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Antioxidant Status of a Polyherbomineral Formulation (Gly-13-C) in STZ-Diabetic Rats

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Abstract: In the present investigation, antidiabetic and antioxidant activities of a polyherbomineral formulation, Gly-13-C was evaluated in streptozotocin-induced diabetic male Wistar rats and compared with that of glibenclamide (600 μg kg^{-1}), a reference drug. After induction of diabetes by a single intraperitoneal injection of streptozotocin (65 mg kg^{-1}), diabetic animals were treated with different doses of Gly-13-C (100, 200 mg kg^{-1} and 400 mg kg^{-1} b.wt.) for 21 days. Various biochemical parameters-fasting plasma glucose, albumin fructosamine levels, liver enzymatic antioxidants like superoxide dismutase, catalase, non-enzymatic antioxidants like vitamin C and E, lipid peroxidation in liver and glycogen biosynthesis in liver and skeletal muscle were measured. Study results showed that Gly-13-C 200 and 400 mg kg^{-1} b.wt. demonstrated better glycemic control and significantly protected liver tissue against oxidative damage than reference drug.

Key words: Streptozotocin-diabetic rats, antidiabetic, antioxidant status, albumin fructosamine assay, lipid peroxidation

INTRODUCTION

Multiple defects have been implicated in the pathophysiology of diabetes mellitus (Tiwari and Madhusudana-Rao, 2002). Several therapeutic agents with an ability to target a specific pathobiological pathway involved in causing diabetes have been designed to fight hyperglycemia. Sulfonylurea class of agents such as glipizide, glyburide, glimepiride produce their hypoglycemic action by squeezing existing pancreatic beta cells to release insulin (Insulin secretagouge). Biguanides such as metformin stimulate peripheral utilization of elevated blood glucose and thereby reduce insulin resistance. Glitazones such as troglitazone, pioglitazone, rosiglitazone primarily affect the genes involved in glucose and lipid metabolism through adipocytes differentiation by modulating Peroxisome Proliferator-Activated Receptor-gamma (PPARγ), a steroid superfamily nuclear receptors and increase glucose uptake in such tissues (Martha and John, 2001). Thus, these individual classes of oral hypoglycemic agents selectively modulate a specific pathological pathway suggesting that they are single target specific. For instance, to manage post-prandial hyperglycemia at digestive level, α-glucosidase inhibitors such as acarbose, miglitol and voglibose are used. They inhibit only degradation of carbohydrates into monosaccharides and their subsequent absorption from the intestine (Martha and John, 2001). However, these agents do not modulate other therapeutic targets such as pancreatic beta cells or PPARγ nuclear gene to offer better glycemic control.

In addition, there are a number of hypothesis on the origin of diabetic complications. Numerous experimental evidences suggest that oxygen free radicals such as superoxide anion, hydroxyl radical etc., are capable of damaging all major macromolecules including nucleic acid, proteins and lipids, thereby interrupting their normal physiologic functions which may contribute to the initiation and progression of diabetic complications (Matteucci and Giampietro, 2000; Baynes and Thorpe, 1997). Hyperglycemia increases oxidant production by multiple pathways rather than a single dominant pathway. By products of this will trigger signaling cascades such as activation of protein kinase C which in turn activate NAD(P)H oxidase via glucose induced De novo diacyl glycerol synthesis, production of hexosamine and increased glucose flux via polyol pathway further leading to the free radical generation (Inoguchi et al., 2000).

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Non-enzymatic glycation products of glucose and albumin (advanced glycation end-products, or AGEs) have been implicated in the development of vascular complications. It is clear that hyperglycemia is a primary factor that initiates and promotes diabetic complications (Jakus and Rietbrock, 2004). It is now thought that Advanced Glycation Endproducts (AGEs) contribute to accelerated micro- and macrovasculopathy observed in diabetes. It has been well documented that AGEs progressively accumulate on the tissues and organs which develop chronic complications of diabetes mellitus, such as retinopathy, nephropathy, neuropathy and also macrovascular disease atherosclerosis (Jakus and Rietbrock, 2004). Since diabetes mellitus is a multifactorial disorder, addressing a single pathological factor may not cause reversal of almost all the aspects associated with diabetes mellitus. This suggests that the efficacy of these synthetic hypoglycemic agents have been compromised principally due to their single-target oriented action. This could be the reason that even after so much of advancement in understanding the disease process and availability of a wide range of therapeutic agents, the disease is still progressing. Therefore, the multifactorial pathogenicity of diabetes mellitus demands alternative strategies with multi-model therapeutic approach (Tiwari and Madhusudana-Rao, 2002).

