Research Article

Effect of Treatment with 'Nishamalaki' Powder on Glycemic Control and Markers of Erythrocyte Oxidative Stress in Diabetic Rats Compared to Troglitazone

Guruprasad Rao[1]*, Surekha Bhat[1], S. Gurumadhva Rao[2], P. Gopalakrishna Bhat[3]
[1]Department of Biochemistry, Melaka Manipal Medical College, Manipal University, Manipal, India
[2]Department of Pharmacology, Ras Al Khaimah Medical and Health Sciences University, United Arab Emirates.
[3]Manipal Life Sciences Centre, Manipal University, Manipal, India
***Corresponding author's E-mail:** gpraoudupi@gmail.com

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ABSTRACT

The 1:1 (w/w) mixture of powdered rhizome of *Curcuma longa* (turmeric) and the dried fruits of *Emblica officinalis* (Indian gooseberry) known as 'nishamalaki', described in traditional Indian medicine as a therapeutic agent in diabetes mellitus was studied for its effect on glycemic control and erythrocyte parameters of oxidative stress in rats with streptozotocin-induced diabetes mellitus in comparison with troglitazone, a thiazolidinedione compound known to possess antioxidant properties and the sulfonylurea drug, glyburide. Streptozotocin-induced diabetic rats were divided into the following treatment groups; nishamalaki powder treated (0.9 g/kg, NT group, n=12), troglitazone treated (36 mg/kg, TT group, n=12) and glyburide treated (4 mg/kg, GT group, n=12). The animals received the respective treatments for 30 days, at the end of which plasma glucose, glycated hemoglobin and erythrocyte parameters of oxidative stress were measured in all animals and the results compared with those of the untreated diabetic control (DC, n=12) and normal control (NC, n=12) rats. Nishamalaki treatment resulted in significant lowering of plasma glucose and glycated hemoglobin in diabetic rats ($p<0.001$ NT vs DC) comparable to that of the GT and TT groups. Erythrocyte membrane lipid peroxidation was lowered to a comparable extent by the three drugs ($p<0.001$ vs DC). NT rats showed greater improvement in erythrocyte reduced glutathione (GSH) level and glutathione peroxidase (GSH-Px) activity (both $p<0.001$ vs DC) than GT rats ($p>0.05$ vs DC for GSH and $p<0.05$ vs DC for GSH-Px) and this was comparable to the results of TT rats. Erythrocyte superoxide dismutase (SOD) activity was restored to a similar extent by the three drugs ($p<0.01$ vs DC). Glycemic control exerted by nishamalaki powder in diabetic rats is comparable to that of glyburide and troglitazone. Antioxidant protection offered by this preparation compares favourably with troglitazone and is greater than glyburide suggesting a role for this preparation in the management of diabetes mellitus.

Keywords: Diabetes mellitus, erythrocyte, nishamalaki, troglitazone, lipid peroxidation, antioxidant.

INTRODUCTION

The development of long term complications in diabetes mellitus has been linked to the extent of hyperglycemia and the duration of the disease. Uncontrolled diabetes mellitus results in oxidative stress and a number of mechanisms or pathways by which hyperglycemia, the major contributing factor of increased reactive oxygen species (ROS) production, causes tissue damage or diabetic complications have been identified. These include hyperglycemia-enhanced polyol pathway, hyperglycemia-enhanced formation of advanced glycation end products (AGEs), hyperglycemia-activated protein kinase C (PKC) pathway, hyperglycemia-enhanced hexosamine pathway and hyperglycemia-activated Poly-ADP ribose polymerase (PARP) pathway.[1] All of these pathways, in association to hyperglycemia-induced mitochondrial dysfunction and endoplasmic reticulum stress, promote ROS accumulation that, in turn, promotes cellular damage and contribute to the diabetic complications development and progression. ROS can directly damage lipids, proteins or DNA and modulate intracellular signaling pathways, such as mitogen activated protein kinases and redox sensitive transcription factors causing changes in protein expression and therefore irreversible oxidative modifications.[2] Damage to membrane lipids caused by reactive oxygen species can be assessed by measuring malondialdehyde (MDA) in biological samples. Measurement of cellular antioxidants such as reduced glutathione (GSH), glutathione peroxidase and superoxide dismutase (SOD) also serves as a marker of oxidative stress.

