bhasma 25 and 50 mg/kg, i.g. The standard treated group received metformin 500 mg/kg, i.g. The assessment of blood glucose was carried out on 0, 1, 3, 12, 24 h, 3rd day and 7th day after administration of drugs.

Effect of vanga bhasma on oral glucose tolerance test (OGTT) in normal rats

After treatment for 7 days with vanga bhasma, the oral glucose tolerance test was performed in overnight fasted (18 h) normal rats[13-15]. Rats were divided into four groups (n = 5) and i.g. administered vehicle, vanga bhasma (25 and 50 mg/kg), and metformin (500 mg/kg). Glucose (4 g/kg) was fed i.g., 3 h after the last dose of test drug/vehicle on day 7[13]. Blood was withdrawn from the retro orbital plexus at 0, 30, 60 and 120 min of glucose administration and the serum was estimated for fasting glucose level.

Effect of vanga bhasma on serum glucose in hyperglycemic rats

The alloxan treated (diabetic) rats were divided into four different groups (n = 5). Rats were fasted overnight and optimum care was exercised to avoid coprophagia. Vehicle treated group received 4% gum acacia in normal saline while test group received vanga bhasma (25 and 50 mg/kg, i.g.) in addition to alloxan. Standard treated

diabetic group received metformin (500 mg/kg, i.g). The assessment of blood glucose was carried out on 0, 1, 3, 12, 24 h, 3rd day and 7th day after administration of drugs.

Statistical analysis

The data were analyzed with unpaired 't' test and two-way ANOVA followed for by Bonferroni multiple comparison post hoc test wherever necessary. A statistical difference of $P<0.05$ was considered significant in all cases.

RESULTS

Authentication of vanga bhasma

In the phased spot test, the colour changes appearing in the spot of vanga bhasma were noted in three phases. In the first phase (5 min) a wide dark brown solid spot appeared followed by immediate formation of glittering grey central spot which continued in the second phase (20 min). In the third phase 24 h after the first phase, the dark brown spot started fading away in the center of the spot leaving behind a yellow solid spot as big as the glittering grey spot, which appeared in first phase. The entire pattern of colour changes in the different phases [Fig. 1] was matching to the standard plate no.1 mentioned in the literature, which suggested that vanga bhasma was authentic and reliable for undergoing further studies.

Effect of vanga bhasma on serum glucose in normoglycemic rats

Two-way ANOVA did not show any significant effect of vanga bhasma on blood glucose levels in normal rats ($P > 0.05$). Post hoc test indicated that vanga bhasma at 25 and 50 mg/kg did not exhibit significant reduction in the blood glucose in normal rats. The standard drug, metformin ($P > 0.05$) also did not influence serum glucose level [Table 1].

Effect of vanga bhasma on oral glucose tolerance test (OGTT) in normal rats

Two-way ANOVA showed that vanga bhasma treatment for 7 days has significant ($P < 0.0001$) effect on OGTT after glucose load administration and post hoc test indicated that vanga bhasma (25 and 50 mg/kg) normalized the impaired glucose tolerance with significant ($P < 0.05$-0.001) reduction in serum glucose levels up to 120 min after glucose load. The standard drug, metformin significantly ($P < 0.01$-0.001) also normalized the impaired glucose tolerance compared to control and showed reduction in the blood glucose from 60 min onwards [Table 2].

Effect of vanga bhasma on serum glucose in hyperglycemic rats

Two tailed Unpaired 't' test indicated that single intravenous dose of alloxan induced significant hyperglycemia ($P < 0.0001$) in 48 h of treatment in the experimental rats which was confirmed by the presence of high serum glucose levels (>250 mg/dl) [Fig. 2]. The rats exhibiting serum glucose less than 250 mg/dl at 0h were excluded from the study.

Two-way ANOVA showed that vanga bhasma has significant ($P < 0.0001$) influence on blood glucose levels in alloxan induced hyperglycemic rats. Post hoc test indicated that long term administration of vanga bhasma (25 and 50 mg/kg) significantly ($P < 0.05$-0.001) reduced the blood glucose levels in alloxan hyperglycemic animals only after 24 h and on 3rd and 7th day. The standard drug, metformin also showed the reduction in the blood glucose levels in alloxan hyperglycemic rats [Table 3].

