

Role of gymnemic acid in ameliorating altered liver metabolism in streptozotocin induced diabetic ratsLakshmi S.¹, Ancy A.¹, Anju T.R.^{1,2*}¹Molecular Neurobiology and Cell Biology Unit, Centre for Neuroscience, Department of Biotechnology, Cochin University of Science and Technology, Cochin-682022 Kerala, India.²Department of Biotechnology, Newman College, Thodupuzha- 685585, Kerala, India.

Abstract: The liver plays a pivotal role in glucose homeostasis and any impairment in glucose metabolism can cause liver dysfunctions. Hence in managing conditions like diabetes mellitus, it is important to monitor hepatic signaling too. This gives momentum for phytotherapy for diabetes management due to its multifactorial effects. Gymnemic acid, the active constituent of *Gymnema sylvestre*, is a potent antihyperglycemic agent with effects on other organ functions too. The present study evaluated the therapeutic effect of gymnemic acid in managing the hepatic functional alterations in diabetes. Enzyme kinetics of malate dehydrogenase (MDH) and Real-Time PCR analysis of hepatocyte growth factor (HGF) and hypoxia-inducible factor 1 α (HIF1 α) mRNA expression were carried out in the liver of diabetic rats. We observed that liver MDH activity was differentially altered in cytosolic and mitochondrial extracts of diabetic rats. Treatment with gymnemic acid helped in reducing the MDH activity in the cytosolic fraction and increasing the activity in the mitochondrial fraction. In addition, the down-regulated HGF and HIF1 α expression in diabetic conditions were reversed by treatment with gymnemic acid. The evident hepatoprotective action of gymnemic acid in diabetes condition can be exploited for future therapeutic interventions for diabetes.

Key words: Diabetes mellitus; Gymnemic acid; Malate Dehydrogenase; Hepatocyte Growth Factor; Hypoxia inducible factor

Introduction

Diabetes mellitus, a metabolic disorder characterized by hyperglycemia, arises as a consequence of insulin secretion deficiency or insulin resistance. Hyperglycemia is associated with alteration of glucose and lipid metabolism in liver. Impairment of glucose homeostasis can even lead to liver disease in diabetes. This includes abnormal liver enzymes, nonalcoholic fatty liver disease, cirrhosis, hepatocellular carcinoma and acute liver failure (Tolman *et al.*, 2007). Complications due to therapeutic treatment of diabetes triggered the need for development of novel antidiabetic agents (Maritim *et al.*, 2003).

Phyto products from various species represent sustainable starting material for the preparation of new bioactive substances. Phyto-therapy in diabetes management has been applied since time immemorial. *Gymnema sylvestre* is a medicinal plant that is used for diabetics (Alarcon-Aguilaria *et al.*, 1998) and the usage has gained further importance as leaves of the plant contain certain active components which help in regeneration of islets of

Langerhans (Shanmugasundaram *et al.*, 1990; Baskaran *et al.*, 1990). The extract also has hypolipidaemic effect both in non-diabetic and high fat diet fed rats (Wang *et al.*, 1998). Though there is scientific and medicinal evaluation of plant to support its antidiabetic effect, information on the effect of the plant on the liver is lacking.

Net glucose uptake and its metabolism in hepatocytes depend on intracellular metabolic status, which is determined by metabolic enzymes and transcription factors. Malate dehydrogenase (MDH) is an enzyme directly involved in glucose metabolism. Since MDH is an important enzyme in glucose metabolism, variation in its quantitative and qualitative nature contributes to the pathological status of diabetes (Seema *et al.*, 1996). Hepatocyte growth factor (HGF) is potent mitogen for hepatocytes (Bertola *et al.*, 2007). In humans, circulating HGF positively correlates with insulin and glucose (Hiratsuka *et al.*, 2005), hence can be used to study alterations in diabetic condition. Increasing evidence in both experimental and

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clinical studies suggests that oxidative stress plays a major role in pathogenesis of diabetes (Brownlee 2001; Rosen *et al.*, 2001; Bonnefont-Rousselot 2002; Ceriello 2003). Free radicals are formed disproportionately in diabetes by glucose oxidation, non-enzymatic glycation of proteins and subsequent oxidative degradation of glycated proteins. Hypoxia inducible factor (HIF-1) dysfunction is the end result of reactive oxygen species-induced modification of its coactivator p300 by glycolytic metabolite methylglyoxal (Thangharajah *et al.*, 2010).

