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Evaluation of anti-diabetic activity of Nishamlaki on streptozotocin induced type II diabetic rats

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Abstract

Diabetes is a major health problem particularly in India. In spite of many drugs available, uncontrolled diabetes remains a challenge. Moreover, some of the antidiabetic drugs are on the verge of withdrawal due to adverse effects. So, there is an acute need for a new effective and safe drug. Present study was planned to evaluate anti-diabetic efficacy of Nishamalaki in diabetic wistar rats.Method- Nishamalaki was prepared from powder of *Curcuma longa* and fresh juice of *Emblica officinalis* according to ayurvedic literature and administered with honey. Diabetes was induced in wistar rats by injection of 60 mg/kg of Streptozotocin and 110 mg/kg Nicotinamide IP. 30 rats showing blood glucose above 250 mg/dl were divided into 5 groups Group I(Control)-Saline, Group II(Control)- Vehicle honey, Group III – Nishamalaki Prophylactic, Group IV- Nishamalaki treatment & Group V- Pioglitazone, given orally for 30 days. Blood glucose levels checked at days 0, 15, 30 & Cholesterol on day 30. Results - Nishamalaki treatment achieved significant ($p<0.01$) lowering of blood glucose in diabetic rats comparable to that of the Pioglitazone treated group. Nishamalaki also reduced serum cholesterol levels.

Conclusion - Antidiabetic efficacy of Nishamalaki in diabetic rats is comparable to Pioglitazone. It has also improved the lipid profile in diabetic rats.

Keywords: Madhumeha, Haridra, Curcuma longa, BSL, Lipid profile

Introduction

Diabetes Mellitus (DM) is a disorder of metabolism and its incidence is increasing. It has become a global health problem in spite of advances in modern science. India has been projected by WHO as the country with the fastest growing population of Diabetic patients. It is estimated that the total number of people with diabetes will raise from 50.8 million in 2010 to 87.0 million by 2030.[1]HO projects that diabetes will be the 7th leading cause of death in 2030. [2] According to Diabetes Atlas published by the International Diabetes Federation (IDF), by 2030 every 5th diabetic will be an Indian.[3]

Type I & type II are two main forms of diabetes encountered clinically. Out of these two, type 2 diabetes is the most prevalent type of diabetes.[iv] Diabetes is preceded by a phase of impaired glucose tolerance (IGT). These patients are called as pre-diabetics. Development of Type 2 diabetes is characterised by variable degree of insulin resistance, impaired insulin secretion and increased glucose production. Diabetes, mainly if not controlled, affects many systems like- heart, eyes, kidney & CNS. Type 2 DM is commonly associated with other medical disorders including obesity, hypertension and hyperlipidemia and this cluster is known as metabolic syndrome, which pre-pones the occurrence of complications. [4]

Lifelong treatment has to be taken for Diabetes mellitus. Many drugs are available to target specific metabolic derangement, yet diabetes is not controlled in some patients. Moreover, adverse reactions to the medications are also encountered. No drug is available in modern medicine to prevent/ postpone diabetic complications.[5] Thus, in uncontrolled diabetes, ADRs due to antidiabetic agents and paucity of drugs for prevention of complications- still remain as challenge for the clinicians. Some anti-diabetic drugs like Pioglitazone are on the verge of withdrawal. [6,7,8] Therefore, there is a need of effective and safe drug in DM. Ayurvedic drugs are claimed to be useful against all limitations mentioned above.[9] So, Ayurvedic agents are commonly used as non prescription agents along with modern medicinal drugs by the patients.[10] In Ayurveda, Diabetes is known as Madhumeha which is vataj variety of prameha (increased Urination). [11] Diabetes mellitus (Madhumeha) is treated with Emblica officinalis (amla) and Curcuma longa along with other herbs like- gugul, triphala, devdaru etc. [12] Present study was planned to evaluvate the therapeutic efficacy of Nishamalaki, a combination of *Emblica officinalis* (amla) and *Curcuma longa* in type 2 diabetes in wistar Rats.

Material And Methods

Animals

30 Wistar rats of either sex having average weight of 150-200g were used for the study. Housing was done in standard cage (3 to 4 animals per cage). The animals were given food and water ad libitum and were exposed to12 hr. light and dark cycle. Study started after obtaining the approval (BVDUMC/2679/2012-13) from Institutional Animal Ethics Committee of the Bharati Vidyapeeth University Medical College, Pune (CPCSEA - 258).

