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Antidiabetic and Antioxidant Efficacy Of a Powdered Mixture Of Curcuma Longa and Emblica Officinalis In Diabetic Rats In Comparison With Glyburide

Corresponding Author:
Dr. Guruprasad Rao,
Associate Professor of Biochemistry, Melaka Manipal Medical College, 576104 - India

Submitting Author:
Dr. Guruprasad Rao,
Associate Professor of Biochemistry, Melaka Manipal Medical College, 576104 - India

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Author(s): Rao G , Bhat S, Rao GS, Bhat GP

Abstract

Introduction and objective: Powdered rhizome of Curcuma longa (turmeric) and the dried fruits of Emblica officinalis (Indian gooseberry) are used as an adjunct in diabetes treatment in traditional Indian medicine. The 1:1 (w/w) mixture of these two known as nishamalaki was evaluated for its effect on glycemic control and erythrocyte parameters of oxidative stress in rats with streptozotocin-induced diabetes mellitus in comparison with the sulfonylurea drug, Glyburide.

Methods: One group of streptozotocin-induced diabetic rats was treated with nishamalaki powder (0.9 g/kg, NT group, n=12) and another group was treated with glyburide (4 mg/kg, GT group, n=12) respectively 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.

Results: Nishamalaki treatment achieved significant lowering of plasma glucose and glycated hemoglobin in diabetic rats ($p<0.001$ NT vs DC) comparable to that of the glyburide treated group. Erythrocyte membrane lipid peroxidation was lowered to a comparable extent by the both drugs ($p<0.001$ vs DC). Nishamalaki treated rats showed greater improvement in erythrocyte reduced glutathione (GSH) level and glutathione peroxidase (GSH-Px) activity (both $p<0.001$ vs DC) than glyburide treated rats ($p>0.05$ vs DC for GSH and $p<0.05$ vs DC for GSH-Px).

Conclusion: Antidiabetic efficacy of nishamalaki in diabetic rats is comparable to glyburide and it offers better antioxidant protection than glyburide.

Introduction

Oxidative stress is known to occur in uncontrolled diabetes mellitus. Chronic hyperglycemia has been reported to result in oxidative stress through various mechanisms including glucose autoxidation, glycoxidation of proteins, increased rate of polyol pathway and advanced glycation end products among others (Baynes JW & Thorpe SR, 1999; Hunt JV & Wolff SP, 1991). Hyperglycemia is considered to be responsible for the establishment of micro and macrovascular complications observed in diabetes. Numerous authors have suggested that excess generation of highly reactive oxygen and nitrogen species is a key component in the development of complications caused by hyperglycemia. Overproduction and/or insufficient removal of these reactive species result in vascular dysfunction, damage to cellular proteins, membrane lipids and nucleic acids leading to chronic complications. (de M Bandeira S et al, 2013). 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) and glutathione peroxidase also serves as a marker of oxidative stress.

Since ancient times, many herbal remedies, individually or in combination have been used to treat diabetes mellitus in traditional Indian medicine. There is a considerable amount of data from both animal and human trials suggesting efficacy of Ayurvedic interventions in managing diabetes (Elder C, 2004). India has about 45000 plant species and among them, several thousands have been claimed to possess medicinal properties. Research conducted in last few decades has shown that plants mentioned in ancient literature or used traditionally for diabetes have anti-diabetic properties (Grover JK et al, 2002). Since diet forms the mainstay in the management of diabetes mellitus, there is scope for exploiting the antidiabetic potency of vegetables and fruits, especially those which possess both hypoglycemic and antioxidant properties.

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 (Illustration 1). Turmeric has been used as an ethnomedicine from time immemorial in Ayurvedic medicine. 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 (Luthra PM et al, 2001).

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 (Illustration 2) is highly nutritious and is an important dietary source of Vitamin C, minerals and amino acids. (Katiyar CK et al, 1997). Extracts from amla fruits have been evaluated for anticancer, antioxidant, cytoprotective properties among many others (Rajeshkumar NV et al, 2003, Bhattacharya SK et al, 2000, Sai Ram M et al, 2002).

