ORIGINAL ARTICLE

Evaluation of antihyperglycemic activity of modified *Nisha Amalaki yoga* in swiss albino mice

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Abstract

Background: Juvenile diabetes or insulin dependent diabetes mellitus is a chronic condition. Ayurvedic herbs are relatively low cost, more suitable and have negligible side effects than synthetic oral anti-hyperglycemic agents. *Guduchi, Aamalaki,* and *Haridra* are reported as highly potent anti-diabetic herb in Ayurveda and *Nisha-Aamalaki* is popularly known by the Ayurvedic fraternity for its therapeutic properties on Prameha represented as diabetes mellitus. **Aim:** The aim of the study was to evaluate the modified *Nisha Amalaki yoga* (MNA) for its anti-hyperglycemic effect in glucose overloaded albino mice. **Materials and Methods:** Anti-hyperglycemic potential of MNA Yoga was evaluated in Swiss albino mice. **Statistical Analysis:** The results were statistically interpreted using Student's "*t*"-test for paired and unpaired data to assess the statistical significance and the significant level was set at $P < 0.05$. **Results:** MNA showed on blood sugar level (BSL) in glucose overloaded mice at different time intervals. Marked increase in BSL in glucose overloaded mice in control group at all the time intervals in comparison to their initial values. MNA *yoga* and standard drug did not produce any significant anti-hyperglycemic effect at 30 min in comparison to values observed in control group. **Conclusion:** MNA showed anti-hyperglycemic effect found in MNA and standard treated mice. MNA treated mice produced more anti-hyperglycemic effect at some extent followed by standard treated in glucose overloaded hyperglycemic mice at 120 min.

Key words: Antihyperglycemic activity, *NishaAamalaki*, Swiss albino mice

INTRODUCTION

Pharmacological studies for Ayurvedic drugs are necessary to judge and revalidate the efficacy of Ayurvedic drugs in living organisms by animal experiments. Juvenile diabetes or insulin dependent diabetes mellitus is a chronic condition in which the pancreas produces little or no insulin. Insulin is a hormone needed to allow sugar (glucose) to enter cells to produce energy.[1] As Ayurvedic herbal preparations, which are relatively low cost, significantly potent, and more suitable than synthetic oral anti-hyperglycemic agents for attenuation of hyperglycemia in case of diabetes mellitus.[2] From the Ayurveda perspective, for a disease of *Sahaja* in nature, wherein *Dhatukshaya* is seen, the classical approach is applied, that is, the use of *Shodhana* therapy followed by use of *Rasayana* (Rejuvenating, antioxidants, and anti-ageing) to protect the body tissues from destruction. Considering the conditions in children with *Prameha*, the drugs used for the treatment for *Prameha* should have *Pramehahara* and *Rasayana* properties. Till date no any experimental; work reported on Modified *NishaAmalaki* (MNA) *yoga,* therefore in the present study, sample of MNA *yoga* was tested for its antihyperglycemic activity in experimental animals.

Aims and Objectives

The aim of the study was to evaluate the MNA *yoga* for its anti-hyperglycemic effect in glucose overloaded albino mice.

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MATERIALS AND METHODS

Animals

Swiss albino mice of either sex weighing 30 ± 2 g were obtained from Animal house attached to Pharmacology laboratory of Institute of Teaching and Research in Ayurveda, Jamnagar, for experiments and maintained under standard experimental and husbandry conditions.

The 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. 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. Animals were fed with Amrut brand rat pellet feed supplied by Pranav Agro Mills Pvt. Limited and drinking water *ad libitum*. The experiments were carried out after obtaining permission from Institutional Animal Ethics Committee (Approval No: IAEC/24/2018/28).

Test Formulations

MNA *yoga* was prepared in the Pharmacy Gujarat Ayurved University, Jamnagar.

Dose Selection and Schedule

Dose for experimental study was calculated by extrapolating the human dose to animals based on the body surface area ratio by referring to the table of Paget and Barnes.[3]

Human dose of MNA *yoga* = 6 g/day

=Human Dose × 0.0026 (conversion factor for mice)

=6 g (6000 mg) × 0.0026 (conversion factor for 20 g mice)

=15.6 mg/20 g = 780 mg/kg body weight of albino mice.

The test drugs were suspended in distilled water with suitable concentration and administered according to the body weight of the animals by oral route with the help of gastric oral cannula.

Instruments Used

Gluneo-lite Glucometer (K-LifeSurgicals, New Delhi, India) with *Ez Smart* strips, weighing scale, disposable needle and syringe, mono pan balance, and mortar and pestle.

Experimental Study

The anti-hyperglycemic activity was carried out by modifying method of Pilkhwal *et al*.[4] for the test drugs as per the following protocols.

Anti-hyperglycemic Activity

Swiss albino mice of either sex were randomly divided into three groups of six animals each as follow,

Group I Control group, received distilled water (10 ml/kg, po).