The present study evaluated the alterations in liver metabolism by studying MDH activity in mitochondrial and cytoplasmic fractions and expression of HGF and HIF 1 α mRNA in diabetic condition. The effect of insulin and the herbal extract of *Gymnema sylvestre* were analyzed to investigate the beneficial effect of gymnemic acid on impairment of free radical scavenging system, carbohydrate uptake and metabolism in liver of streptozotocin (STZ) induced diabetic rats. Our present study on the effect of gymnemic acid in regulating the altered liver function under diabetic condition will help in exploring the novel therapeutic possibilities of gymnemic acid for diabetes treatment.

Materials and Methods

Biochemicals used in the present study; gymnemic acid was purchased from Synthite Industries Ltd., Kerala, India and streptozotocin from Sigma Chemical Co., USA. All other reagents were of analytical grade purchased locally. Tri-reagent kit was purchased from Sigma Chemical Co., USA. ABI PRISM High Capacity cDNA Archive kit, Primers and TaqMan probes for Real-Time PCR analysis were purchased from Applied Biosystems, Foster City, CA, USA.

Animals

Male Wistar adult rats were used for all experiments. They were housed in separate cages under 12 hours light and 12 hours dark periods and were maintained on standard food pellets and water *ad libitum*. Male Wistar rats, weighing 250 to 300g body weight were housed for 1 to 2 weeks before experiments were performed. All animal care and procedures were in accordance with Institutional and National Institute of Health guidelines and Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines. All efforts were made to minimize the number of animals used and their suffering.

Diabetes induction

Diabetes was induced in rats by a single intrafemoral injection of STZ, which was freshly dissolved in 0.1 molar-citrate buffer (pH 4.5) under anaesthesia (Junod *et al.*, 1969). Streptozotocin was given at a dose of 55mg/Kg body weight (Hohenegger & Rudas, 1971; Arison *et al.*, 1967). Control rats were injected with citrate buffer alone. The diabetic state of animals was assessed by measuring blood glucose concentrations 72 hours after streptozotocin treatment. The rats with a blood sugar level above 250 mg/dl were selected as diabetic rats.

Determination of anti-diabetic potential of gymnemic Acid.

Animals used in this study were randomly divided into the following groups. Each group consisted of 6-8 animals.

- a. Group 1: Control
- b. Group 2: Diabetic
- c. Group 3: Diabetic rats treated with insulin
- d. Group 4: Diabetic rats treated with Gymnemic acid (200mg/kg body weight)

The insulin treated diabetic group (Group 3) received subcutaneous injections (45.5mg/kg body weight IU) of Lente and Plain insulin daily during the entire period of the experiment (Sasaki & Bunag, 1983). The last injection was given 24 hr before sacrificing the diabetic rats. The animals were then sacrificed on 21st day by cervical dislocation. The liver was dissected out quickly over ice and the tissue samples and plasma were kept at -80° C until assay. Blood glucose was estimated using Glucometer.

MDH Assay

Liver mitochondria were isolated by the modified method of Johnson and Lardy, 1947. Subcellular fractions of liver were separated by differential centrifugation (Prokhorova, 1951). Mitochondrial, nuclear and cytosolic fractions were separated and purified in several stages. Total protein concentration was estimated by the method of Lowry *et al.*, 1951 using bovine serum albumin as standard. MDH was assayed according to Mehler *et al.*, 1948 in cytoplasmic and mitochondrial fraction and the kinetic parameters were measured. The reaction mixture contained phosphate buffer (pH 7.4), NADH, oxaloacetate and tissue extract. The reaction mixture of 1ml was assayed at 340nm in a spectrophotometer by measuring the decrease in optical density due to the oxidation of NADH measured at 15s intervals for 1 min at room temperature. A unit of enzyme activity is equal to the change in the optical density of 0.1 for 100s at

334 nm. Kinetic parameters such as V_{max} and K_m were calculated from the data of MDH activity measured at substrate concentrations of 0.04–0.15 mM.