There are several limitations for the use of existing modern therapeutic agents to control all aspects of the disorder including associated life-threatening side effects such as development of hypoglycemia, secondary failure, weight gain, gastrointestinal disturbances and liver toxicity (Modak *et al.*, 2007). For instance, glitazones are linked to liver toxicity (troglitazones), including a number of deaths from hepatic failure and raising the symptoms and risk of heart disease leading to heart failure (rosiglitazone) (Modak *et al.*, 2007).

More recently, glibenclamide in the state of elevated blood glucose level has shown to stimulate ROS production in β cells line MIN6 through protein kinase C (PKC)-dependent activation of NAD(P)H oxidase in a dose dependent manner and this may contribute to β cell dysfunction (Tsubouchi *et al.*, 2004).

Therefore, future therapeutic strategies require a combination of various types of agents that can target multiple pathologic factors to achieve euglycemia and more importantly, delay diabetic complications occurring as a result of oxidative stress in diabetics. Traditional medicinal plants with a diverse array of active principles and pharmacological actions are being scientifically validated as a potential alternative to synthetic agents for the treatment of diabetes. The ancient Indian literature has prescribed various herbs and metals to treat diabetes mellitus. This has led to an increasing demand of research on anti-diabetic natural products that produces minimal or no side effects (Modak *et al.*, 2007).

Polyherbal formulations have plant-based pharmacological agents which may exert synergistic, potentiative, agonistic/antagonistic actions by virtue of its diverse active principles within themselves. These pharmacological principles work together in a dynamic way to produce maximum therapeutic efficacy with minimum side effects (Tiwari and Madhusudana-Rao, 2002). Thus, a new polyherbomineral formulation containing eleven medicinal plant extracts viz., *Pterocarpus marsupium*, *Azadirachta indica*, *Swertia chirata*, *Curcuma longa*, *Gymnema sylvestre*, *Emblica officinalis*, *Eugenia jambolana*, *Salacia reticulate*, *Trigonella foenum graecum*, *Vinca rosea*, *Aegle marmelos* and two minerals viz., processed stannum and processed asphltum (shilajit) named Gly-13-C has taken up in the present study to evaluate its antidiabetic and antioxidant potential.

MATERIALS AND METHODS

Chemicals: STZ was purchased from Sigma-Aldrich (St. Louis, MO, USA). All other chemicals and reagents used were of analytical grade.

Selection of animal and animal care: Healthy male albino Wistar rats (100-150 g) were procured from the Central Animal Facilities of the Indian Institute of Science, Bangalore, India. Animals were housed at our Institute's animal house facilities until they gained significant weight (250±10 g) suitable for the present investigation. Animals were housed in polypropylene cages and maintained under standard conditions (12 h light/dark cycle, 22±2°C and 55±5% relative humidity). They were fed with standard rat pellet diet and water *ad libitum*. The animals were maintained in accordance with CPCSEA (Committee for the Purpose of Control and Supervision of Experimental Animals) guidelines for the care and use of laboratory animals. The study protocol was approved by Institutional Animal Ethics Committee (IAEC/KLECP/BNG/11/2007). The study was conducted at the pharmacology laboratory of K.L.E. Society's College of Pharmacy, Bangalore, Karnataka, India during June 2008- February 2009.

Experimental induction of diabetes: Overnight fasted animals (deprived of food for 16 h but water *ad libitum*) were rendered hyperglycemic by a single intraperitoneal (i.p.) injection of STZ (65 mg kg^{-1} b.wt.) in 0.1 M cold sodium citrate buffer (pH 4.5). The animals were allowed to drink 5% dextrose solution overnight to counteract the hypoglycemia-induced mortality occurring due to sudden

outburst of insulin from pancreatic β cells after STZ injection. A week after single i.p. injection of STZ, diabetic animals having fasting plasma glucose greater than 250 mg dL^{-1} were selected and considered for further investigations (Murugesh *et al.*, 2006).

Experimental protocol: The diabetic animals were randomly divided into five groups of six animals each. Another set of normal animals (n = 6) served as negative control.