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[16]. 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 living state thereby becoming bio-compatible. The same metal processed with different herbs acts on different organs in the human body[17]. The present study was conducted to evaluate the antidiabetic activity of vanga bhasma in alloxan-induced hyperglycemic rats to scientifically validate its traditional use in diabetes.

The results of the investigations revealed that treatment with a daily single dose of vanga bhasma for seven 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 day and 7th day during the period of administration of vanga bhasma in normal rats. Similarly, metformin did not affect the basal serum glucose levels. This is in accordance with the reports which demonstrated that metformin does not produce hypoglycemia in non-diabetic state[18]. These investigations suggest that vanga bhasma, *per se,* has no hypoglycemic effect. In glucose loaded normal rats, hypoglycemia was observed at 120 min after glucose load in rats treated with vanga bhasma for seven days. This indicates the efficacy of vanga bhasma to suppress the elevated blood glucose levels.

Excessive hepatic glycogenolysis and gluconeogenesis associated with decreased utilization of glucose by tissues 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[19]. In alloxan-hyperglycemic rats, vanga bhasma at a dose of 25 and 50 mg/kg showed a dose-dependent decrease in glucose levels and brought to near normal on 7th day of treatment. The effect of the drug was also found time dependant [Table 3]. The effect of vanga bhasma was comparable to metformin. It is possible that vanga bhasma like metformin might 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[20-22]. Recently, treatment with vanga 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 vanga bhasma is not elucidated in the study.

It is noteworthy to mention here that the vanga bhasma used in the present study was prepared according to method described in Siddha Yog Sangraha[23]. In this method, it is mentioned that potency of vanga bhasma was augmented (bhavna) with addition of herbs like *Butea monosperma* and *Aloe vera* juice. Hence, it is possible that observed antidiabetic effect may be attributed to these herbs along with tin metal. Literature has also mentioned the antidiabetic effect of these herbs in animal experiments[24,25]. However, the detailed and long term administration studies are required to comment on the efficacy and exact mechanism of antihyperglycemic effect of vanga bhasma.

To conclude, it can be stated that, vanga bhasma possesses significant antihyperglycemic effect against alloxan induced hyperglycemic rats only after long term administration.

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TABLE 1. EFFECT OF VANGA BHASMA ON NORMAL GLYCEMIC RATS

Groups	Blood glucose (mg/dl)						
	0h	1h	3h	12h	24h	3 days	7 days
Vehicle	137±8.08	127±12.18	107±6.31	113±2.82	120±6.24	117±3.63	131±9.43
Vanga Bhasma 25	130±5.53	155±7.16	119±4.47	111±2.28	127±6.62	116±5.86	94±1.98
Vanga Bhasma 50	127±2.98	116±3.52	76±10.76	126±13.57	157±15.47	121±4.54	123±7.38
Metformin	121±9.47	114±6.15	103±6.24	150±7.19	144±15.18	124±7.51	153±11.61

Normoglycemic rats were treated with vehicle or vanga bhasma (25 and 50 mg/kg, i.g.) or metformin (500 mg/kg, i.g.) for 7 days. The serum glucose was estimated at 0, 1, 3, 12 h, 24 h, 3rd day and 7th day to study *per se* hypoglycemic effect of vanga bhasma. Values are the mean ± SEM (n = 5 rats).

TABLE 2. EFFECT OF VANGA BHASMA ON ORAL GLUCOSE TOLERANCE TEST

Groups	Blood glucose (mg/dl)			
	0 min	30 min	60 min	120 min
Vehicle	118±5.50	185±4.85	175±4.92	128±12.52
Vanga Bhasma 25	120±7.66	193±14.69	129±3.38@	103±1.72*
Vanga Bhasma 50	121±5.78	210±4.85	126±4.59@	94±2.90**
Metformin	122±5.68	198±7.17	120±1.77@	93±7.13*

Normoglycemic rats were treated with vehicle or vanga bhasma (25 and 50 mg/kg, i.g.) or metformin (500 mg/kg, i.g.) for 7 days and on day 7 glucose load was given after 3 h of last dose of vanga bhasma. The serum glucose was estimated at 0, 30, 60, 120 min after glucose load to study of vanga bhasma on oral glucose tolerance. Values are the mean ± SEM (n = 5 rats), *P < 0.05, **P < 0.01, @P < 0.001 compared to vehicle.

