ejpmr, 2015,2(6), 177-181

SJIF Impact Factor 2.026

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 3294-3211

EJPMR

ANTIHYPERGLYCEMIC POTENTIAL OF *LAGHU MALINI VASANTA RASA*- ZINC CONTAINING AYURVEDIC HERBOMINERAL PREPARATION.

Walunj Manisha[1*], Gunjal A[2],Nariya M[3], PK Prajapati[1]

[1]Dept. of Rasa Shastra & Bhaishajya Kalpana Including Drug Research, Institute for Post Graduate Teaching & Research In Ayurveda, Gujarat Ayurved University, Jamnagar, India.
[2]Dept. of Kayachikitsa Including Drug Research, Institute for Post Graduate Teaching & Research In Ayurveda, Gujarat Ayurved University, Jamnagar, India.
[3]Pharmacology Laboratory, Institute for Post Graduate Teaching & Research In Ayurveda, Gujarat Ayurved University, Jamnagar, India.

***Correspondence for Author: Dr. Walunj Manisha**
Dept. of Rasa Shastra & Bhaishajya Kalpana Including Drug Research, Institute for Post Graduate Teaching & Research In Ayurveda, Gujarat Ayurved University, Jamnagar, India.

Article Received on 22/08/2015 **Article Revised on 15/09/2015** **Article Accepted on 07/10/2015**

ABSTRACT

Context: *Laghu Malini Vasanta Rasa* is a renowned formulation for its rejuvenation effect. In textual reference of drug, it is not indicated in diabetes per se. As per Ayurvedic treatment protocol for disease, rejuvenation (*Dhatu Yapana*/ *Santarpana*) therapy is essential in diabetic patients. This justifies exploit of this particular formulation in management of diabetes traditionally by Ayurveda practitioners. **Aims:** To evaluate anti hyperglycemic effect of *Laghu Malini Vasanta Rasa*in albino mice. **Methods and material:** Swiss albino mice of either sex were divided randomly in three groups; control group, *Laghu Malini Vasanta Rasa*(32.5 mg/kg) treated group and standard drug as glibenclamide (0.65 mg/kg). One hour after test drug administration and vehicle to control group, mice were administered orally with glucose (5g/kg) solution in distilled water and blood glucose level was recorded at 30min, 60min and 120min of post glucose overload. **Results:** Administration of glucose solution produced highly significant increase in blood glucose level in control group at 30, 60, 90 and 120 mins when compared to its initial blood glucose. *Laghu Malini Vasanta Rasa* produced statistically highly significant decrease in blood glucose level at 30 min, 60 min and 120 min intervals while non-significant decrease at 90 min interval in comparison to control group. **Conclusion:** From the present study, it is concluded that *Laghu Malini Vasanta Rasa* possesses significant antihyperglycemic potential which substantiates its use as an alternative medicine in management of type 2 Diabetes mellitus.

KEYWORDS: Antihyperglycemic effect, *Laghu Malini Vasanta Rasa*, rejuvenation, *Yashada*, Zinc.

INTRODUCTION

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action or both.[1] The number of people with diabetes and pre-diabetes are exponentially increasing worldwide due to population growth, aging, urbanization, unhealthy eating habits, increasing prevalence of obesity and physical inactivity.[2] As per WHO projection, diabetes will be 7th leading cause of death by 2030.[3] The prevalence is expected to double between years 2005–2030, with the greatest increases expected in low and middle income developing countries of the African, Asian, and South American regions.[4] Diabetes is also associated with a host of life threatening and potentially disabling macro and micro-vascular complications.[5] Hence, there is also a much larger burden in the form of lost productivity as a result of restricted daily activity. Ninety percent of those with diabetes have type-2 diabetes, characterized by insulin resistance, hyper insulinaemia, β-cell dysfunction and subsequent β–cell failure.[6] Conventional oral hypoglycemic drugs and insulin analogues which are in use either as monotherapy or combination therapy for the disease are associated with several side effects based on theirmechanism of action.[7-9] A single, cost-effective, oral, antidiabetic treatment with minimal side effects is the need of thetime.Hence traditional systems of medicines and several metals including zinc are now being evaluated for their beneficial effects in both type 1 and type 2 Diabetes mellitus.[10] *Laghu Malini Vasanta Rasa* is a classical herbomineral preparation described in management of chronic fever. Drug is said to possess anabolic effect and can be used effectively in chronic diseases where along with treating ailments rejuvenation