In Ayurvedic practice, different herbal preparations are used in combination to treat diabetes to maximise the beneficial effects and perhaps to lower the risk of adverse 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 (Shah Virendra Keshav, 1995). There are no studies reported with this preparation on erythrocyte antioxidant status in diabetes along with its hypoglycemic effect in comparison with standard drugs.

Objectives of the study: The objectives of the present study were 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 the sulfonylurea drug glyburide.

Methods

Animals: Adult male Wistar albino rats aged 3-4 months, weighing 150-300g were used for the studies. The animals were maintained under standard hygienic conditions. The animals were food and water ad libitum and were 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.

Preparation of nishamalaki powder: Rhizomes of Curcuma longa and fruits of Emblica officinalis (without seeds) were procured locally and after drying in the shade, they were 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 (Ghosh MN, 1984).

Induction of diabetes in rats: Diabetes mellitus was induced in rats with a single subcutaneous injection of streptozotocin (40 mg/kg) following a 24 hr fast. 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. 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

1. Plasma glucose by glucose oxidase-peroxidase

method (Trinder P, 1969)
2. Glycated hemoglobin by affinity chromatography (Klenk DC et al, 1982)

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

1. Malondialdehyde (MDA), a product of membrane lipid peroxidation measured as thiobarbituric acid reactive substances (TBARS) (Jain SK et al, 1990)
2. Reduced glutathione (GSH) by reaction with 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) (Beutler E et al, 1963)
3. Glutathione peroxidase (GSH-Px) assayed using the method of Paglia DE & Valentine WN, 1967.

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 **(Illustration 3)** comparable to that achieved by treatment with glyburide ($p<0.001$ vs DC both groups). DC rats showed significantly high levels of glycated hemoglobin than NC rats ($p<0.001$). Nishamalaki treatment also brought about significant lowering of glycated hemoglobin **(Illustration 4)** which reflects long term glycemic control and in this regard it was comparable to glyburide ($p<0.001$ vs DC both groups). DC rats showed significantly high levels of erythrocyte TBARS 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 glyburide treatment (**Illustration 5**, $p<0.001$ vs DC for both groups). Glycated hemoglobin levels in the nishamalaki treated diabetic rats showed a significant positive correlation with TBARS levels ($r = 0.6$, $p<0.05$). DC rats showed significantly decreased levels of erythrocyte GSH than NC rats ($p<0.001$). Nishamalaki treated rats showed greater improvement in GSH level (**Illustration 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 ($p<0.01$, **Illustration 6**). When the activity of GSH-Px in erythrocytes was assayed, we observed that DC rats showed significantly decreased activity of the enzyme than NC rats ($p<0.001$). However, treatment with nishamalaki increased the enzyme activity significantly ($p<0.001$ vs DC, **Illustration 7**) 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 also showed a significant negative correlation with activities of GSH-Px ($r = -0.82$, $p<0.01$).

Discussion

The erythrocytes are highly specialized cells that possess higher activities of antioxidant enzymes compared to other cell types. Circulating red cells are mobile free radical scavengers and provide antioxidant protection to other tissues and organs. It has been suggested that blood can be studied as an indicator of overall body oxidative status (Giustarini D et al, 2003).

In our studies, streptozotocin-induced untreated diabetic rats (DC group) sustained a marked degree of fasting hyperglycemia and elevated glycated hemoglobin levels at the end of one month after induction of diabetes. In DC rats, there were significant decreases in GSH levels and activity of the antioxidant enzyme GSH-Px 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 peroxidative damage which was manifest as a significant increase in the levels of TBARS. Erythrocytes are particularly at a risk of undergoing free radical damage because of their continuous challenge with high oxygen tension and previous studies have shown increased erythrocyte membrane lipid peroxidation and decreased level of GSH and GSH-Px activity in diabetic patients (Sailaja YR et al, 2003).