Group II MNA *yoga* (780 mg/kg, po) (MNA).

Group III Standard treated group, Glibenclamide (0.65mg/kg, po) (Std).

The animals were fasted overnight prior to the experiment and in the morning the fasting initial reading of blood sugar level (BSL) was measured with the help of One touch Gluneo-lite Glucometer, using Ez Smart stripsGluco test strips as per user's guideline by collecting the blood sample from tail vein following aseptic conditions. Then the vehicle, test drugs and standard drug were administered to respective groups of animals as per the body weight. After 1 h of drug administration, glucose (5 g/kg, po) solution in distilled water was administered to all groups orally to induce hyperglycemia. Thereafter, BSL was recorded at 30 min, 60 min, 90 min and 120 min of post glucose overload for accessing the anti-hyperglycemic activity of test drug.

Statistical Analysis

Results were presented as Mean ± SEM, difference between the groups was statistically determined by Student paired "*t*" test with initial values of respective groups and unpaired "*t*" test with control group. $P < 0.05$ was considered as significant value.

RESULTS

Anti-hyperglycemic Activity

Tables 1-4 revealed effect of test drug on BSL in glucose overloaded mice at different time intervals. Marked increase in BSL in glucose overloaded mice in control group at all the time intervals in comparison to their initial values. MNA *yoga* and standard drug did not produce any significant anti-hyperglycemic effect at 30 min in comparison to values observed in control group.

Table 1: Effect of test drugs on blood sugar level in glucose overloaded Swiss albino mice

Groups	Blood glucose (mg/dl)			
	Initial	30 min	Actual change	%Change
Control	151.83±54.21	190.67±50.26	38.84↑	25.58↑
MNA	146.14±38.46	201.57±49.35**	55.43↑	37.93↑
Std	158.57±24.37	177.43±36.01	18.86↑	11.89↑

MNA: Modified *NishaAmalaki.* Mean±SEM, ↑-increase ↓- decrease **p<0.02, when compared to respective initial values (Paired 't' test).

Table 2: Effect of test drugs on blood sugar level in glucose overloaded Swiss albino mice

Groups	Blood glucose (mg/dl)			
	Initial	60 min	Actual change	% Change
Control	151.83±54.21	191.33±51.82	39.5↑	26.01↑
MNA	146.14±38.46	146.43±27.11	0.29↑	0.19↑
Std	158.57±24.37	120±21.92*###@@	38.57↓	24.32↓

MNA: Modified *Nisha Amalaki.* Mean±SEM, ↑-increase ↓- decrease *p<0.05, when compared to respective initial values (Paired 't' test); ###p<0.01, when compared to control group (Unpaired 't' test); @@p<0.01, when compared to control group (One way ANOVA followed by Dunnett's multiple 't' test).

Table 3: Effect of test drugs on blood sugar level in glucose overloaded Swiss albino mice

Groups	Blood glucose (mg/dl)			
	Initial	90 min	Actual change	%Change
Control	151.83±54.21	184±48.88	32.17↑	21.18↑
MNA	146.14±38.46	128.57±51.34***#@	17.57↓	12.02↓
Std	158.57±24.37	123.43±22.7*##@	35.14↓	22.16↓

MNA: Modified *Nisha Amalaki.* Mean±SEM, ↑-increase ↓- decrease *p<0.05, ***p<0.01, when compared to respective initial values (Paired 't' test); #p<0.05, ##p<0.02, when compared to control group (Unpaired 't' test); @p<0.05, when compared to control group (One way ANOVA followed by Dunnett's multiple 't' test).

Table 4: Effect of test drugs on blood sugar level in glucose overloaded Swiss albino mice

Groups	Blood glucose (mg/dl)			
	Initial	120 min	Actual change	% Change
Control	151.83±54.21	165.33±43.24	13.5↑	8.89↑
MNA	146.14±38.46	124.57±34.89***	21.57↓	14.76↓
Std	158.57±24.37	139.29±22.79**	19.28↓	12.16↓

Data: Mean±SEM, ↑-increase↓- decrease *P<0.05, **P<0.02, ***P<0.01, when compared to respective initial values (Paired "t" test); #P<0.05, ##P<0.02, ###P<0.01, when compared to control group (Unpaired 't' test); @P<0.05, @@P<0.01, when compared to control group (One way ANOVA followed by Dunnett's multiple "t" test)

At 60 min time intervals, MNA *yoga* reversed the hyperglycemia to almost values observed in initial and produce marked effect in comparison to control group. In control group there was 39.5% increase in glucose level in mice while test drug show attenuation of blood glucose level in mice.

At 90 min time intervals, MNA *yoga* reversed the hyperglycemia produce significant anti-hyperglycemic effect in comparison to initial value. While in comparison to control group, MNA *yoga* produced significant anti-hyperglycemic effect at 90 min and non-significant effects at 120 min.