is also required.[11]There is no obvious textual reference for the exploit of this formulation particularly in management of diabetes but asper renowned ancient seers of *Ayurveda- Acharya Caraka*[12] and *Acharya Susruta*[13]in the management of diabetes, rejuvenation (*Rasayana*)therapy is essential; as with the advancement of disease there is deranged metabolism of carbohydrates, proteins and fat. This justifies use of formulation in management of diabetes by some practitioners by tradition *Yashada Bhasma* (incinerated zinc) is used as an alternative to *Rasaka* (a chief ingredient of formulation) for preparation of drug owing to non-availability of genuine *Rasaka*.[14] *YashadaBhasma*has been well acclaimed for its antidiabetic activity in classical text[15] as well as researches carried out also sustain this fact.

Present study has been undertaken to find out whether this formulation which is in use in diabetes for its rejuvenation effect; is having role in glycemic control by evaluating its antihyperglycemic effectexperimentallyto substantiate its therapeutic efficacy in type 2 diabetes mellitus.

MATERIALS AND METHODS

Test drug

Ingredients of *Laghu Malini Vasanta Rasa* have been enlisted in table1.

Firstly *Yashada Bhasma* was prepared in three stages viz. *Shodhana* in *Churnodaka* (lime water) media (seven quenching in lime water), *Jarana* by *Apamarga* (*Achyranthes aspera* Linn.) *Panchanga* (whole part) and *Marana* by *Kumari* (*Aloevera* Linn.) *Swarasa* (expressed juice) as *Bhavana Dravya* (levigating media). Total seven *Puta* were required in EMF with peak temperatureof 650ºC to attain chief desired characteristics of *Bhasma* like- white in colour with yellowish tint.

To prepare *Laghu Malini Vasanta Rasa*, fine powder(85 sieve) of *Yashada Bhasma*(2 parts) and *Maricha (Piper nigrum*Linn.)(1part) were mixed in porcelain *Khalva*(mortar and pestle), freshly prepared cow butter in 50% quantity of ingredients was added to the mixture and levigation was done for 6 hr. Then levigated mass was transferred to wet grinder and repeated levigation with lemon juice was carried out till greasiness of mixture got minimized. Fine powder (85 sieve) of *Laghu Malini Vasanta Rasa* and *Pippali (Piper longum*Linn.) in equal proportion were taken and mixed homogenously. This mixture was transferred to stainless steel vessel. Then 30% honey was added as binding agent and converted into granules with the help of a 20 mesh sieve, taken into a SS tray and kept in the oven at 50ºC until complete drying. Granules were passed through 16 station rotary tablet machine to prepare tablets of 250 mg.

Animals

Swiss albino mice (*Musmusculus*) of either sex weighing 30±5 g were obtained from Animal house attached to Pharmacology Laboratory of IPGT&RA, Gujarat Ayurveda University, Jamnagar. Six animals were housed in each cage made of poly-propylene with stainless steel top grill. The dry wheat (post hulled) waste was used as bedding material and was changed every morning. All the selected animals were kept under acclimatization for 7 days before experimentation. The animals were exposed to 12 h light and 12 h dark cycle with the relative humidity of 50-70% and the ambient temperature during the period of experimentation was 22±3°C.All animals were kept on same environmental conditions. Amrut brand rat pellet feed supplied by Pranav Agro Ltd. was provided throughout the study period. The drinking water was given *ad libitum* in polypropylene bottles with stainless steel sipper tube. The animals were fasted overnight before experimentation. The experimental protocols were approved by Institutional Animal Ethics Committee (IAEC/15/2013/34) in accordance with the guideline formulated by CPCSE, India.