The use of combined herbal preparations to treat diabetes is a common practice in Ayurvedic system of medicine. It was reported that the total extracts of the dried powdered combination of fruits of Momordica charantia (MC) and Emblica officinalis and rhizomes of Curcuma longa in equal proportions, produced better blood glucose lowering action than MC extract alone in normal and streptozotocin-diabetic rats. It was suggested that the combination of these three plant extracts significantly potentiates the action of MC (Sankaranarayanan J & Jolly CI, 1993). The

Ayurvedic medicine “Rajanyamalakadi” containing Curcuma longa, Emblica officinalis and Salacia oblonga showed significant antidiabetic, hypolipidemic and antioxidant effects in type II diabetic patients over a period of 3 months (Faizal P et al, 2009). We therefore decided to test the antioxidant and antidiabetic efficiacy of nishamalaki powder which has equal proportions of powdered rhizomes of CL and dried fruits of EO in diabetic rats.

From our results it was apparent 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 the standard drug 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 (Nanda GC et al, 1998). A clinical study with Nishamalaki was carried out in diabetic patients and encouraging results were obtained (Gopakumar K et al, 1995). 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 (Singh AK et al, 1991). 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 (Yadav RK et al, 2001). Arun N and Nalini N (2002) administered dried powder of turmeric rhizomes at a dose of 1 g/kg to alloxan-diabetic rats for 21 days, which caused a decrease of about 30% in blood glucose value. Ali Hussain HEM (2002) reported that turmeric powder decreased fasting plasma glucose as well as plasma glucose levels during a glucose tolerance test in streptozotocin-diabetic rats. Flavonoids from EO exerted highly potent hypoglycemic and hypolipidemic actions in rats (Anila L & Vijayalakshmi NR, 2000). 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 (Sabu MC & Kuttan R, 2002). 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 (Tank R et al, 1989). The ingestion of 6 g CL increased postprandial serum insulin levels in healthy subjects (Wickenberg J et al, 2010). Turmeric reportedly potentiated action of insulin on glucose metabolism in rat epididymal fat cell assay (Khan A et al, 1990) 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 by Arun N and Nalini N (2002).

Nishamalaki treatment significantly decreased level of TBARS in erythrocytes of diabetic rats in our study, which was in fact slightly less than even that of normal control value. This effect was comparable to that of glyburide. Curcumin is known to be a potent inhibitor of lipid peroxidation and scavenger of superoxide radicals (Sreejayan N & Rao MNA, 1996), singlet oxygen (Das KC & Das CK, 2002) and nitric oxide (Sreejayan N & Rao MNA, 1997). Treatment with powdered rhizome of CL decreased lipid peroxidation in erythrocytes of streptozotocin-diabetic rats (Ali Hussain HEM, 2002). 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 (Arun N & Nalini N, 2002). The accumulation of lipid peroxidation products in diabetic serum was reduced significantly by curcumin (Sajithlal GB et al, 1998). It has been suggested that curcumin supplementation could improve diabetes-induced endothelial dysfunction significantly in relation to its potential to decrease superoxide production (Rungseesantivanon S et al, 2010). 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. 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 (Ghosal S et al, 1996). Methanolic extract of EO was found to inhibit lipid peroxide formation and to scavenge hydroxyl and superoxide radicals in vitro (Sabu MC & Kuttan R, 2002). Administration of active tannoids of EO to rats for 7 days decreased lipid peroxidation in the brain (Bhattacharya A et al, 1999). 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. 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 (Arun N & Nalini N, 2002). 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 (Illustration 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. 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 (Arun N & Nalini N, 2002). 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 (Arun N & Nalini N, 2002). 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 (Suryanarayana P et al, 2004). 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 (Suryanarayana P et al, 2007). 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.