- **Group 1:** Normal animals (negative control)
- **Group 2:** STZ-diabetic animals (positive control) +2% gum acacia (2 mL kg^{-1})
- **Group 3:** STZ-diabetic + Gly-13-C (100 mg kg^{-1} b.wt.)
- **Group 4:** STZ-diabetic + Gly-13-C (200 mg kg^{-1} b.wt.)
- **Group 5:** STZ-diabetic + Gly-13-C (400 mg kg^{-1} b.wt.)
- **Group 6:** STZ-diabetic + Glibenclamide (600 µg kg^{-1} b.wt.) (Sathishsekar and Subramanian, 2005)

The test and standard drugs were suspended in 2% gum acacia solution as vehicle and administered orally for 21 days using intragastric tube. Body weights of animals were assessed on daily basis.

Sample collection: For acute study, blood samples of 12 h fasted animals in all groups were collected on day 0 by puncturing retro-orbital plexus at 0 h under mild diethyl ether anesthesia and immediately centrifuged at 6000xg for 10 min using cooling centrifuge (Remi, India). Plasma was then separated from the erythrocytes sediment and stored in refrigerator until further analysis. In acute study, group I normal animals were also treated with Gly-13-C (400 mg kg^{-1}) to evaluate the hypoglycemic potential of Gly-13-C in normal animals. Exactly after 4 h, blood samples of all groups were drawn again and processed further as mention earlier and fasting plasma glucose was determined. For chronic study, all diabetic animals in the respective groups were treated daily for 21 days as per experimental protocol. Blood samples were collected at intervals of day 7, 14 and 21 and fasting plasma glucose for each sample was determined. At the end of experimental period, animals were sacrificed under the influence of overdosed diethyl ether anesthesia. Liver and skeletal muscle were quickly excised and ringed with ice cold saline and blotted dry. Each liver sample was minced and homogenized in 50 mM tris buffer containing 2 mM EDTA, pH 7.4 to get a 10% w/v homogenate using Teflon homogenizer (Remi, India) and centrifuged at 10,000xg for 10 min at 4°C. (cooling microfuge, Remi, India) and stored at 0 -4°C until analyzed. The supernatant was used for various biochemical estimations. For glycogen estimation, liver and skeletal muscle homogenate (10% w/v) were prepared in 0.05 M phosphate buffer at pH 7.0. A portion of liver tissue was stored in 10% formalin after washing with cold normal saline for histopathological studies.

Biochemical estimations: Fasting plasma glucose was estimated by ortho-toluidine method (Godkar and Godkar, 2003). Plasma albumin was extracted using HCl-ethanol reagent according to the method of Fernandez *et al.* (1966). Total plasma albumin and extracted plasma albumin were estimated by bromocresol green (BCG) method using commercial kit (Liquixx Albumin Cal B, Erba diagnostic Mannheim, Daman).

Extracted plasma albumin was used to perform fructosamine assay on day 0 and 21 as an index of glycated albumin according to the method proposed by Johanson *et al.* (1983) and modified by Ohkawara *et al.* (2002) using dihydroxy acetone as a standard (Phillipou *et al.*, 1988). Briefly, reaction was started by adding 500 µL of extracted albumin sample to 2.5 mL NBT reagent (0.5 mmol L^{-1} in 0.2 M carbonate buffer, pH 10.8) at 37°C and time was noted. The difference in the absorbance at 540 nm (blue color complex) between 10 and 15 min after the start of the reaction was measured using UV-visible spectrophotometer, Jasko V-530. The same procedure was followed for standard caliberator dihydroxy acetone (1 mmol L^{-1} freshly prepared) to calculate fructosamine value. Fructosamine levels were calculated using formula:

Albumin fructosamine (mmol 1 L^{-1}) = (OD of test at 15th min OD of test at 10th min)/(OD of std. at 15th min - OD of std. at 10th min)×Calibrator concentration

Fructosamine concentration corrected for total plasma albumin concentration using formula:

[Fructosamine] Corrected for total plasma albumin = (Fructosamin value for extracted albumin (mmol L^{-1})/Extracted albumin)×Total plasma albumin