Gene expression studies in liver of control and experimental rats

RNA was isolated from the liver of the experimental rats using the Tri-reagent (Sigma Chemical Co., USA) according to the procedure of Chomczynski & Sacchi. Total complementary DNA synthesis was performed using the ABI PRISM complementary DNA archive kit in 0.2ml microfuge tubes. The reaction mixture of 20 ml contained 0.2 mg total RNA, 10 X RT buffer, 25 X dNTP mixture, 10 X random primers, MultiScribe RT (50 U/ml) and RNase-free water. The complementary DNA synthesis reactions were carried out at 25°C for 10 min and 37°C for 2 h using an Eppendorf Personal Cycler. Real-time PCR assays were performed in ninety-six-well plates in an ABI 7300 Real-time-PCR instrument (Applied Biosystems). The specific primers of HGF and HIF1 α , were purchased from Applied Biosystems. The TaqMan reaction mixture of 20 ml contained 25 ng of total RNA-derived complementary DNA, 200nm each of the forward primer, reverse primer and TaqMan probe for assay on demand and endogenous control β -actin and 12.5 ml of Taqman 2X Universal PCR Master Mix (Applied Biosystems), and the volume was made up with RNase-free water. The following thermal cycling profile was used (forty cycles): 50°C for 2 min, 95°C for 10 min, 95°C for 15s and 60°C for 1 min. Fluorescence signals measured during amplification were considered positive if fluorescence intensity was 20-fold greater than the standard deviation of baseline fluorescence. The

$\Delta\Delta CT$ method of relative quantification was used to determine the fold change in expression. This was done by normalizing the resulting threshold cycle (CT) values of the target mRNA to the CT values of the internal control β -actin in the same ($\Delta CT = CT_{Target} - CT_{\beta\text{-actin}}$). It was further normalized with the control ($\Delta\Delta CT = \Delta CT - CT_{Control}$). The fold change in expression was then obtained as $2^{-\Delta\Delta CT}$ and the graph was plotted using $\log 2^{-\Delta\Delta CT}$.

Statistics

Statistical evaluations were done by ANOVA using InStat (Ver.2.04a) computer programme. Graphs were made using SIGMA PLOT (Ver 2.03), Sigma Plot software (version 2.0, Jandel GmbH, Erkrath, Germany). Relative Quantification Software was used for analyzing Real-Time PCR results.

Results

Body weight of control and experimental rats

The body weight was significantly decreased ($p < 0.001$) in the diabetic rats when compared to control. After insulin treatment and Gymnemic acid (GA) supplementation for 20 days, the body weight was significantly reversed ($p < 0.001$) when compared with diabetic rats. (Table 1)

Blood glucose

Blood glucose level of all rats before streptozotocin administration was within the normal range. Streptozotocin administration in rats led to a significant increase ($p < 0.001$) in blood glucose level when compared to control group. Insulin and GA treatments significantly reversed ($p < 0.001$) the increased blood glucose level when compared to diabetic group. (Table 2)

Table 1. Body weight (gm) of experimental rats

Experimental groups	0 day	7 th day	14 th day	21 th Day
Control	220	223.3 \pm 1.7	228.3 \pm 1.7	233.3 \pm 1.7
Diabetic	233.3 \pm 6.7	226.7 \pm 8.3	221.7 \pm 8.3	216.7 \pm 8.3
D+I	231.6 \pm 6	230 \pm 5	223.3 \pm 4.4	228.3 \pm 4.4
D+G	230 \pm 5.8	226 \pm 6	225 \pm 7.6	228.3 \pm 4.4

Values are mean of \pm S.E.M of 3-4 separate experiment. Each group consists of 3-4 rats