Conclusion

In conclusion, we can state that nishamalaki preparation has antidiabetic efficacy comparable to glyburide, offers better antioxidant protection than glyburide and 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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Illustrations

Illustration 1

Rhizomes of Curcuma longa

Illustration 2

Fruits of Emblica officinalis

Illustration 3

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

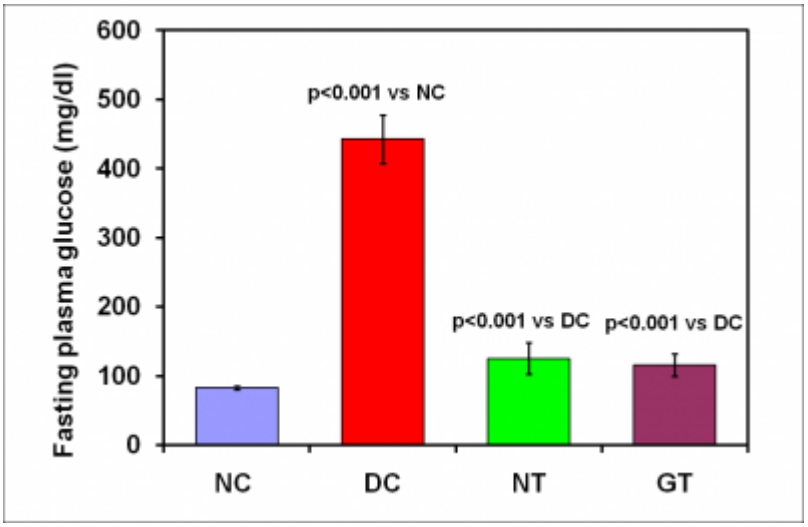


Illustration 4

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

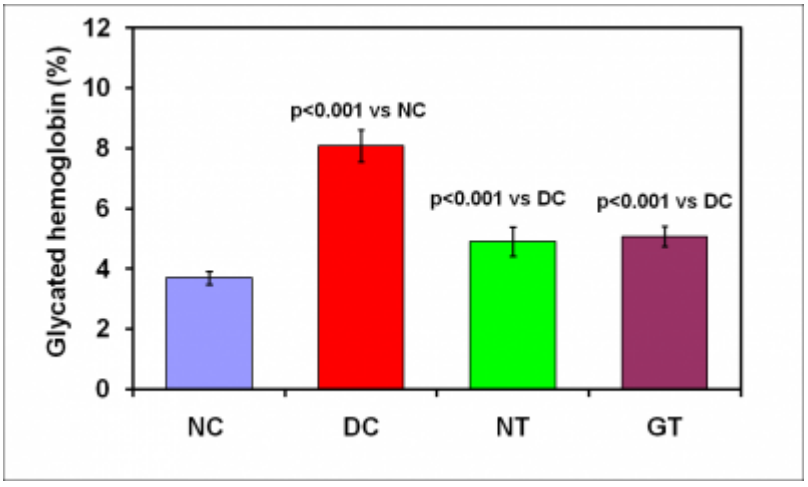


Illustration 5

Erythrocyte malondialdehyde levels measured as thiobarbituric acid reactive substances (TBARS, nmoles/g Hb) in the different study groups at the end of the 30 days treatment period. Values are mean ± SEM. NC: normal control (n=12), DC: untreated diabetic control (n=12), NT: nishamalaki treated (n=12), GT: glyburide treated (n=12).