Glycogen content of liver and skeletal muscle was measured according to Carroll *et al.* (1956) with slight modification (Plummer, 2007). In this method, released glycogen was hydrolyzed with strong acid to convert it into glucose. Briefly, 2 mL of KOH (30 % w/v) was added to 2 mL of 10% w/v tissue homogenate and heated in boiling water bath for 20 min. After cooling test tubes in ice bath, 0.2 mL of saturated Na_2SO_4 was added and mixed thoroughly. Liberated glycogen was precipitated by addition of ethanol (90% w/v). After centrifugation at 1000xg for 20 min, precipitate was collected. Precipitated glycogen was then dissolved in 5 mL of distilled water with gentle warming and made the volume up to 10 mL

and mixed. Two milliliter of this solution was added to the equal volume of HCl (1.2 mmol L^{-1}) and kept in boiling water bath for 2 h. From this, 2 mL solution was pipetted out into 4 mL of anthrone reagent (2 g^{-1} L in conc. H_2SO_4) and heated again for 10 min at 100°C and then cooled in ice bath. Deep green color developed was measured at 560 nm against blank using UV-visible spectrophotometer, Jasko V-530. Glucose (100 µg mL^{-1}) was used as standard. Enzymatic antioxidants-Superoxide dismutase (SOD) activity in liver homogenate was estimated following the modified method of Kakker *et al.* (1984) and liver catalase (CAT) activity was determined using the method suggested by Sinha (1972). Liver homogenate was also analyzed for non-enzymatic antioxidants- Vitamin C according to the method of Roe and Kuether (Varley *et al.*, 1991) and Vitamin E levels following the method of Barker and Frank (Varley *et al.*, 1991). Lipid peroxidation in liver homogenate was measured as Malondialdehyde (MDA) following the modified method described by Reddy and Lokesh (1992).

Total protein contents in plasma and liver tissue were measured according to Lowery method as modified by Pomory (2008).

For histopathological studies, 5 µm thickness sections of liver were cut, stained with hematoxylin and mounted following standard protocol. Hepatic cellular architecture, vacuole formation, necrotic and inflammatory changes in hepatocytes were studied.

Statistical analysis: Statistical analysis was carried out using Graph Pad Prism version 3.0 (GraphPad Software Inc., San Diego, Calif., USA). Data were expressed as mean±SEM. Statistical comparison between different groups were done using One-way Analysis of Variance (ANOVA) followed by Tukey-Kramer multiple comparison test. $p < 0.05$ was considered significant.

RESULTS

Fasting plasma glucose: Table 1 shows the effect of Gly-13-C on elevated plasma glucose levels during acute study. All treatment groups have demonstrated the significant reduction in elevated plasma glucose at 4 h when compared with 0 h plasma glucose. The treatment with Gly-13-C in a dose of 100 mg kg^{-1} ($p < 0.01$), 200 mg kg^{-1} ($p < 0.001$) and 400 mg kg^{-1} b.wt. ($p < 0.001$) resulted in a significant decrease in plasma glucose when compared with their respective 0 h plasma glucose values, while diabetic animals treated with glibenclamide (600 µg kg^{-1} b.wt.) has shown a significant reduction of plasma glucose ($p < 0.01$) that was comparable to the action of Gly-13-C 100 mg kg^{-1} b.wt. Gly-13-C in a dose of 400 mg kg^{-1} on the plasma glucose of normal animals did not produce hypoglycemia.

Table 2 shows the effect of Gly-13-C on fasting plasma glucose at an interval of day 7, 14 and 21 in STZ induced diabetic animals. The diabetic animals treated

Table 1: Effect of Gly-13-C on plasma glucose at 4 h on STZ-induced diabetic animals

Group	Treatment	Fasting plasma glucose (mg dL^{-1}) 0	4
		(h)	
Normal control	Gly-13-C, 400 mg kg^{-1} b.wt.	82.00±4.70	76.84±4.36[ns]
Diabetic control	2% gum acacia, 2 mL kg^{-1} b.wt.	309.88±13.60	303.09±14.42[ns]
Gly-13-C	100 mg kg^{-1} b.wt.	287.46±12.11	209.59±22.53**
Gly-13-C	200 mg kg^{-1} b.wt.	293.21±2.52	177.72±13.03***
Gly-13-C	400 mg kg^{-1} b.wt.	298.82±20.57	154.43±5.47***
Glibenclamide	600 µg kg^{-1} b.wt.	299.41±4.81	220.64±3.71**

Values are expressed as Mean±SEM (n = 6). **$p < 0.01$, ***$p < 0.001$, ns: not significant