Table 2: Blood glucose levels of experimental rats

Experimental groups	0 day (before STZ injection)	3 rd Day initial	10 th day	14 th day	21 th day
Control	91.3 \pm 2.6	93 \pm 2.9	96.3 \pm 2.6	98.3 \pm 2.4	103 \pm 1.15
Diabetic	87.4 \pm 2.3	266.6 \pm 4.1	320.3 \pm 7.9	306.3 \pm 7.5	281.3 \pm 4.2
D+I	89.1 \pm 0.8	255 \pm 3.6	271 \pm 1.7	218.6 \pm 2.2	102.3 \pm 3.5
D+G	91.6 \pm 2.9	250 \pm 1.7	285.3 \pm 2.6	233.6 \pm 1.76	128 \pm 1.2

Values are mean of \pm S.E.M of 3-4 separate experiment. Each group consists of 3-4 rats

Enzyme kinetics of liver malate dehydrogenase in the cytoplasmic fraction of experimental groups

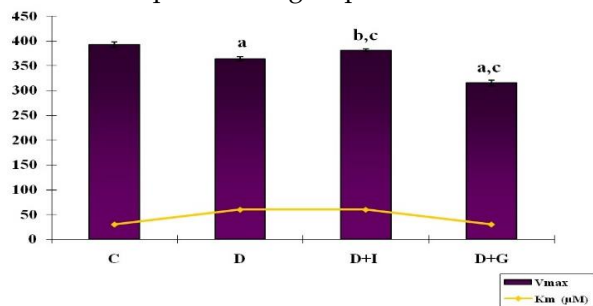
V_{max} of cytoplasmic MDH in the liver was significantly decreased ($p < 0.001$) in diabetic rats. In insulin treated diabetic rats V_{max} significantly increased ($p < 0.001$) compared to diabetic rats. GA treatment showed a significant decrease in V_{max} of cytoplasmic MDH compared to diabetic rats. (Table- 3 Figure- 1a, Figure- 1b). K_m of cytoplasmic MDH in the liver was significantly increased ($p < 0.001$) in diabetic rats and it was significantly reversed ($p < 0.001$) to near control in GA treated diabetic rats. K_m did not show any change in insulin treated diabetic rats when compared with the diabetic rats, (Table- 3 Figure- 1a and 1b).

Table 3: Liver MDH activity in the cytoplasmic fraction of experimental groups

Experimental Groups	V_{max} (U/mg protein)	K_m (mM)
C	392.80 ± 2.5	0.03 ± 0.001
D	363.75 ± 2.0 ^a	0.06 ± 0.005 ^a
D + I	381.40 ± 2.5 ^{b,c}	0.06 ± 0.003 ^a
D+ G	315.15 ± 1.5 ^{a,c}	0.03 ± 0.001 ^c

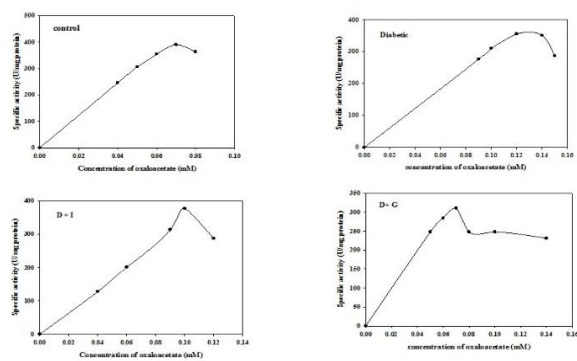
Values are mean ± S.E.M of 3-4 separate experiments. Each group consists of 3-4 rats. ^a $p < 0.001$ ^b $p < 0.05$ when compared to control; ^c $p < 0.001$ when compared to diabetic group.

Figure 1a: Liver MDH activity in cytoplasmic fraction in experimental group



Values are mean ± S.E.M of 3-4 separate experiments. Each group consists of 3-4 rats. ^a $p < 0.001$ ^b $p < 0.05$ when compared to control; ^c $p < 0.001$ when compared to diabetic group.

Figure 1b: Representative graph of liver MDH activity in the cytoplasmic fraction in experimental group



Values are mean ± S.E.M of 3-4 separate experiments. Each group consists of 3-4 rats.