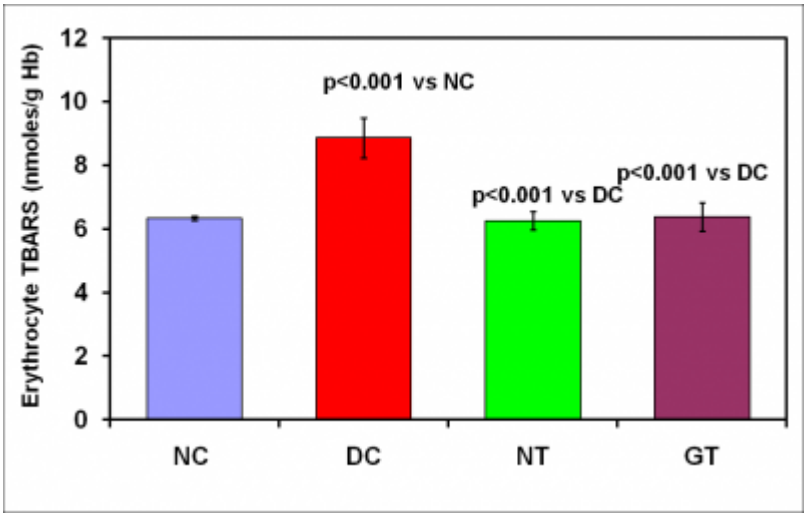


Illustration 6

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

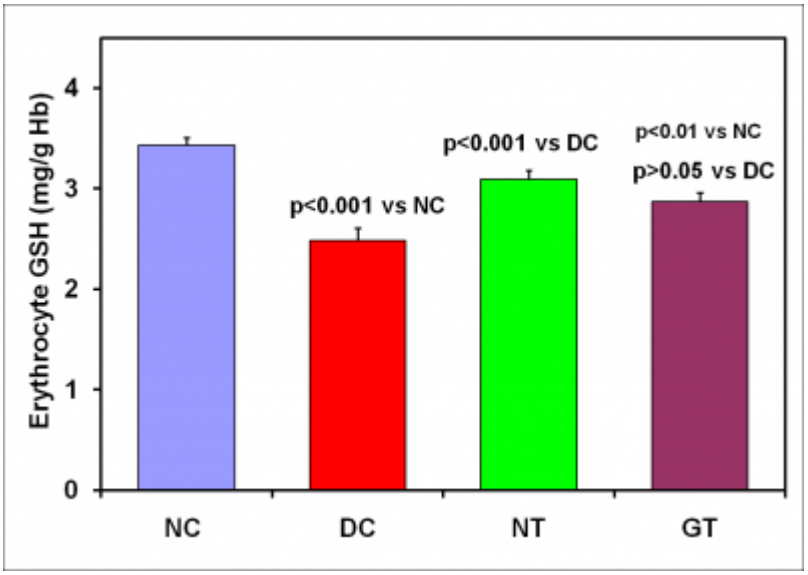
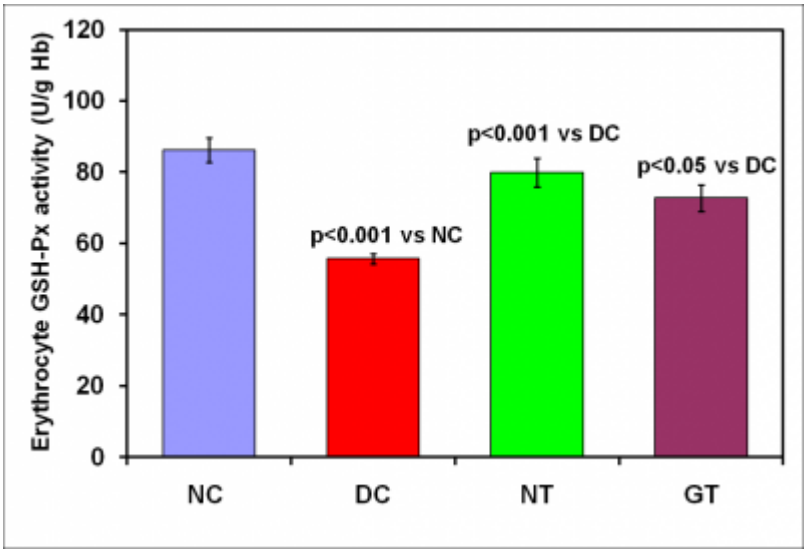


Illustration 7

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



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