Modern medicines, despite offering a variety of effective treatment options for diabetes mellitus, can have several adverse effects including hypoglycemia. In view of these adverse effects and limitations of intensive treatment of hyperglycemia in preventing diabetic complications, which is linked to oxidative stress, it has been proposed that simultaneous targeting of hyperglycemia and oxidative stress could be more effective than intensive treatment of hyperglycemia alone in the management of diabetes mellitus.[3] This has spurred interest in the use of antioxidants in the treatment of diabetes. Ayurveda, the traditional Indian system of medicine, offers a balanced and holistic multi-modality approach to treating diabetes. Since diet forms the mainstay in the management of diabetes mellitus, there is scope for exploiting the antidiabetic potency of vegetables and fruits which may hold promise as potential antidiabetic agents. Plant products possessing both hypoglycemic and antioxidant properties will be particularly useful in the management of diabetes mellitus. Of considerable interest is the

adoption of Ayurveda by the mainstream medical system in some European countries (e.g. Hungary), emphasizing the increasing worldwide recognition being received by this modality.[4]

Curcuma longa (CL) [Family: Zingiberaceae, common names: turmeric, nisha (Sanskrit), haldi (Hindi)], a native of southern Asia and cultivated extensively throughout the warmer parts of the world, is a perennial herb, with a short stem and tufted leaves. The rhizomes which are short and thick, constitute the turmeric of commerce (Figure 1). Turmeric is used in Ayurvedic medicine in the treatment of a variety of conditions. The various beneficial activities of *Curcuma longa* that have been scientifically probed include antioxidant, anticancer, anti-inflammatory, antidiabetic, lipid lowering and wound healing activities among many others.[5]

Figure 1: Rhizomes of turmeric (Curcuma longa)

Emblica officinalis (EO) [Family: Euphorbiaceae; syn. *Phyllanthus emblica* (Latin), Indian gooseberry (English), Amalaki (Sanskrit), Amla (Hindi)] is a small or medium-sized deciduous tree commonly found in subtropical and tropical parts of India, China and Indonesia. The amla fruit (Figure 2) is highly nutritious and contains the high level of heat and storage-stable vitamin ascorbic acid.[6] Extracts from amla fruits have been evaluated for antidiabetic, hypolipidemic, antibacterial, antioxidant, antiulcerogenic, hepatoprotective, gastroprotective, and chemopreventive properties.[7]

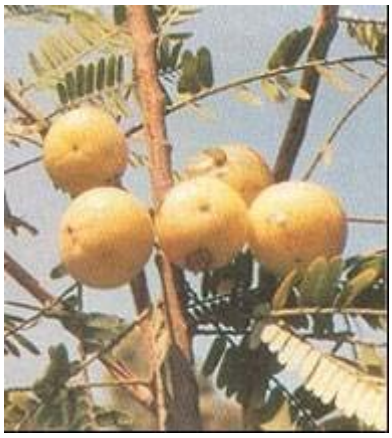

Figure 2: Fruits of Indian gooseberry (*Emblica officinalis*)

In Ayurvedic practice, different herbal preparations are used in combination to treat diabetes to maximise the beneficial effects. A 1:1 w/w mixture of powder of dried rhizome of CL and the powder of dried fruits (excluding the seeds) of EO called 'nishamalaki' is described by Sushruta as a treatment for diabetes mellitus.[8] There are no studies reported with this preparation on erythrocyte antioxidant status in diabetes along with its hypoglycemic effect in comparison with standard drugs. In our study we decided to use two pharmacologic agents as control groups against which we wished to compare the effect of treatment with nishamalaki powder. One of the drugs we used was troglitazone, which is a thiazolidinedione (TZD) compound containing the active chromane ring of α-tocopherol in its structure. This structural feature gives troglitazone both antidiabetic and antioxidant properties.[9] TZDs are potent and selective agonists for the nuclear receptor, peroxisome proliferator-activated receptor-gamma (PPARγ), a transcription factor that regulates expression of specific genes especially in adipose tissue. Activation of these receptors regulates the transcription of insulin-responsive genes involved in the control of production, transport and use of glucose.[10] They reduce insulin resistance not only in type 2 diabetes but also in nondiabetic conditions associated with insulin resistance such as obesity.[11] The other standard oral hypoglycemic drug we used for comparison was the sulfonylurea compound glyburide.