Enzyme kinetics of liver malate dehydrogenase in the mitochondrial fraction of experimental groups

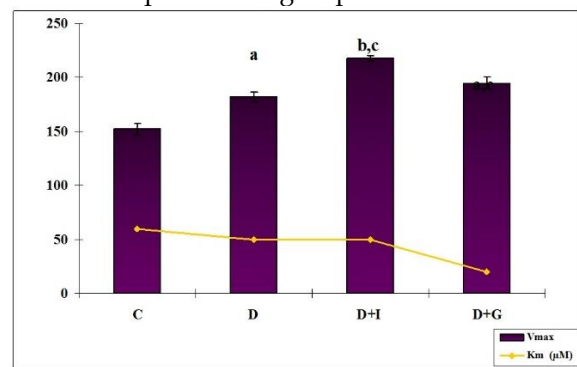
V_{max} of mitochondrial MDH in the liver was significantly increased ($p < 0.001$) in diabetic rats and it was also found to increase ($p < 0.001$) in case of insulin treated diabetic rats but there was a significant reversal ($p < 0.001$) to near control in GA treated diabetic rats, (Table- 4 Figure- 2a and 2b). K_m of mitochondrial MDH in the liver showed little variation in diabetic and insulin treated diabetic rats. A significant change was seen in gymnemic acid treated group, (Table- 4, Figure- 2a and 2b).

Table 4: Liver MDH activity in the mitochondrial fraction of experimental groups

Experimental Groups	V_{max} (U/mg protein)	K_m (mM)
C	152.05 ± 1.0	0.06 ± 0.005
D	181.95 ± 1.5 ^a	0.05 ± 0.002
D + I	217.75 ± 2.0 ^{a, c}	0.05 ± 0.001
D+ G	194.95 ± 3.0 ^{a, c}	0.02 ± 0.001

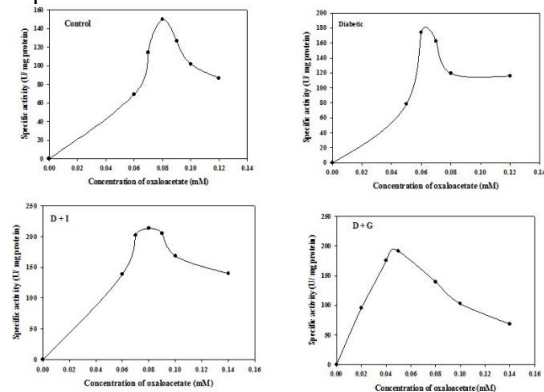
Values are mean ± S.E.M of 3-4 separate experiments. Each group consists of 3-4 rats. ^a $p < 0.001$ ^b $p < 0.05$ when compared to control; ^c $p < 0.001$ when compared to diabetic group.

Figure 2a: Liver MDH activity in mitochondrial fraction of experimental groups



Values are mean ± S.E.M of 3-4 separate experiments. Each group consists of 3-4 rats. ^a $p < 0.001$ ^b $p < 0.05$ when compared to control; ^c $p < 0.001$ when compared to diabetic group.

Figure 2b: Representative graph of liver MDH activity in mitochondrial fraction of experimental groups



Values are mean ± S.E.M of 3-4 separate experiments. Each group consists of 3-4 rats.

Real Time PCR analysis of hepatocyte growth factor (HGF) and hypoxia inducible factor (HIF-1 α) mRNA in the liver of control and experimental rats.

Real-time PCR analysis showed that the HGF mRNA in the liver was significantly downregulated ($p < 0.001$) in diabetic rats when compared to control and it was significantly reversed ($p < 0.001$) to near control in GA and insulin treated diabetic rats. (Table 5, Figure- 3). Real-time PCR analysis showed that the HIF- 1 α mRNA in the liver was significantly downregulated ($p < 0.001$) in diabetic rats when compared to control and it was significantly reversed ($p < 0.001$) to near control in GA treated diabetic rats. An upregulation was observed in case of insulin treated diabetic rats. (Table- 5, Figure- 4).