Table 2: Effect of Gly-13-C on the fasting plasma glucose in STZ-induced diabetic animals

Groups	Fasting plasma glucose (mg dL^{-1}) 0	7	14	21
			(day)	
Normal control	82.00±4.70	-	-	-
Diabetic control	309.88±13.60	311.06±12.15	318.29±13.92	321.38±14.63
Gly-13-C, 100 mg kg^{-1} b.wt.	287.46±12.43	240.12±8.69[a]**	209.29±10.77[b]***	167.26±11.43[c,d]***
Gly-13-C, 200 mg kg^{-1} b.wt.	293.22±22.53	218.44±7.95[a]***	169.17±11.52[b]***	124.04±5.83[c,d]***
Gly-13-C, 400 mg kg^{-1} b.wt.	298.82±20.57	202.21±11.0[a]***	143.51±4.83[b]***	88.05±5.10[c,d]***
Glibenclamide, 600 µg kg^{-1} b.wt.	299.41±4.81	256.34±9.87[a]*	219.03±8.21[b]***	185.10±8.97[c,d]***

Values are expressed as Mean±SEM (n = 6). *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$. ns: Not significant. a: When day 0 plasma glucose compared with that of day 7, b: When plasma glucose on day 7 compared with that of day 14, c: When plasma glucose on day 14 compared with that of day 21 and d: When plasma glucose on day 7 compared with that of day 14

Table 3: Effect of Gly-13-C on body weight (g) of STZ-induced diabetic animals

| | | After induction | |
Group	Before induction	Day 0	Day 21
Diabetic control	245.83±2.38	215.0±3.42[b]***	179.17±2.39
Gly-13-C, 100 mg kg⁻¹	246.66±2.47	218.33±1.05[b]***	212.50±2.47****
Gly-13-C, 200 mg kg⁻¹	247.50±2.81	217.5±2.14[b]***	219.17±2.1****
Gly-13-C, 400 mg kg⁻¹	249.16±2.38	215.83±2.01[b]***	236.66±2.11[(a,c)]***
Glibenclamide, 600 µg kg⁻¹	251.66±1.66	220.83±1.54[b]***	213.30±1.66****

Values are expressed as Mean±SEM (n = 6). *p<0.05, **p<0.01 and ***p<0.001. ns = not significant. a: When compared with vehicle treated diabetic animals, b: When compared with weights before induction of diabetes, c: When compared with GBC treated group

with Gly-13-C 100, 200, 400 mg kg⁻¹ b.wt. and glibenclamide (600 µg kg⁻¹ b.wt.) have shown significant reduction in fasting plasma glucose on day 7 (p<0.05 for Gly-13-C 100 mg kg⁻¹ and glibenclamide 600 µg kg⁻¹, p<0.01 for Gly-13-C 200 mg kg⁻¹, p<0.001 for Gly-13-C 400 mg kg⁻¹) when compared with their fasting plasma glucose on day 0. The reduction in fasting plasma glucose was significant on day 14 (p < 0.001 for all treatments) and on day 21 (p < 0.001 for all treatments) when compared with their fasting plasma glucose on day 7 and 14, respectively.

Body weight: Table 3 shows the effect of STZ and Gly-13-C treatment on the body weight of experimental animals. In our findings, a single i.p. injection of STZ (65 mg kg⁻¹ b.wt.) resulted in a significant loss of body weight (p<0.001) of all experimental animals when compared with their weights prior to diabetes induction. Vehicle treated diabetic animals showed a significant loss of body weight on day 21 when compared with body weights on day 0 (p<0.001). All treatment groups showed a significant gain in body weight (p<0.001) on day 21 when compared to vehicle treated diabetic animals.

Plasma albumin concentration: Figure 1 shows the effect of Gly-13-C on the plasma albumin concentration on day 21 in the STZ-induced diabetic animals. Gly-13-C 200 and 400 mg kg⁻¹ doses significantly restored the plasma albumin levels (p<0.05 and p<0.001, respectively) when compared to positive control animals. Gly-13-C 100 mg kg⁻¹ and standard drug glibenclamide (600 µg kg⁻¹) failed to elevate plasma albumin significantly. In addition, the increased albumin levels in Gly-13-C 400 mg kg⁻¹ treated diabetic animals were significant when compared with glibenclamide treated (p<0.001) diabetic animals.