Objective of the study

The objective of the present study was to evaluate the effect of treatment with nishamalaki powder on parameters of glycemic control and erythrocyte oxidative stress in streptozotocin-induced diabetic rats in comparison with troglitazone and glyburide.

ANIMALS AND METHODS

Adult male Wistar albino rats aged 3-4 months, weighing 150-300g were used in the studies. The animals were maintained under standard hygienic conditions, provided food and water a*d libitum* and exposed to proper light and dark cycle (12 hours each of light and darkness). The experimental protocol was approved by the Institutional Animal Ethics Committee of the Manipal University and met the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals, Ministry of Social Justice and Empowerment, Government of India (Registration number of the animal house – 94/1999/CPCSEA).

Preparation of nishamalaki powder

Curcuma longa and *Emblica officinalis* plants procured locally were identified and allotted voucher specimen numbers PP532 and PP533 respectively by experts in the department of Pharmacognosy, Manipal College of Pharmaceutical Sciences, Manipal University, Manipal, India. Rhizomes of *Curcuma longa* and fruits of *Emblica officinalis* (without seeds) were dried in the shade and ground into a fine powder using an electrical blender.

They were weighed separately and mixed to obtain a mixture with equal amounts (1:1 w/w) of both the preparations. The dose used in the study was 0.9 g/kg (consisting of 0.45 g/kg each of the two preparations). Acute toxicity studies were conducted with nishamalaki preparation and it was well tolerated and no toxicity was observed upto a dose of 2g/kg. The dosage administered to diabetic rats was determined using the surface area ratios of humans to rats.[12]

Induction of diabetes in rats

Diabetes mellitus was induced in rats with a single subcutaneous injection of streptozotocin (40 mg/kg, in 0.01M citrate buffer pH 4.5) following a 24 hr fast with free access to water. Fasting blood samples were collected from the animals 48 hours after the injection. All animals showed a plasma glucose level exceeding 250 mg/dl and were included in the diabetic study groups.

Study groups

The rats were divided into the following groups and they were treated with the respective drugs as indicated.

1. Normal control (NC): non diabetic, did not receive any treatment (n=12)

2. Untreated diabetic control (DC): diabetic rats administered 2% gum acacia as vehicle (n=12)

3. Nishamalaki treated (NT): diabetic rats treated with nishamalaki powder (0.9 g/kg as 2% gum acacia suspension, n = 12)

4. Troglitazone treated (TT): diabetic rats treated with troglitazone (36 mg/kg as 2% gum acacia suspension, n=12)

4. Glyburide treated (GT): diabetic rats administered glyburide (4 mg/kg as 2% gum acacia suspension, n = 12)

The treatment commenced 24 hours after confirmation of diabetes mellitus and the drugs were orally administered once daily for 30 days. At the end of 30 days, fasting blood samples were collected from all the study groups and were processed to measure parameters of glycemic control and erythrocyte oxidative stress.

Measurement of parameters of glycemic control and oxidative stress

a. Glycemic control parameters measured

i. Plasma glucose by glucose oxidase-peroxidase method[13]

ii. Glycated hemoglobin by affinity chromatography[14]

b. Parameters reflecting oxidative stress and antioxidants in the red blood cells measured

i. Malondialdehyde (MDA), a product of membrane lipid peroxidation measured as thiobarbituric acid reactive substances (TBARS)[15]

ii. Reduced glutathione (GSH) by reaction with 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) [16]

iii. Glutathione peroxidase (GSH-Px) assay[17]

iv. Superoxide dismutase (SOD) assay[18]

Values were expressed as mean ± SEM. Statistical analysis of the results was carried out using One-way analysis of variance (ANOVA) with Bonferroni's correction and correlation of the Prism software package.