Table 5: Real Time PCR analysis of HGF and HIF-1 α mRNA expression in the liver of experimental groups

Experimental Groups	Log RQ (HGF)	Log RQ (HIF- 1 α)
C	0	0
D	-1.79 \pm 0.11 ^b	0.77 \pm 0.15 ^a
D+ I	-4.15 \pm 0.46 ^a	0.53 \pm 0.14 ^b
D+ G	-4.58 \pm 0.57 ^a	1.10 \pm 0.12 ^a

Values are mean \pm S.E.M of 3-4 separate experiments. Each group consists of 3-4 rats.

^a $p < 0.001$ ^b $p < 0.01$ when compared to control

Figure 3: Real time PCR analysis of mRNA in the liver of experimental groups

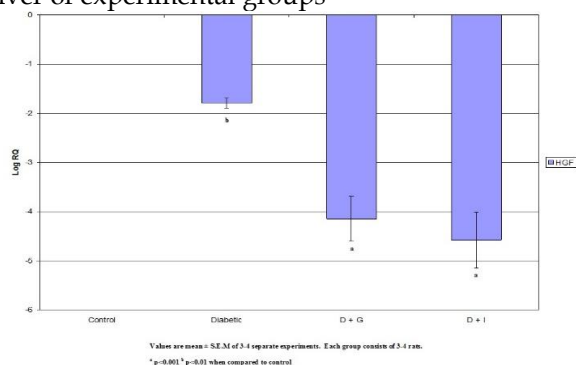
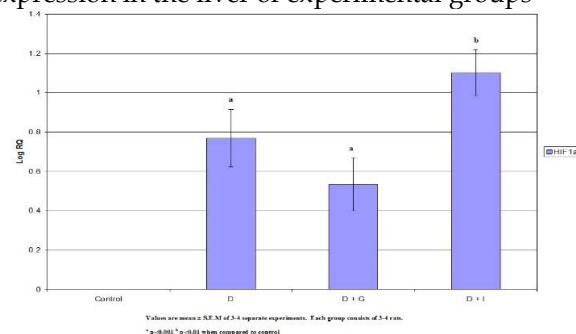


Figure 4: Real time PCR analysis of mRNA expression in the liver of experimental groups



Discussion

Diabetes is a hyperglycemic disorder that affects the liver, brain, kidney, heart and other organs. Prolonged exposure to chronic hyperglycemia in diabetes can lead to many severe disorders. Poorly controlled diabetes results in structural and functional changes in the liver. As liver plays a major role in the carbohydrate metabolism, diabetes results in severe alterations in hepatic functioning. The present study investigated the effects of Gymnemic Acid treatment on diabetes-associated liver metabolic complications and the possible mechanisms involved. STZ-induced diabetic rats have proved to be useful in trying to determine the underlying cause of liver complications during diabetes (Thomas, 1999). In accordance with the previous reports, in the present study, STZ-induced diabetes was characterized by hypoinsulinaemia, hyperglycemia and decreased body weight (Kumar *et al.*, 2011; Junod *et al.*, 1969) mediated through oxidative stress (Matkovics *et al.*, 1997; Kavalali *et al.*, 2003) and selective destruction of beta cells. Treatment with Gymnemic Acid significantly improved hyperglycemia and prevented body-weight loss, which indicated amelioration in diabetic complications.

Shigematsu *et al.*, 2001 reported that the *Gymnema sylvestre* suppresses the glucose absorption in the small intestine of rats, reduces plasma glucose increment in the oral sucrose tolerance test, lowers blood glucose level and alleviates diabetic symptoms in type-2 diabetes. However, Kanetker *et al.*, 2007 reported that *Gymnema sylvestre* exerts its hypoglycemic effects through increasing secretion of insulin, promoting regeneration of islets cells, increasing utilization of glucose through increased activities of enzymes responsible for utilization of glucose by insulin dependent pathways and by inhibition of glucose absorption from intestine. The present study also supports the foresaid mechanisms.

There was an increase in blood glucose level in diabetic rats when compared to control group. The increased blood glucose level was due to the decreased circulating insulin level, a result of marked destruction of insulin secreting pancreatic islet β -cells by STZ (Junod *et al.*, 1969). Treatment using insulin and GA showed restorative effect on blood glucose level by increasing the insulin level in the serum. Previous reports showed that gymnemic acid isolated from the leaves of *G. sylvestre* have antihyperglycemic, glucose uptake inhibitory and gut glycosidase inhibitory activity

(Kimura, 2006). Gymnemic acid of leaf and callus extracts of *G. sylvestre* significantly increases the regeneration of β -cells in treated rats compared to the control rats.