Dose fixation

With clinical dose of tablet of *Laghu Malini Vasanta Rasa* considered as 250 mg[16] dose for mouse was calculated by extrapolating the human dose to animal dose(32.5 mg/kg)based on the body surface area ratio using the table of Paget and Barnes (1964).[17]Test drug suspension was prepared in 0.5% CMC solution. Glibenclamide at the dose of 0.65 mg/kg was considered as reference standard control. The drugs were administered by oral route with the help of oral feeding canula.

Antihyperglycemic activity[18]

Swiss albino mice of either sex were selected and randomly divided into three groups each consist of six mice per group. Selected animals were acclimatized for seven days prior experiment. Group (I) kept as normal control and received distilled water (10 ml/kg, po) (NC). Group (II) kept as test drug treated group and received *Laghu Malini Vasanta Rasa* (32.5mg/kg, po) (LMV). Group (III) served as standard control group treated with glibenclamide (0.65mg/kg, po) (GB).

The animals were fasted overnight prior to the experimentation. In the next day morning, initial fasting BSL was measured with the help of Touch Ez Smart Glucometer (CE0537), by using one touch EzGluco test strips as per user's guidelines. Blood was collected from mice tail vein under light ether anesthesia following aseptic conditions. Then distilled water, test drug and standard drug were administered to respective groups of animals as per the body weight. One hour after test drug administration, mice were administered orally with glucose (5g/kg) solution in distilled water. Thereafter BSL was again recorded at 30min, 60min and 120min of

post glucose overload for accessing the anti-hyperglycemic activity of test drug.

Statistical analysis

The data are expressed as mean±standard error of mean for six mice per experimental group. The data generated during the study were subjected to Student't' test for paired and unpaired data to assess the statistical significance between the groups at $P<0.05$.

RESULTS AND DISCUSSION

In non-diabetic person an increase in glucose concentration is accompanied by a prompt increase in insulin and prompt decrease in glucagon concentrations. In the prandial state intestinal glucose absorption adds to the amount of glucose in the system over and above the fasting level.

During prandial state, hepatic glucose output is inhibited and other tissues (other than brain)start utilizing or storing glucose. This is facilitated by a rapid increase in insulin and decline in glucose level.[19]Higher rates of glucose appearance as a result of continued hepatic glucose output even in prandial state have been reported in type 2 DM.[20]Along with this reduced peripheral uptake also contributes to prandial plasma glucose level in type 2 DM patients.[21]

The results of present study revealed that administration of glucose (5 g/kg, po) solution in mice produced highly significant increase in blood glucose level in control group at 30, 60, 90 and 120 mins when compared to its initial blood glucose level (Table 2 and 3) which suggest hyperglycemia in mice. Standard drug glibenclamide produced statistically highly significant decrease in glucose level at 30, 60, 90 and 120 mins intervals when compared to control group as well as its initial readings.

Laghu Malini Vasanta Rasa produced statistically highly significant decrease in blood sugar level at 30 min, 60 min and 120 min intervals when compared to control group. At 90 min interval drug produced statistically non-significant decrease in glucose level in comparison to control group. The post prandial blood sugar level in drug treated group was increased after glucose administration but that was significantly less in comparison to control group suggestive of antihy-perglycemic potential of the test drug.

Yashada Bhasma- chief ingredient of formulation has been reported to improve glucose tolerance.[22] This is achieved by two mechanisms- inhibition of intestinal alpha glucosidase enzyme and thereby decreasing glucose absorption.[23]Secondly zinc accumulates in pancreatic beta cells due to beta cell specific zinc transporter 8 and results in increased glucose stimulated insulin secretion.[24] This increased glucose stimulated insulin secretion by zinc might contribute to increase glucose uptake. Analogous action could be possible for *Yashada Bhasma* as study has revealed presence of zinc oxide nanoparticles within *Yashada Bhasma*.[25] Various studies conducted on zinc supplements pertaining to their role in DM, showed that zinc not only promotes insulin action but also increases its stability.[26]It helps to improves insulin signaling by PTP1B inhibition[27] and increases glucose uptake by GLUT4 translocation in adipocytes.[28] Besides these actions, zinc has got pleiotropic role in regulation of glucose metabolism by its insulin mimetic,[29] hypoglycaemic,[30] antioxidant action byinhibition of lipid perioxidation[31] and beta cell protective[32] actions.