Glibenclamide showed significant anti-hyperglycemic effect at 60, 90, and 120 min in comparison to its initial values while significant effect produced at 60 and 90 min time interval in comparison to glucose overloaded control mice.

In the present study, anti-hyperglycemic effects of MNA *yoga* were studied in glucose overloaded mice. The results obtained presented as consolidated data are shown in Table 5.

Anti-hyperglycemic Activity

Overall, anti-hyperglycemic effect found in MNA and standard drug treated mice. Glibenclamide has pronounced anti-hyperglycemic effect followed by MNA *yoga* in glucose overloaded hyperglycemic mice.

Table 5: Consolidated data of effect of test drugs on blood sugar level in glucose over loaded hyperglycemic mice

Duration (Min)	Control	MNA	Standard Control
30	NSI[#]	SI[#]	NSI[#]
60	NSI[#]	NSE[#]NSD[@]	SD[#] HSD[@]
90	NSI[#]	HSD[#]SD[@]	SD[#]HSD [@]
120	NSI[#]	SD[#] NSD[@]	HSD[#] NSD[@]

[#]In comparison to initial, [@]In comparison to control group, NSI: Non significant increase, NSD: Non significant decrease, SD: Significant decrease, SI: Significant increase, HSD: Highly significant decrease, HSI: Highly significant increase

DISCUSSION

One of the earliest mechanisms of anti-hyperglycemic drugs is noted as insulinotropic effect. These drugs promote insulin secretion from the β-cells of Islet of Langerhans. Drugs with this mechanism of anti-hyperglycemic action have the tendency to produce hypoglycemia if the dose is increased and in normoglycemic animals also. If the drugs have this type of activity they produce good hypoglycemic activity even in normoglycemic mice.

Modified *Nisha Amalaki Yoga* is a simple combination of three *Ayurvedic* herbs- *Amalaki* (*Emblica officinalis*), *Haridra* (*Curcuma longa*) and *Guduchi* (*Tinospora cordifolia*). *Amalaki* is excellent antioxidant, antihyperglycemic and has rejuvenation properties. It boosts body immunity, reduces stress levels, and improves glucose metabolism. *Haridra* is also having antihyperglycemic property and improves glucose metabolism.[5] *Guduchi* has also antioxidant, antihyperglycemic, strength improving and rejuvenation properties. Previous works on *Guduchi* in regard of Diabetes Mellitus has proved its antidiabetic property[6] and it may help in percolation of insulin at cellular level and increase insulin uptake thereby modifying the Blood Sugar Levels.[7]

Promotion of insulin binding to the target tissues especially to the insulin receptors can be another mechanism. Some of the recently induced sulfonylurea derivatives promote insulin release by blocking the ATP-sensitive potassium channel. (The reference standard used in this study – Glibenclamide is a sulfonylurea derivative) Inhibition of K^+ efflux results in the depolarization of the insulin containing granules and the release of the insulin. The depolarization leads to opening of a voltage-gated calcium channel enhancing the calcium content in the insulin containing granules this promotes insulin secretion.[8] Some of the drugs act by promoting the exo-cytosis of insulin from their granules by direct action on the binding proteins. Some of the drugs of the above group indirectly inhibit the secretion of glucagon the hormone secreted by α-cells of the islet of Langerhan's which plays active role in the mobilization of glucose from different source and its mechanism of action and activity profile is opposite to the activity profile of insulin.

In the present study, anti-hyperglycemic effects of MNA *yoga* were studied in glucose overloaded mice. The results obtained presented as consolidated data are shown in Table 5.

Present study indicates that, MNA *yoga* treated group and standard treated group has statistically significant antihyperglycemic effect if administered 1 h before glucose overload. Nisha Amalaki Yoga increases peripheral glucose utilization and decreases hepatic glucose synthesis and/or increase in insulin secretion,[9] due to this reduced blood sugar level may be achieved. The glucose overloaded mice of anti-hyperglycemic study similar to glucose tolerance test. α-glucosidase and α-amylase enzymes are responsible for catabolism of dietary complex sugars in to simple carbohydrates, that is, glucose. In the present experimental mice, glucose itself has been used for induction of hyperglycemia; hence, antihyperglycemic effect in present experiment is not due to these mechanism of action of the test drug. Thus, drug is capable of attenuating hyperglycemia after 1 h of drug administration. Although drug possess α-glucosidase and α-amylase inhibitory properties[10] still present experiment further support the potential of other mechanisms of action of MNA *yoga* for attenuation of hyperglycemia such as insulin mimetic property, enhancement of peripheral tissue glucose uptake, improvement of insulin sensitivity, regulation (reduction) of Hepatic glucose production, and regulation of glucose production by kidneys.[11]

CONCLUSION

The anti-hyperglycemic effect found in MNA and standard treated mice. MNA treated mice produced more anti-hyperglycemic effect at some extent followed by standard treated in glucose overloaded hyperglycemic mice at 120 min. MNA can be used as supportive medicine as it is having anti-hyperglycemic activity.

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