RESULTS

The untreated diabetic control (DC) group of rats showed significantly elevated plasma glucose level as compared to normal control (NC) rats ($p<0.001$) at the end of 30 day period of the experiment. Nishamalaki treated diabetic rats showed significant reduction plasma glucose (Figure 3) comparable to that achieved by treatment with troglitazone and glyburide ($p<0.001$ vs DC for all three groups). DC rats showed significantly high levels of glycated hemoglobin than NC rats ($p<0.001$). Nishamalaki treatment also brought about significant decrease in glycated hemoglobin (Figure 4) which reflects long term glycemic control and in this regard it was comparable to both troglitazone and glyburide ($p<0.001$ vs DC all three groups). DC rats showed significantly high levels of erythrocyte TBARS (which reflects the extent of lipid peroxidation) than NC rats ($p<0.001$). Nishamalaki treatment decreased TBARS significantly in diabetic rats in comparison to diabetic controls and this effect was comparable to that shown by troglitazone and glyburide treatment (Figure 5, $p<0.001$ vs DC for all three groups). Glycated hemoglobin levels in the DC rats and in NT group of diabetic rats showed a significant positive correlation with TBARS levels ($r = 0.7$, $p<0.05$ and $r = 0.6$, $p<0.05$ respectively). DC rats showed significantly decreased levels of erythrocyte GSH than NC rats ($p<0.001$). Nishamalaki treated rats showed greater improvement in GSH level (Figure 6, $p<0.001$ vs DC) than glyburide treated rats ($p>0.05$ vs DC, not significant). Glyburide treated rats in fact showed significantly lower GSH levels than normal controls ($p<0.01$ vs NC, Figure 6). The effect of treatment with nishamalaki on erythrocyte GSH was comparable to that of troglitazone. We observed that DC rats showed significantly decreased activity of the enzyme GSH-Px than NC rats ($p<0.001$). However, treatment with nishamalaki increased the enzyme activity significantly ($p<0.001$ vs DC, Figure 7) comparable to the effect of troglitazone treatment, whereas glyburide treated rats showed a lesser degree of increase in the activity ($p<0.05$ vs DC). Glycated hemoglobin levels in the nishamalaki treated diabetic rats showed a significant negative correlation with activity of GSH-Px ($r = -0.82$, $p<0.01$). Activity of the SOD was significantly decreased in DC rats as compared to NC rats ($p<0.001$). Nishamalaki treatment improved the SOD activity significantly ($p<0.01$ vs DC, Figure 8) and in this regard it was comparable to both troglitazone and glyburide. We observed a negative correlation between glycated hemoglobin and SOD activity in NT groups of rats ($r = -0.63$, $p<0.05$).

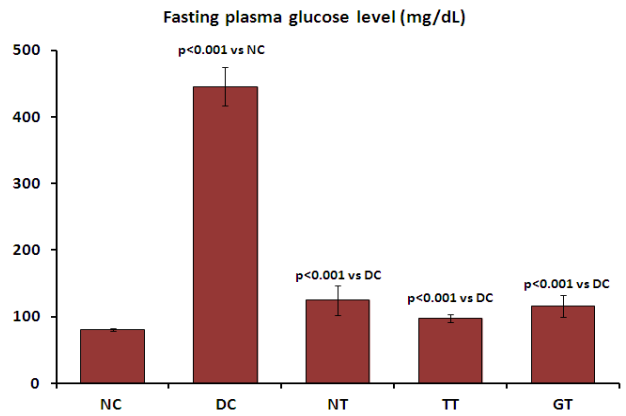

Figure 3: Fasting plasma glucose levels in the different study groups at the end of the 30 day treatment period. Values are mean ± SEM. NC: normal control (n=12), DC: untreated diabetic control (n=12), NT: nishamalaki treated (n=12), TT: troglitazone treated (n=12), GT: glyburide treated (n=12).

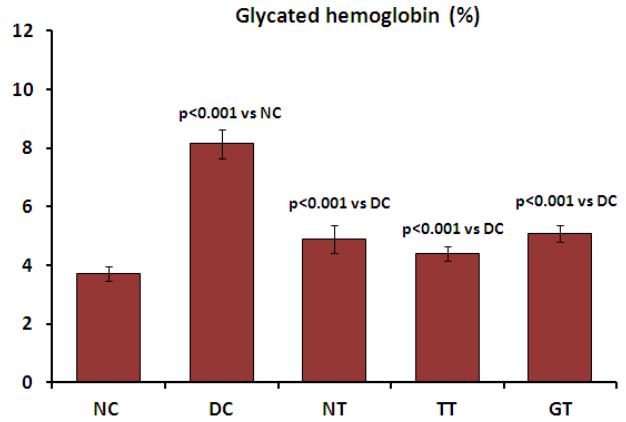

Figure 4: Glycated hemoglobin levels in the different study groups at the end of the 30 day treatment period. Values are mean ± SEM. NC: normal control (n=12), DC: untreated diabetic control (n=12), NT: nishamalaki treated (n=12), TT: troglitazone treated (n=12), GT: glyburide treated (n=12).