Blood sugar lowering effect of zinc has been aided in this particular formulation by *Piper longum*, and *Piper nigrum*.[33] Ethanolic extract of piper longum fruit had shown capacity to correctcarbohydrate metabolizing pathways-decrease in glucose 6 phosphatase, glycogen phosphorylase and fructose-1,6-biophosphate activities and increase inglucose 6 phosphate dehydrogenase and hexokinase activities resulting in increase in liver glycogen content and plasma insulin level.[34]Piperine an alkaloid from both Piper nigrum and Piper longum has been proven to potentiate antihyperglycemic action due to its bioenhancer effect when used with antihyperglycemic drug.[35]Honey a binding agent in this formulation, consists of predominantly fructose and glucose. The results indicate that fructose within honey enhances hepatic glucose uptake, glycogen synthesis and storage via activation of hepatic glucokinase, glycogen synthase respectively[36] and stimulates insulin secretion[37] thereby improving glycemic control.

Anti-hyperglycemic effect of individual drugs within formulation seems to be responsible for observed therapeutic effect of *Laghu Malini Vasanta Rasa* in present study. However, further study need to be carried out for exact mechanism of action of *Laghu Malini Vasanta Rasa* on BSL.

Table 1.Details of ingredients of *Laghu Malini Vasanta Rasa* tablet

Drug	Botanical name	Part used	Proportion
Yashada	Incinerated zinc	*Bhasma*	1 part
Maricha	*Piper nigrum* Linn.	Fruit	2 parts
Navaneeta	Freshly prepared butter		50% of ingredients
Nimbu	*Citrus acida*Linn.	Fruit	Q.s.
*Pippali*as adjuvant	*Piper longum*Linn.	Fruit	In equal proportion to laghuMaliniVasanta rasa
Honey as binding agent			30% of total ingredients

Table 2. Effect of test drugs on blood glucose levelin anti-hyperglycemic study in mice

Group	Blood glucose level (mg/dl)				
	Initial	30 min	% change to initial	60min	% change to initial
C	97.00±4.21	276.2±7.78$^{\#\#\#}$	179.20±5.94↑	185.40±4.79$^{\#\#\#}$	88.40±5.41↑
GB	88.33±3.70	82.0±3.36$^{\#\#@@}$	6.33±1.453↓	76.67±3.03$^{\#\#\#@@}$	11.67±1.36↓
LMV	76.4±2.97	182±11.53$^{\#@@}$	107.0±15.95↑	114.75±19.24$^{@}$	40.0±20.11↑

Data: Mean ± SEM, ↑ increase, ↓ decrease
$^{\#}$P<0.05, $^{\#\#}$P<0.01, $^{\#\#\#}$P<0.001 when compared to respective initial values (Paired 't' test)
$^{@}$P<0.01, $^{@@}$P<0.001 when compared to control group (Unpaired 't' test)

Table 3: Effect of test drugs on blood glucose level in anti-hyperglycemic study in mice

Group	Blood glucose level (mg/dl)				
	Initial	90 min	% change to initial	120min	% change to initial
Control	97.00±4.21	117.60±3.43$^{\#}$	20.60±7.06↑	106.80±2.76	9.80±5.02↑
GB	88.33±3.70	65.33±1.82$^{\#\#@@}$	23.00±2.74↓	60.0±1.71$^{\#\#@@}$	28.33±3.25↓
LMV	76.4±2.97	95.75±10.65	20.25±9.72↑	79.75±5.39$^{@}$	4.25±5.12↑

Data: Mean ± SEM, ↑ increase, ↓ decrease
$^{\#}$P<0.05, $^{\#\#}$P<0.001 when compared to respective initial values (Paired 't' test)
$^{@}$P<0.01, $^{@@}$P<0.001 when compared to control group (Unpaired 't' test)

CONCLUSION

From the present study, it is concluded that *Laghu Malini Vasanta Rasa* possesses significant antihyperglycemic potential which substantiates its use as an alternative medicine in management of type 2 Diabetes mellitus.