Preparation of Nishamalaki

Good quality rhizomes of *Curcuma longa* and Fresh fruits of *Emblica officinalis* were obtained from local market & authenticated by Ayurvedic college of Bharati Vidyapeeth University, Pune. Amla fruits were cut into small pieces crushed and crude extract was prepared. Rhizomes of Curcuma longa were ground into a fine powder using an electrical blender**.**This powder was sieved through 80 mm mesh. Amla extract was added to the Curcuma powder just sufficient to soak it. This mixture was then trichurated till fine paste is formed. The mixture was spread in the shed till complete drying. It was then powdered & stored in clean dry glass container. The dosage administered to diabetic rats was determined using the surface area ratio of humans to rats.[xv] The final product Nishamalaki was given to Rats in the dose of 0.9 g/kg with honey.

Induction of diabetes

Nicotinamide (110mg/kg) was given by IP route half an hour before streptozotocin to all the rats.[xvi] Diabetes mellitus was induced with a single intra-peritoneal injection of Streptozotocin (STZ) dissolved in citrate buffer (0.01 M, pH 4.5) in the dose 65 mg/kg following a 24 hr fast.[13]The animals were allowed to drink 5% w/v glucose solution overnight to overcome the drug induced hypoglycaemia. Fasting blood samples were collected from the animals 48 hours after the injection. All animals showing plasma glucose exceeding 250 mg/dl were included in the study. Animals were divided into five groups of 6 rats each. For Nishamalaki prophylactic group, NA treatment was started with STZ

Group I (Diabetic Control)-Saline treated ,
Group II (Diabetic Control) - Vehicle honey treated,
Group III – Nishamalaki Prophylactic ,
Group IV- Nishamalaki Therapeutic,
Group V- Pioglitazone (2.7 mg/kg/day)

Drugs treatment was given orally daily for 30 days. Blood glucose levels were checked at 0, 15 & 30 days by glucose oxidase-peroxidase method. Blood cholesterol was checked on 30th day.

Statistical Significance

Values were expressed as mean ± SEM. ANOVA followed by Student's 't' test was used for Statistical significance. P value < 0.05 was considered as statically significant. Statistical analysis was done with Graph pad prism 6.

Results

Effect of NA on blood sugar level

Within 48 hours of injection of STZ, rat blood sugar levels were increased ranging from 250-650 mg%. Then animals were randomly divided into different groups. So, average baseline levels were not similar. (Figure-1& Figure- 2), Shows the effect of drugs on blood sugar level in comparison to saline and honey control group. It is seen that prophylactic NA was maximally effective ($p<0.01$), followed by therapeutic NA ($p<0.05$). Pioglitazone also reduced the blood sugar level ($p<0.05$). Effect on blood sugar level on day 15 and day 30 are similar.