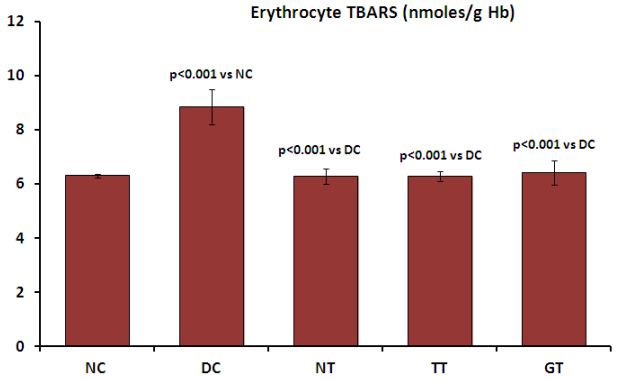

Figure 5: Erythrocyte membrane lipid peroxidation product malondialdehyde (MDA) measured as thiobarbituric acid reactive substances (TBARS) in the different study groups at the end of the 30 day treatment period. Values are mean ± SEM. NC: normal control (n=12), DC: untreated diabetic control (n=12), NT: nishamalaki treated (n=12), TT: troglitazone treated (n=12), GT: glyburide treated (n=12).

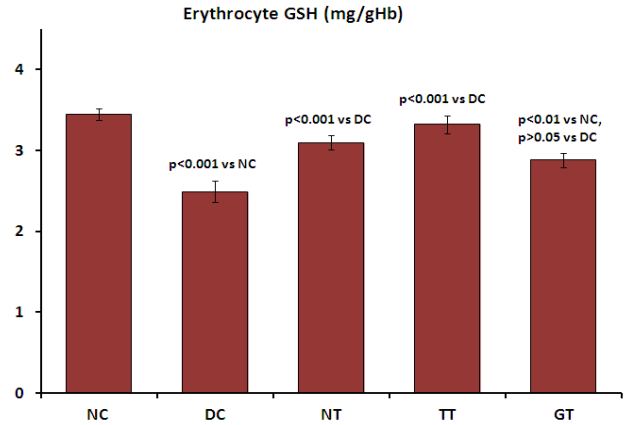

Figure 6: Erythrocyte reduced glutathione (GSH) levels in the different study groups at the end of the 30 day treatment period. Values are mean ± SEM. NC: normal control (n=12), DC: untreated diabetic control (n=12), NT: nishamalaki treated (n=12), TT: troglitazone treated (n=12), GT: glyburide treated (n=12).

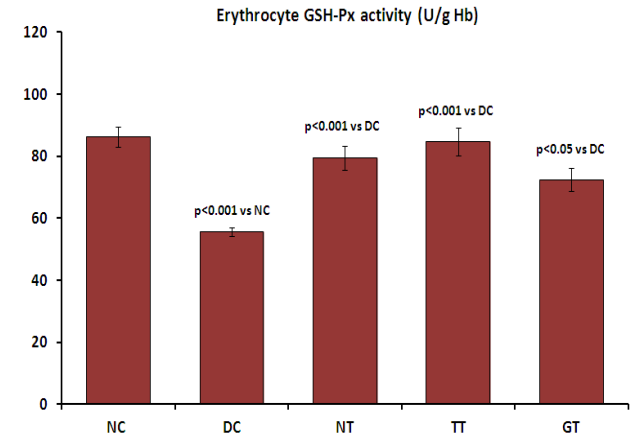

Figure 7: Erythrocyte glutathione peroxidase (GSH-Px) activity in the different study groups at the end of the 30 day treatment period. Values are mean ± SEM. NC: normal control (n=12), DC: untreated diabetic control (n=12), NT: nishamalaki treated (n=12), TT: troglitazone treated (n=12), GT: glyburide treated (n=12).