REFERENCES

1. The expert committee on the diagnosis and classification of diabetes mellitus. Report of the expert committee on the diagnosis and classification of diabetes mellitus. Diabetes care., 1997; 20(7): 1183-97.
2. Wild S, Roglic G, Green A, Sicree R, King H. Global Prevalence of Diabetes: estimates for the year 2000 and projections for 2030. Diabetes Care., 2004; 27(5): 1047–53.
3. Who.int.Diabetes Fact Sheet N^{0}312 [updated January 2015; cited on 19 sep 2015]. available from http://www.who.int/mediacentre/factsheets/ fs312/en/index.html.
4. Wild S, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. Diabetes Care., 2004; 27: 1047–53.
5. Levin M, Pfeifer M. Uncomplicated Guide to Diabetes Complications. 3rd edition. Virginia: American Diabetes Association., 2009.
6. Stumvoll M, Goldstein BJ, Van Haeften TW. Type 2 diabetes: principles of pathogenesis and therapy. Lancet., 2005; 365(9467): 1333–46.
7. Morral N.Novel targets and therapeutic strategies for type 2 diabetes. Trends in Endocrinology and Metabolism., 2003; 14(4): 169–75.
8. Moller DE. New drug targets for type 2 diabetes and the metabolic syndrome. Nature., 2001; 414(6865): 821–27.
9. Philis-Tsimikas. Type 2 diabetes: limitations of current therapies. Consultant., 2009; 7: S5–11.
10. Umrani RD, Paknikar KM. *Jasada bhasma*, a Zinc-Based Ayurvedic Preparation: Contemporary Evidence of Antidiabetic Activity Inspires Development of a Nanomedicine. Evidence-based Complementary and Alternative Medicine : eCAM 2015; 2015: article ID 193156, 9 pages available from http://www.ncbi.nlm.nih.gov/pubmed/25866533.
11. Sastri Laxmipati, Commenter. Yoga Ratnakara, Jwaradhikara, Reprint edition, Chaukhambha Prakashana, Varanasi, 2002; 245.
12. Jadavaji Trikamji Acharya, editor. Charaka Samhita, *Chikitsasthana,Prameha Chikitsa Adhyaya*, 6/15-17, reprint edition,Chaukhambha Prakashana, Varanasi, 2008; 446.
13. Sharma Anant Ram, editor. Susruta Samhita, *Sutrasthana, Avaraniya Adhyaya*, 33/3-4, Reprint edition, Chaukhambha Prakashana, Varanasi, 2008; 259.
14. Anonymus. The Ayurvedic Formulary of India. part 1. 2nded. New Delhi: Ministry of Health and Family welfare, Department of AYUSH Government of India publication., 2001; 271.
15. Sharma S. Rasa Tarangini. *Sisakadi Vidnyaniya ekonvinsha Taranga* ,19 /121,translated by Shri Kashinatha Shastri,11th Edition, Motilala Banarsidas, New Delhi, 2009; 479.
16. Walunj MB, Patgiri BJ, Prajapati PK and *From* 5th World Ayurveda Congress 2012 Bhopal, Madhya Pradesh, India. PA01.58.Role of laghu Malini Vasanta rasa in management of Madhumeha, type 2 diabetes mellitus. Ancient science of life., 2012; 32(Suppl 1): S 108.
17. Paget GE, Barnes JM. Evaluation of drug activities. In: Laurence DR, Bacharach AL, editors. Pharmacometrics. vol. 1. London: Academic press., 1964; 50.