Albumin fructosamine assay: Figure 2 shows the effect of Gly-13-C on the fructosamine levels corrected for actual total albumin concentration in diabetic animals on day 21 in different treatment groups. Gly-13-C 100 mg kg⁻¹and glibenclamide 600 µg kg⁻¹ treatments did not exhibit any significant change in fructosamine levels. Gly-13-C 200
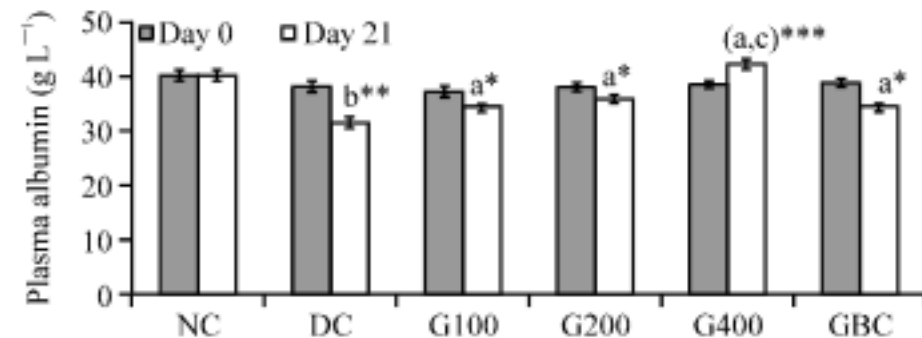

Fig. 1: Effect of Gly-13-C on plasma albumin concentration in STZ-induced diabetic animals. Values are expressed as mean±SEM (n = 6). *p<0.05, **p<0.01 and ***p<0.001. NC: Normal control, DC: Diabetic control, G100: Gly-13-C 100 mg⁻¹ kg, G200: 200 mg⁻¹ kg and G400: 400 mg⁻¹ kg, GBC : glybenclamide. (a) when compared with vehicle treated DC, (b) when compared with NC, (c) when compared with GBC treated group
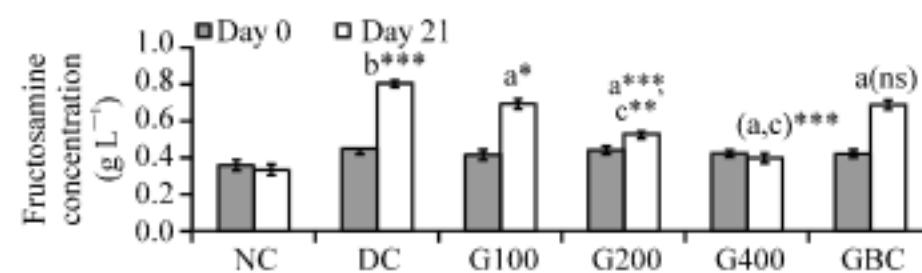

Fig. 2: Effect of Gly-13-C on fructosamine concentration in STZ-induced diabetic animals. Values are expressed as Mean±SEM (n = 6). *p<0.05, **p<0.01 and ***p<0.001. ns: not significant. (a) when compared with vehicle treated DC, (b) when compared with NC, (c) when compared with GBC treated group

and 400 mg kg⁻¹ treated diabetic animals showed a significant inhibition of plasma albumin glycation in terms of decreased fructosamine levels when compared with vehicle treated (p<0.001 for both doses) and glibenclamide 600 µg kg⁻¹ treated diabetic animals (p<0.01 for Gly-13-C 200 mg kg⁻¹, p<0.001 for 400 mg kg⁻¹).