Figure:1 – Effect of Nishamalaki on STZ induced diabetic rats on 15 th day

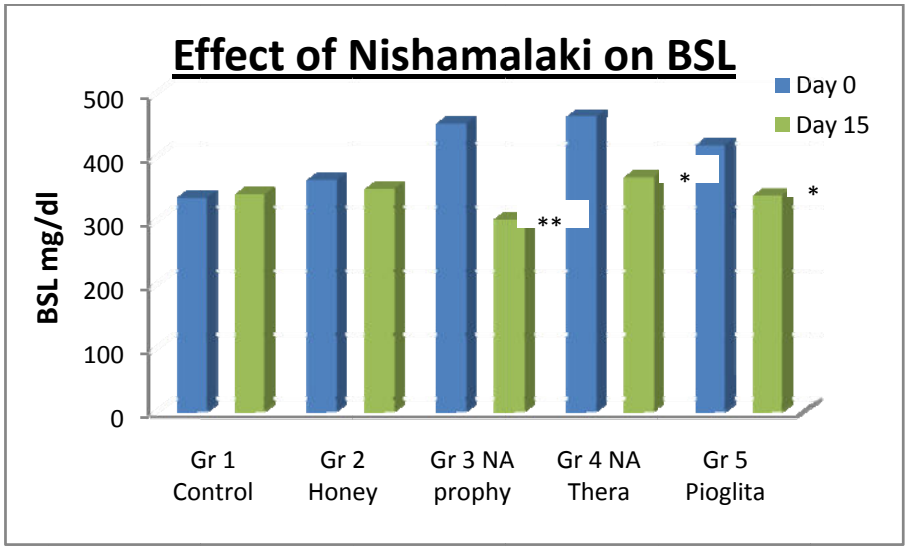
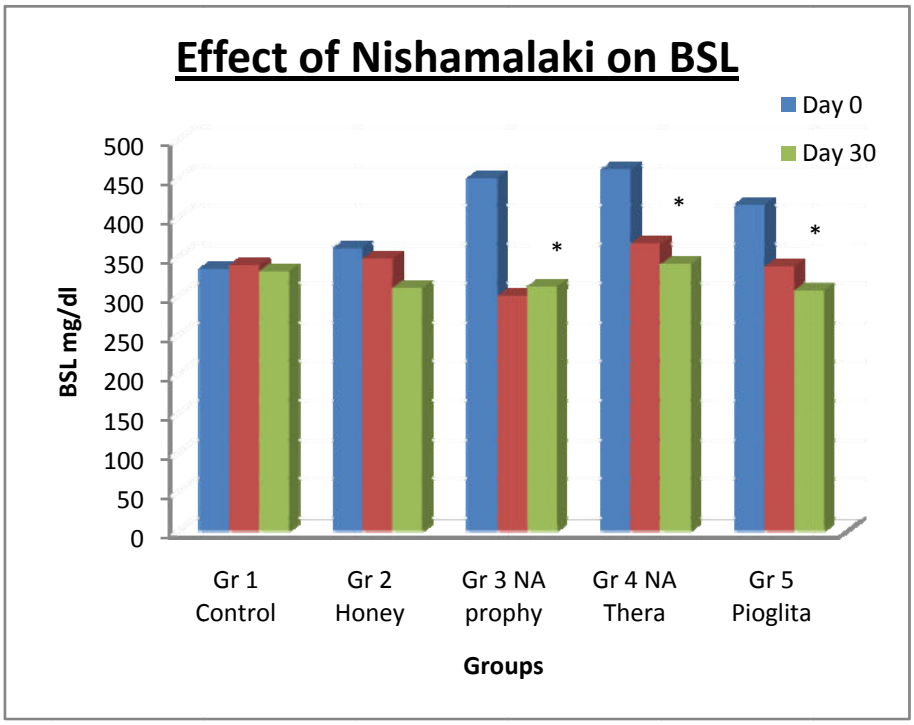


Figure:2 – Effect of Nishamalaki on STZ induced diabetic rats on 30 th day



Effect of NA on blood cholesterol level

Blood cholesterol levels, checked on the day 30, showed significant reduction in NA treated group (Figure-3).Prophylactic NA was more effective than therapeutic. Pioglitazone did not change cholesterol levels significantly.

Figure:3 – Effect of Nishamalaki on Cholesterol in STZ induced diabetic rats on 30 th day