Diabetic rats showed a significant decrease in body weight when compared with control and are in agreement with the previous reports (Junod *et al.*, 1969; Willsky *et al.*, 2011). The decreased body weight in the diabetic rats was a result of excessive breakdown of tissue proteins (Salahuddin *et al.*, 2010; Poongothai *et al.*, 2011). Treatment of diabetic rats with insulin and GA improved body weight significantly when compared with the control group which indicates prevention of muscle tissue damage due to hyperglycemic condition.

Streptozotocin induced diabetes proved to be accompanied by a significant reduction in the activity of cytosolic MDH with a slight increase in mitochondrial MDH activity. In the diabetic rats, fasting plasma glucose concentrations were greater than control levels despite insulin injections and the activities of malate dehydrogenase, which plays a crucial role in the malate-aspartate shuttle, were decreased remarkably in the cytosolic fraction. The present study on liver tissue observed a decreased cytosolic MDH with a slight increase in mitochondrial MDH activity. The reduced cytosolic MDH activity can disrupt the normal malate aspartate shuttle thereby reducing the mitochondrial transport of NADH. The slight increase in mitochondrial MDH activity may be a response of the hepatocytes to the increased energy demand. This can only be considered as a short-term adaptive reaction by means of which the organism and its organs maintain homeostasis, for it is the common feature of all compensatory adaptive reactions to be a combination of its physiological functions. This reflects a depression of energy metabolism in hepatocytes of the diabetic rats. In insulin treated diabetic group, the cytoplasmic MDH activity was reversed to near control level which triggered a high mitochondrial MDH activity. This accounts for a compensatory adaptation of the body to balance the glucose homeostasis and meeting the ATP demand. In the present study, we observed a reduced MDH activity in the cytosolic fraction and an increased activity in the mitochondrial fraction of diabetic rats treated with gymnemic acid. *G. sylvestre* leaf extracts have been shown to reduce hyperglycemia and change enzyme activities in experimentally induced diabetic animals (Shanmugasundaram *et al.*, 1983), and these glucose-decreasing effects being mediated by increase in insulin secretion

(Sugihara *et al.*, 2000). Acyl moieties of GA were reported to have interactions with Glycerol-3-phosphate dehydrogenase, the key enzyme involved in hepatic metabolism of glycerol, there by inhibiting its action (Ishijima *et al.*, 2008). Similarly, in the present study also the acyl moieties of GA might have interacted with the cytosolic MDH thereby reducing its activity and suggest the possibility that GA may have some physiological effects on glucose, glycerol, and lipid metabolism via interaction with MDH. The dramatic increase in mitochondrial MDH activity irrespective of the reduced cytoplasmic MDH activity in GA treated diabetic rats might have resulted in active ATP production through TCA cycle. This can help in overcoming the oxidative stress induced by diabetes. But the underlying mechanism behind this ironical observation is not fully known. Pharmacological and clinical studies suggest that the hypoglycemic activity of *Gymnema* may be mediated through stimulation of insulin release (possibly by pancreatic regeneration or repair), stimulation of enzymes responsible for glucose uptake and utilization and/or inhibition of intestinal absorption of glucose (Shanmugasundaram, 1990; Baskaran, 1990).

The observed increase in mitochondrial MDH activity can be partly due to mitochondrial permeability transition (MPT), a reversible phenomenon whereby the mitochondrial inner membrane becomes freely permeable. The abnormal permeability can induce the disappearance of transmembrane potential and the release of various factors. Mitochondrial swelling mainly results from insufficient cell energy in conditions like hypoxia and factors such as bacteriotoxin, viral infection, ionizing radiation, osmotic pressure injury and alimentary deficiency (Bernardi *et al.*, 1999). Slight swelling in physiological condition manifests an increase in mitochondrial functional compensation (Green & Reed, 1998). The increase of mitochondrial membrane permeability increased the mitochondrial Ca^{2+} content and induced MDH function.