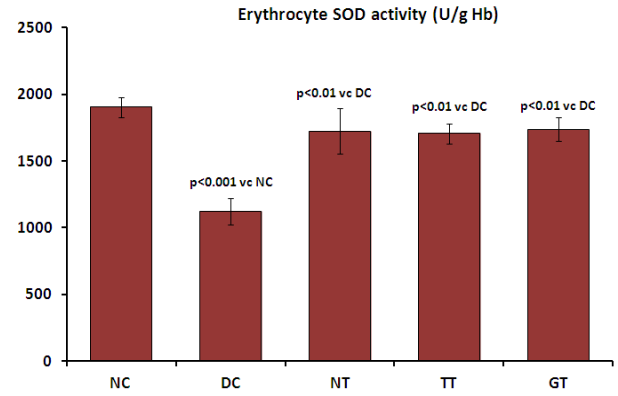

Figure 8: Erythrocyte superoxide dismutase (SOD) activity in the different study groups at the end of the 30 day treatment period. Values are mean ± SEM. NC: normal control (n=12), DC: untreated diabetic control (n=12), NT: nishamalaki treated (n=12), TT: troglitazone treated (n=12), GT: glyburide treated (n=12).

DISCUSSION

Erythrocytes possess higher activities of antioxidant enzymes such as Cu,Zn-SOD, GSH-Px, glutathione reductase and catalase compared to other cell types. Most of the nonenzymatic antioxidant capacity of whole blood is likewise localized in the erythrocytes. Circulating red cells are mobile free radical scavengers and provide antioxidant protection to other tissues and organs.[19] The easy accessibility, finite lifespan and relative simplicity of erythrocytes make them an attractive model to study the oxidative stress status of the body in diabetes mellitus.

In our studies, streptozotocin-induced untreated diabetic control rats (DC group) showed significant decreases in GSH levels and activities of the antioxidant enzymes GSH-Px and SOD in erythrocytes, showing a generalized decline in ability of erythrocytes to tackle ROS generated in the diabetic state resulting in increased susceptibility of erythrocyte membrane lipids to peroxidation which was manifest as a significant increase in the levels of MDA. The positive correlation between glycated hemoglobin levels and lipid peroxidation in untreated diabetic control rats confirms the link between sustained hyperglycemia and increased oxidative stress in diabetes.

The use of combined herbal preparations to treat diabetes is a common practice in Ayurvedic system of medicine. A polyherbal Ayurvedic formulation known as 'Dihar', containing extracts from eight different herbs including CL and EO showed significant glucose-lowering effect in streptozotocin-diabetic rats besides demonstrating antioxidant effects.[20] Oral administration of hyponidd, a herbomineral formulation composed of the extracts of ten medicinal plants including *Momordica charantia*, CL and EO at a dose of 200 mg/kg for 45 days resulted in significant lowering of blood glucose, improved glucose tolerance and significantly increased levels of hepatic glycogen in streptozotocin-diabetic rats.[21] We therefore decided to test the antioxidant and antidiabetic efficacy of nishamalaki powder which has equal proportions of powdered rhizomes of CL and dried fruits of EO in diabetic rats. We used troglitazone for comparing the antioxidant benefits provided by nishamalaki in diabetic rats since troglitazone is an antidiabetic agent with known antioxidant properties.

Our results clearly showed that nishamalaki treatment achieved significant reductions in both fasting plasma glucose level and glycated hemoglobin in diabetic animals. This effect was comparable to that shown by troglitazone and glyburide. Our study corroborates results of several earlier studies with nishamalaki preparation in diabetes mellitus. It was reported that nishamalaki was effective in lowering fasting blood sugar levels as well as reducing symptoms in type 2 diabetic patients.[22] A clinical study with Nishamalaki was carried out in diabetic patients and encouraging results were obtained.[23] In normal fasting as well as alloxan-diabetic rats, combination of extracts of CL and EO exhibited good reduction in blood sugar and a satisfactory response in glucose tolerance test was also observed.[24] A significant improvement in the symptoms along with lowering of blood glucose level was observed in diabetic patients treated with nishamalaki powder in a clinical trial.[25] An earlier study[26] reported that administration of dried powder of turmeric rhizomes at a dose of 1 g/kg to alloxan-diabetic rats for 21 days caused a decrease of about 30% in blood glucose value. Flavonoids from EO exerted highly potent hypoglycemic and hypolipidemic actions in rats.[27] Oral administration of methanolic extract from EO reduced blood sugar level in normal and in alloxan-diabetic rats significantly within 4 hours. Continued daily administration of the drug produced a sustained effect.[28] It has been reported that supplements containing *Emblica officinalis* showed the most consistency in lowering fasting blood sugar or glycated hemoglobin levels in diabetic patients.[29] Our finding supports the traditional view that combination of turmeric and amla can provide benefit to diabetic patients. In combination, these two plant products probably potentiate the actions of each other. The hypoglycemic effect of turmeric has been suggested to be due to increased peripheral glucose utilization, decreased hepatic glucose synthesis and/or increase in insulin secretion.[30] The ingestion of 6 g CL increased postprandial serum insulin levels in healthy subjects.[31] Turmeric reportedly potentiated action of insulin on glucose metabolism in rat epididymal fat cell assay[32] suggesting a possible mechanism of action of turmeric on glycemic control. Furthermore, curcumin, the active ingredient in turmeric was found to have a better hypoglycemic effect than turmeric powder.[26] A study involving type 2 diabetic KK-A^y mice suggested that curcuminoids in turmeric exhibit hypoglycemic effects via PPAR-γ activation as one of the mechanisms.[33]