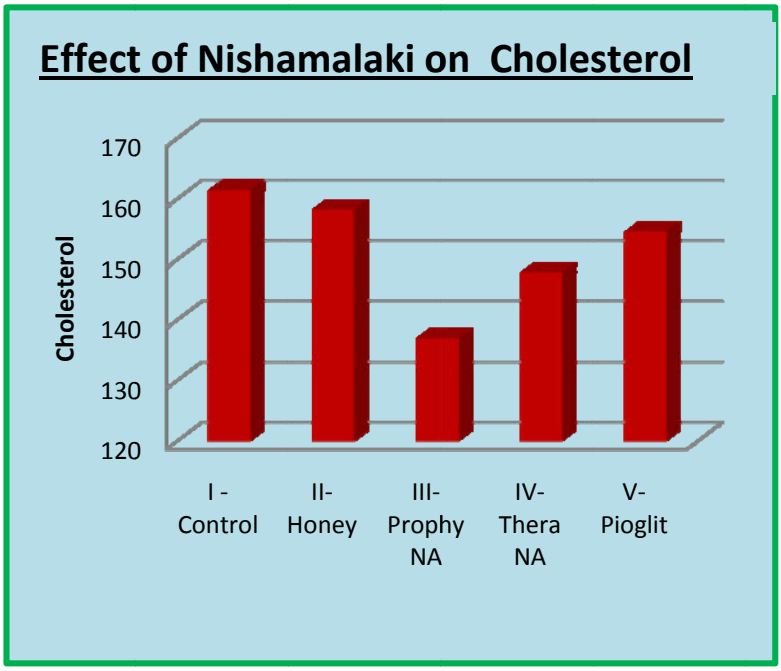


Figure:4 Rizomes of Curcuma Longa

Figure:5 Fresh fruits of Amla

Figure:6 Nishamalaki

Discussion

Insulin resistance coupled with impaired insulin secretion and overproduction of glucose leads to hyperglycaemia, which is central in the pathology of Type II DM. Insulin resistance, also increases flux of free fatty acids from adipose tissue into the circulation.[14] Hyperglycaemia and increased free fatty acids are shown to increase the production of superoxide causing oxidative stress which in turn is responsible for increased polyl flux, formation of advanced glycation end products, activation of protein kinase C and increased hexamine pathway flux-which are responsible for diabetic complications.[15] Insulin resistance and oxidative stress therefore appear to be most important in the pathogenesis of Type II DM.

Though, many drugs are said to be useful in DM according to Ayurvedic text, Nishamalaki is the first medicine with which treatment of DM should be initiated.[16] Nishamalaki is a herbal preparation, obtained from Curcuma longa and Emblica officinalis. According to the ayurvedic text, juice of Amla fruit should be mixed with powder of Curcuma longa, when the preparation is done in this way, six times the amount of Amla is required. We prepared Nishamalaki as per Ayurvedic text. Our NA preparation therefore, had Curcuma and Emblica in 1:6 proportions. Therefore for 1 gm of powdered Curcuma longa, 6 gms of Amla fruit are required. Amongst the drugs available in modern medicine, Thiozolidindione (TZD), group of drugs improve insulin resistance[xxi] and have antioxidant action due to active chromane ring present in its structure. Because of similarity in the mechanism of actions of TZD and Curcuma & Emblica, we compared the effect of NA with Pioglitazone (TZD group agent) in present study.

Streptozotocin(STZ) is probably the most widely used agent for induction of diabetes mellitus in experimental animals. Streptozotocin is a glucosamine nitrosourea compound which enters the beta cell of pancreas via a glucose transporter (GLUT2) and is converted to reactive metabolite which damages DNA of B cell.[17] Production of reactive metabolite causes oxidative stress. To reduce the extent of oxidative damage of B cells, Nicotinamide is administered before STZ. With antioxidant property and reduces oxidative damage.[18] Produces favourable metabolic changes and reduces apoptosis. Thus, combination of STZ and Nicotinamide produce reliable Type II diabetes in animals. [19]

Many studies are carried out to confirm the activity of both the agents singly. Curcuma longa (CL) and Emblica officinalis (EO) share many activities like hypoglycaemic, hypolipidaemic and reducing oxidative stress. xxv,xxvi,xxvii CL in a dose of 1gm/kg to alloxan induced diabeteic ratsxxviii is shown to reduce blood sugar levels by 30%. EO in 1.25 g/10 ml/ kg body weight dose produce similar hypoglycaemic action.[20] According to Ayurvedic text, the hypoglycaemic dose of combination in human is 4gm/day. By extrapolation the dose in rats is 0.9 gm/kg much less in comparison to individual agents which is used in our study. Though most of the actions of CL & EO are based on antioxidant property, it is shown that EO is about 10 times more potent antioxidant than CL.[xxx]so;

the combination is expected to exert potent antioxidant action along with hypolipidaemic and blood sugar reducing effect.
On day 15, all drug treated groups showed significant reduction in BSL when individual groups were compared. BSL reduction was significantly more in prophylactic NA group in comparison to therapeutic NA and Pioglitazone group. This probably is due to protection against B cell damage induced by STZ by its potent antioxidant action. BSL reduction in therapeutic NA and Pioglitazone group was comparable. NA has multiple actions leading to reduction in BSL like improving insulin sensitivity, increases glucose utilization, improves insulin release etc [xxxi] Pioglitazone on the other hand has mainly insulin sensitising action. This could be due to lesser potency of NA in comparison to Pioglitazone; we have observed the effect for 30 days. Since we used whole part of the plant and not the active moiety, it is possible that the combination requires longer duration of treatment to produce the effect. Very significant reduction was obtained with NA in cholesterol levels; Pioglitazone did not produce any change in cholesterol level. Can we find out the mechanism of cholesterol reduction?

Conclusion

Antidiabetic efficacy of Nishamalaki in diabetic rats is comparable to Pioglitazone. It has also improved the lipid profile in diabetic rats. This preparation shows preventive potential in Diabetes and its complications. Further studies are required to explore complete profile of Nishamalaki.

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