In the present study, the expression of both hepatocyte growth factor and hypoxia inducible factor-1 mRNA in the liver of diabetic group was significantly down regulated compared to control, which points to the possibility of high oxidative stress and related tissue damage. Hepatocyte growth factor (HGF), a mesenchyme-derived, multifunctional protein that plays a critical role in cell survival, proliferation, migration, and

differentiation. In the liver, HGF acts in a paracrine fashion on hepatocytes, which express the HGF receptor (Met) but do not express this ligand. Mechanistic and biochemical studies revealed that interaction of Met with insulin receptor (INSR) leads to activation of INSR by Met via formation of a Met-INSR hybrid which subsequently recruits and activates transcription factors involved in the regulation of blood glucose. Thus, signal output by INSR alone in the absence of Met is relatively low (Prat *et al.*, 1991). The down regulation of HGF expression observed in our study can contribute to decreased insulin signalling and hence defective glucose homeostasis. This in turn can contribute to reduction in the energy status of the liver; caused by decreased ability of hepatocytes to utilize glucose and decreased the rate of oxidative phosphorylation in hepatocytic mitochondria. Hypoxia-inducible factor-1 α has central role in degeneration and a transcriptional activator that promotes angiogenesis (Brahimi-Horn & Pouyssegur, 2007). HIF-1 α expression regulates expression of several genes, including, VEGF (Semenza, 2003) and is also induced under normoxic conditions when cells are stimulated with growth factors, inflammatory cytokines, lactate, or prostaglandins (Hunt *et al.*, 2007). HIF activity can be modulated by a number of factors such as hydrogen peroxide and superoxide (Pouyssegur and Mechta-Grigoriou, 2006). Diabetes can cause increased production of ROS (Oberley, 1988; Fridlyand and Philipson, 2005) and disturb antioxidant system and oxidative damage of membrane (Baquer *et al.*, 1998). HIF-1 α gene was downregulated in the study which is attributed to the free radicals generated. Thus, the alterations observed in the expression of transcription factors can contribute to reduced energy utilization and a state of oxidative stress in the liver of diabetic rats, which can act as a trigger for the development of diabetic complications. But these effects were seen to be reversed in the insulin and GA treated rats, i.e. there was an upregulation in the gene expression of HGF and HIF-1 α . This may be due to the partial hepatoprotective effect of the plant extract which could possibly be due to the presence of antioxidant principles.

Gymnemic acid was found to be partially hepatoprotective in accordance with the previous studies. This clearly indicated that it could partially overcome the effects of diabetes. It was, therefore, concluded that *Gymnema sylvestre* could be considered as an effective herbal alternative for the treatment of diabetes mellitus.

Diabetes associated complications result in morbidity and mortality. It is important to elucidate of the risk factors and pathophysiological mechanisms underlying diabetic complications. Deterioration in glucose homeostasis that results from diabetes induced hyperglycemia increases the predisposition to liver diseases. Gymnemic Acid has been shown to decrease the severity of the complications associated with diabetes and improve the blood glucose and insulin levels. The results of this study have demonstrated that the supplementation of gymnemic acid to STZ-induced diabetic rats has beneficial effects which are mediated through alterations in MDH activity and improvement of expression levels of transcription factors involved in glucose metabolism, hepatocyte growth factor and HIF-1 α in the liver. Our findings provide confirmatory evidence for the hepatoprotective role of gymnemic acid and pave way for the better management of diabetes.

Acknowledgement

The authors thank Cochin University of Science and Technology, Cochin for all infrastructural facilities for the research work. The authors acknowledge with gratitude the guidance and mentoring by Late Prof. C. S Paulose.

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Cite this article as:

Lakshmi S., Ancy A., Anju T.R. Role of gymnemic acid in ameliorating altered liver metabolism in streptozotocin induced diabetic rats. *International Journal of Bio-Pharma Research*, Volume 8, Issue 8 (2019) pp. 2749-2757.

 <http://dx.doi.org/10.21746/ijbpr.2019.8.8.1>

Source of support: Nil; **Conflict of interest:** Nil.