Nishamalaki treatment significantly lowered membrane lipid peroxidation in erythrocytes of diabetic rats in our study. This effect was comparable to that achieved by troglitazone and glyburide. Curcumin is known to be a potent inhibitor of lipid peroxidation and scavenger of superoxide radicals,[34] singlet oxygen,[35] and nitric oxide.[36] Treatment with powdered rhizome of CL decreased lipid peroxidation in erythrocytes of streptozotocin-diabetic rats.[37] Treatment with turmeric and curcumin for 21 days decreased TBARS in plasma of alloxan-diabetic rats wherein curcumin showed a better effect than turmeric powder.[26] The accumulation of lipid peroxidation products in diabetic serum was reduced significantly by curcumin.[38] It has been suggested that curcumin supplementation could improve diabetes-induced endothelial dysfunction significantly in relation to its potential to decrease superoxide production.[39] Our finding of decreased lipid peroxidation caused by the mixture of CL and EO in diabetic rats corroborates the findings of these previous workers since turmeric used by them was one of the ingredients in nishamalaki used in our study. Glycated hemoglobin levels in the nishamalaki treated diabetic animals showed a significant positive correlation with TBARS levels showing that improvement

in glycemic status caused by the mixture of CL and EO was associated with decrease in erythrocyte membrane lipid peroxidation. A previous in vitro study has demonstrated that curcumin prevents protein glycosylation and lipid peroxidation caused by high glucose levels using an erythrocyte cell model. The study also suggested that curcumin may inhibit oxygen radical production caused by high glucose concentrations in a cell-free system, and increase glucose utilization in erythrocytes. This provides evidence for a novel mechanism by which curcumin supplementation may prevent the cellular dysfunction associated with diabetes.[40] Our report of decreased lipid peroxidation in erythrocytes of diabetic rats and its negative correlation with glycated hemoglobin supports a role for curcumin in the prevention of hyperglycemia associated oxidative damage seen in diabetes. The other ingredient in nishamalaki, EO is also reported to possess antioxidant activities. Though it is well known that EO fruits are rich in the antioxidant ascorbic acid, studies have shown that the antioxidant activity of amla is also due to presence of tannins.[41] Methanolic extract of EO was found to exhibit scavenging activity against hydroxyl, superoxide and nitric oxide radicals *in vitro.*[42] Administration of active tannoids of EO to rats for 7 days decreased lipid peroxidation in the brain.[43] Our results with nishamalaki on erythrocyte membrane lipid peroxidation in diabetic rats provide further evidence for the antioxidant activity of amla fruits in addition to proving its efficacy as an antidiabetic agent in association with turmeric.

Nishamalaki treatment brought about a greater improvement in erythrocyte GSH than glyburide and comparable to troglitazone. Previous workers have reported that treatment with turmeric (1 g/kg) for 21 days improved plasma GSH level to a point in between that of frankly diabetic and normal states in alloxan-diabetic rats.[26] In our study, GSH level in erythrocytes of diabetic rats treated with nishamalaki (0.9 g/kg, 30 days) was in between that of diabetic and normal value (Figure 6) but membrane lipid peroxidation in this group was normalized. This could be because of the direct inhibitory effect of curcumin on lipid peroxidation and the presence of ample amounts of antioxidants present in the amla fruits of nishamalaki mixture in addition to the increase in erythrocyte GSH caused by this treatment.