Liver and skeletal muscle glycogen content: Figure 3 shows the effect of Gly-13-C on the liver and skeletal muscle glycogen in STZ-induced diabetic animals. There was a significant decrease in the liver (p<0.001) and skeletal muscle (p<0.001) glycogen content in
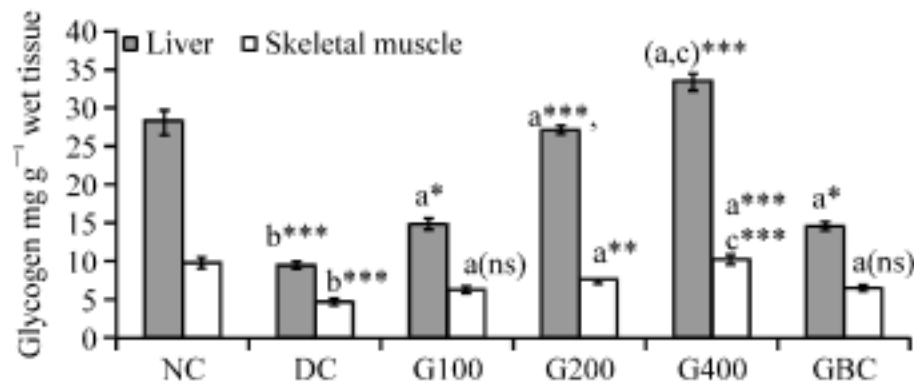
Fig. 3: Effect of Gly-13-C on glycogen content of STZ-induced diabetic animals. Values are expressed as Mean±SEM (n = 6). *$p<0.05$, **$p<0.01$ and ***$p<0.001$. ns: not significant (a) when compared with vehicle treated DC, (b) when compared with NC and (c) when compared with GBC treated group
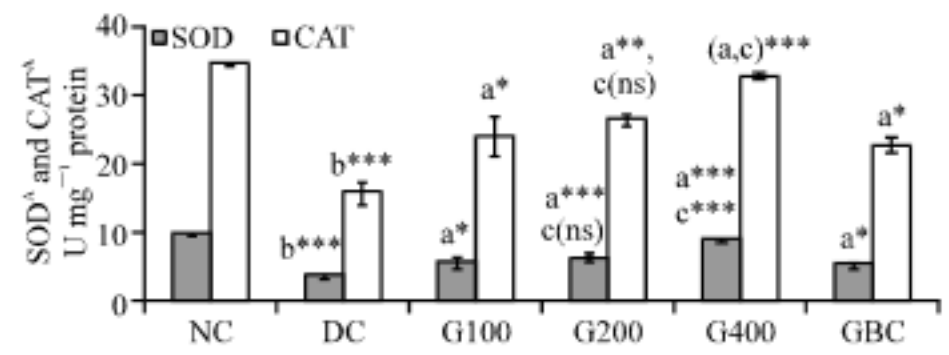

Fig. 4: Effect of Gly-13-C on liver enzymatic antioxidants of STZ-induced diabetic animals. Values are expressed as Mean±SEM (n = 6). *$p<0.05$, **$p<0.01$, ***$p<0.001$. ns: Not significant. (a) when compared with DC, (b) when compared with NC and (c) when compared with GBC treated group. SOD[A]: μmol inhibition of NBT reduction min^{-1}. CAT[B]: μmol of H_2O_2 consumed

STZ-induced diabetic animals when compared with that of negative control animals. The diabetic animals treated with Gly-13-C 100 mg kg^{-1} and glibenclamide (600 μg kg^{-1} b.wt.) have shown a significant increase in glycogen content in liver ($p<0.05$), but there was no significant increase in the glycogen content of skeletal muscle. The treatment with Gly-13-C in a dose of 200 and 400 mg kg^{-1} significantly elevated liver ($p<0.001$ for both doses) and skeletal muscle glycogen ($p<0.01$ for 200 mg kg^{-1}, $p<0.001$ for 400 mg kg^{-1}) when compared with vehicle treated diabetic animals. The liver glycogen levels of Gly-13-C 200 mg kg^{-1} treated diabetic animals were also found to be significant when compared with that of glibenclamide treated diabetic animals ($p<0.01$). The treatment with Gly-13-C 400 mg kg^{-1} showed significantly higher levels of glycogen in both the tissues ($p<0.001$) when compared to glycogen content of diabetic animals treated with glibenclamide (600 μg kg^{-1}).