18. Pilkhwal SS, Sah ML, Juyal V, Pandey S. Hypoglycemic activity of aqueous extract of *Urtica parviflora* Roxb. innormoglycemic rats. Int J Phytomedicine., 2010; 2(1): 47-51.
19. Kapur A. Prandial glucose regulation- Implications in Management of type 2 Diabetes In: Anil Kapur, JK Joshi, editors. Novo nordisk diabetes update 98 proceedings. Bangalore: Novo Nordisk Pharma India Ltd., 1998; 107-8.
20. Firth RG, Bell PM, Marsh HM, Hansen I, Rizza RA. Post prandial hyperglycemia in patients with non insulin dependent diabetes mellitus.J clin invest., 1986; 77(5): 1525-32.
21. DeFronzo RA, Bonadonna RC, Ferrannini E. Pathogenesis of NIDDM-a balanced over review. Diabetes care., 1992; 15(3): 318-68.
22. Umrani RD, Agrawal DS, Paknikar KM. Anti-diabetic activity and safety assessment of Ayurvedic medicine, Jasada bhasma (zinc ash) in rats. Indian Journal of Experimental Biology., 2013; 51(10): 811-22.
23. Ueda E, Yoshikawa Y, Sakurai H, Kojima Y, Kojiwara NM .In vitro alpha Glucosidase inhibitory effect of Zn (II) complex with 6 methyl-2-Piclinmethylamide.Chem. Pharm Bull., 2005; 53(4):451-452.
24. Chimienti F, Devergnas S, Pattou F, Schuit F, Garcia-Cuenca R,Vandewalle B et al. In vivo expression and functional characterization of the zinc transporter ZnT8 in glucose induced insulin secretion. J Cell Sci., 2006; 119: 4199-4206 .
25. Bhowmick TK, Suresh AK, Kane, Joshi AC, Bellare JR. Physicochemical characterization of an Indian traditional medicine, *JasadaBhasma*: detection of nanoparticles containing non-stoichiometric zinc oxide. Journal of Nanoparticle Research., 2009; 11(3): 655–64.
26. Chausmer AB. Zinc, insulin, and diabetes. J Am Coll Nutr., 1998; 17(2): 109–15.
27. Haase H, Maret W. Fluctuations of cellular, available zinc modulate insulin signaling via inhibition of protein tyrosine phosphatises. J Trace ElemMed Biol., 2005; 19(1): 37–42.
28. Ezaki O. IIb group metal ions (Zn2+, Cd2+, Hg2+) stimulate glucose transport activity by post-insulin receptor kinase mechanism in rat adipocytes. The Journal of Biological Chemistry., 1989; 264(27): 16118–22.
29. Rutter GA. Think zinc: New roles for zinc in the control of insulin secretion. Islets., 2010; 2(1): 49–50.
30. Wijesekara N, Chimienti F, Wheeler MB. Zinc, a regulator of islet function and glucose homeostasis . Diabetes Obes Metab., 2009; 11(4): 202–14.
31. Faure P, Benhamou PY, Perard A, Halimi S, Roussel AM. Lipid perioxidation in insulin-dependent diabetic patients with early retina degenerative lesions: Effects of an oral zinc supplementation. Eur J Clin Nutr., 1995; 49(4): 282–88.
32. Emily HO, Courtemanche C, Ames BN. Zinc deficiency induces oxidative DNA damage and increases P53 expression in human lung fibroblasts. Journal of Nutrition., 2003; 133(8): 2543–48.
33. Kaleem M, Sheema, Sarmad H, Bano B. Protective effects of piper nigrum and vinca rosea in alloxan induced diabetic rats. indian j physiol pharmacol., 2005; 49(1): 65–71.
34. Manoharan S, Silvan S, Krishnamoorthy V, Subramanian B. Anti hyperglycaemic and anti lipid perioxidative effects of piper longum (Linn.) dried fruits in alloxan induced diabetic rats. Journal of biological sciences., 2007; 6(1): 161-68.
35. Sama V, Nadipelli M, Yenumulla P. Effect of piperine on antihyperglycemic activity and pharmacokinetic profile of nateglinide. Arzneimittelforschung., 2012; 62(8): 384-8.
36. Erejuwa OO, Sulaiman SA, Wahab MS. Fructose Might Contribute to the Hypoglycemic Effect of Honey .Molecules., 2012; 17(2): 1900-15.
37. Erejuwa OO, Suleiman SA ,Wahab MS, Sirajudeen KN, Salleh MS, Gurtu S. Glibenclamide or metformin combined with honey improves glycemic control in streptozotocin induced diabetic rats. Int. J. Biol. Sci., 2011; 7(2): 244–52.