Treatment with nishamalaki preparation caused a significant increase in activity of red cell GSH-Px in diabetic rats that was better than the action of glyburide and comparable to that of troglitazone. Our results are in agreement with a previous report of a significant increase in erythrocyte as well as hepatic GSH-Px activity in alloxan-diabetic rats treated with turmeric powder.[26] Regeneration of the GSH oxidized in the glutathione peroxidase reaction requires NADPH, which is also required in the polyol pathway. In diabetes mellitus, activity of polyol pathway is increased and there is competition for NADPH between aldose reductase and glutathione reductase. Increased flux of glucose through polyol pathway in diabetes may reduce the effectiveness of the glutathione system in scavenging ROS. Sorbitol formed from glucose by aldose reductase (AR) is converted to fructose by sorbitol dehydrogenase (SDH) in presence of NADH. Increased activity of SDH has been reported in diabetes. Treatment with turmeric powder and curcumin was reported to reverse the observed elevation of SDH activity in liver and plasma of alloxan-diabetic rats thereby slowing down the rate of polyol pathway in diabetes. It was suggested that turmeric treatment, by lowering blood glucose level in diabetic rats, decreased the flux of glucose through the polyol pathway leading to increased NADPH/NADP ratio resulting in increased reduction of GSSG to GSH which in turn led to an elevation in the activity of GSH-Px.[26] In an earlier study, curcumin was shown to inhibit aldose reductase and to suppress sorbitol accumulation in human erythrocytes under high glucose conditions, demonstrating an in vivo potential of curcumin to prevent sorbitol accumulation.[44] It has been reported that the tannoid principles of EO inhibited aldose reductase of rat lens as well as recombinant human AR and inhibited sugar-induced osmotic changes in rat lens.[45] The same authors also reported that Emblica and its tannoids might counter the polyol pathway-induced oxidative stress as there was a reversal of changes with respect to lipid peroxidation, protein carbonyl content, and activities of antioxidant enzymes in diabetic rat lens.[46] Interestingly, the nishamalaki preparation used by us (which contains active ingredients of turmeric as well as the tannoid principles of *Emblica officinalis*) not only improved glycemic status in diabetic rats but also caused a significant increase in erythrocyte GSH. The better availability of GSH and improved GSH-Px activity provide better defence against ROS to the red blood cells. This in turn might have decreased erythrocyte lipid peroxidation in this group of rats. Thus nishamalaki, due to its content of curcumin as well as the tannoid principles of EO has shown a combined effect of exerting glycemic control as well as providing antioxidant protection to diabetic rats. Glycated hemoglobin levels in diabetic rats treated with nishamalaki showed a significant negative correlation with activity of GSH-Px suggesting that the good glycemic control achieved by this treatment might have not only prevented excessive generation of ROS but preserved the activity of this crucial antioxidant enzyme by lowering its nonenzymatic glycation.

Erythrocyte SOD activity was restored to a very significant extent by nishamalaki treatment which was comparable to both troglitazone and glyburide. This is consistent with the earlier report[37] of treatment with powdered rhizome of CL (300 mg/kg for 8 weeks) resulting in increased activities of SOD and catalase in erythrocytes of streptozotocin-diabetic rats. In a similar report, treatment with a polyherbomineral formulation Gly-13-C which contains extracts of CL and EO with many others, resulted in increased hepatic SOD activity in STZ-diabetic rats.[47] Glycated hemoglobin levels in the nishamalaki treated diabetic animals in our study showed a significant

negative correlation with activity of SOD suggesting that improved glycemic control afforded by treatment with nishamalaki may have protected SOD from nonenzymatic glycation and preserved its activity.

CONCLUSION

In conclusion, we can state that treatment with nishamalaki preparation achieved significant glycemic control in streptozotocin diabetic rats comparable to glyburide and troglitazone. Nishamalaki provided better erythrocyte antioxidant protection than glyburide and compared favourably with troglitazone. This preparation of turmeric and amla which is affordable and easily available for everyday use may be suitable as an adjunct in the management of diabetes mellitus. Further studies may elucidate the benefits of combining this preparation with oral hypoglycemic drugs in diabetic patients.

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Corresponding Author's Biography: Dr. Guruprasad Rao

Graduated with an M.Sc in Biochemistry from Kasturba Medical College, Manipal University, Manipal, India and a PhD from Manipal University for the thesis 'Status of oxidative damage and antioxidant profile in diabetes mellitus: effect of antidiabetic treatment'. Serving as faculty member in Melaka Manipal Medical College, Manipal, India for over 17 years. Conducted post-doctoral research on the molecular basis of cataractogenesis in University of Missouri, Columbia, Missouri, USA for two years.