TABLE 3. EFFECT OF VANGA BHASMA ON ALLOXAN-INDUCED HYPERGLYCEMIA

Groups	Blood glucose (mg/dl)						
	0h	1h	3h	12h	24h	3 days	7 days
Alloxan + Vehicle	439±10.47	374±13.77	365±12.75	349±8.03	345±8.55	345±9.09	310±8.40
Alloxan + Vanga Bhasma 25	405±16.91	374±11.5	364±8.62	337±11.17	330±17.54	280±14.55**	151±13.95@
Alloxan + Vanga Bhasma 50	431±14.47	384±4.05	339±10.78	316±10.98	257±10.89@	227±11.56@	143±17.55@
Alloxan + Metformin	391±23.28	373±17.03	350±10.71	330±5.75	283±10.81**	291±3.21*	144±17.75@

Alloxan hyperglycemic rats were treated with vehicle or vanga bhasma (25 and 50 mg/kg, i.g.) or metformin (500 mg/kg, i.g.) for 7 days. The serum glucose was estimated at 0, 1, 3, 12 h, 24 h, 3rd day and 7th day. Values are the mean ± SEM (n = 5 rats), *P < 0.05, **P < 0.01, @P < 0.001 compared to respective controls.

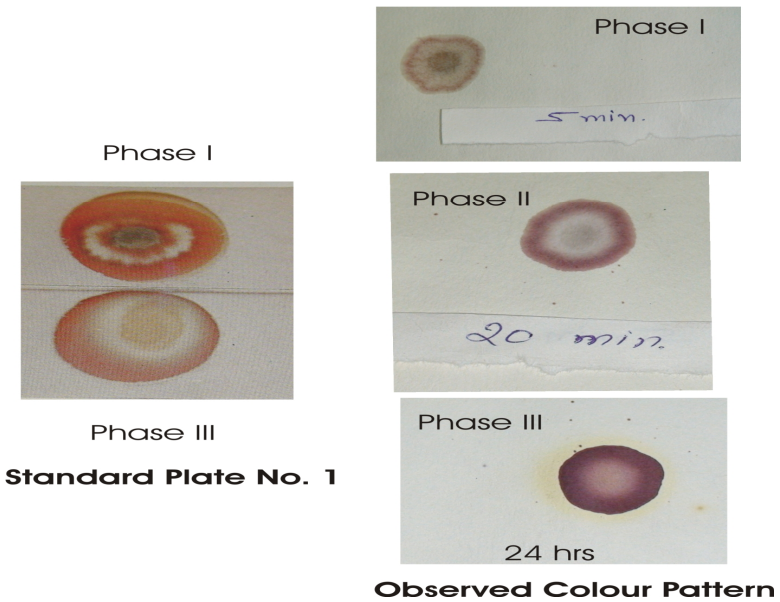

FIGURE 1. NAMBURI PHASED SPOT TEST FOR IDENTIFICATION OF VANGA BHASMA
In the phased spot test, the colour changes appearing in the spot of vanga bhasma on the right side match with the color change pattern in the standard plate shown on left side.

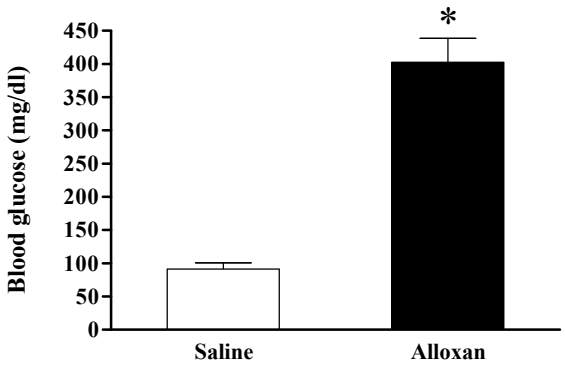

FIGURE 2. ALLOXAN-INDUCED HYPERGLYCEMIA
Rats were treated with single intravenous dose alloxan (65 mg/kg) to induced hyperglycemia. Results are expressed as mean ± SEM (n = 5 rats), @P < 0.0001 compared to saline.

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