Enzymatic antioxidants: Figure 4 shows the activity of SOD and CAT enzymes in Gly-13-C treated diabetic animals. STZ- induced diabetic animals have shown a significant decrease in the SOD ($p<0.001$) and CAT ($p<0.001$) enzyme activities when compared with negative control animals. Administration of GLY-13-C in dose of 100 mg kg^{-1}, 200 mg kg^{-1} and 400 mg kg^{-1} b.wt. significantly normalized the levels of SOD ($p<0.05$, $p<0.001$, $p<0.001$, respectively) and CAT ($p<0.05$, $p<0.01$ and $p<0.001$, respectively) when compared with vehicle treated diabetic animals. The effect of Gly-13-C 100 mg kg^{-1} on SOD ($p<0.05$) and CAT ($p<0.05$) activities was comparable to that of glibenclamide 600 μg kg^{-1}. Moreover, increased SOD and CAT levels of Gly-13-C 400 mg kg^{-1} treated diabetic animals were also significant when compared with glibencalmide 600 μg kg^{-1} treated diabetic animals ($p<0.001$ for SOD and CAT).
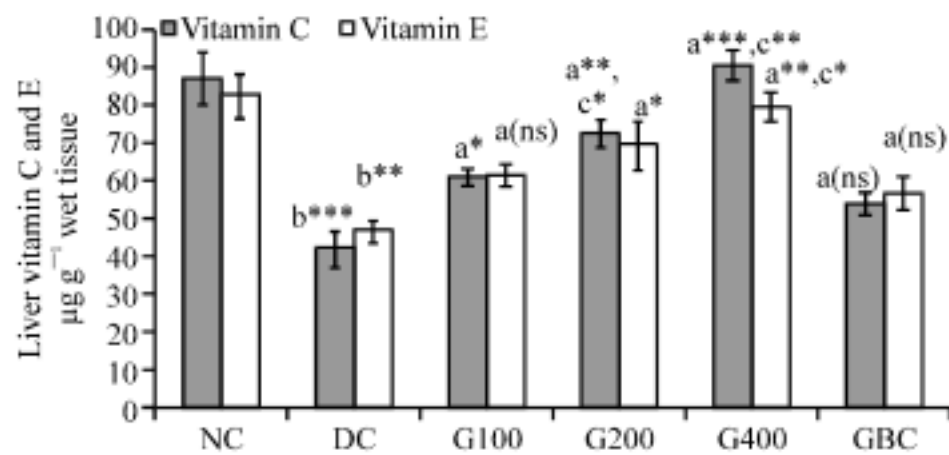

Fig. 5: Effect of Gly-13-C on the liver non-enzymatic antioxidants levels in STZ-induced diabetic animals. Values are expressed as Mean±SEM (n = 6). *$p<0.05$, **$p<0.01$ and ***$p<0.001$. ns: Not significant. (a) when compared with vehicle treated DC, (b) when compared with NC and (c) when compared with GBC treated group

Non-enzymatic antioxidants: Figure 5 shows the effect of Gly-13-C on the endogenous non-enzymatic antioxidants in STZ-induced diabetic animals. Liver vitamin C and vitamin E levels were significantly reduced in STZ-diabetic animals when compared with the levels of normal animals ($p<0.001$). The diabetic animals treated with Gly-13-C 100 mg kg^{-1} showed a significant increase in liver vitamin C levels ($p<0.05$) when compared with vehicle treated diabetic animals. However, this dose of Gly-13-C did not show any significant change in liver vitamin E levels. The increase in the liver vitamin C and vitamin E were not significant in glibenclamide (600 μg kg^{-1} b.wt.) treated diabetic animals when compared with control diabetic animals. The treatment with Gly-13-C 200 mg kg^{-1} in diabetic animals resulted in a significant elevation of liver vitamin C ($p<0.05$) as well as vitamin E ($p<0.05$) levels when compared with control

Table 4: Effect of Gly-13-C on total protein content of plasma and liver in STZ-induced diabetic animals

Groups	Treatments	Total protein content	
		Plasma (g dL^{-1})	Liver mg g^{-1} of wet tissue
Normal control	-	7.01±0.25	202.2±3.58
Diabetic control	2% gum acacia, 2 mL kg^{-1} b.w.	3.43±0.10[b]***	147.7±6.67[b]***
Gly-13-C	100 mg kg^{-1} b.wt.	4.65±0.35**	170.7±5.94**
Gly-13-C	200 mg kg^{-1} b.wt.	5.14±0.21***	177.8±2.37***
Gly-13-C	400 mg kg^{-1} b.wt.	7.23±0.19[(a,c)]***	201.0±3.33[(a,c)]***
Glibenclamide	600 μg kg^{-1} b.wt.	3.43±0.1**	169.3±4.77**

Values are expressed as Mean±SEM (n = 6). *p<0.05, **p<0.01 and ***p<0.001. ns: Not significant. a: When compared with vehicle treated diabetic animals, b: When compared with normal animals and c: When compared with GBC treated group
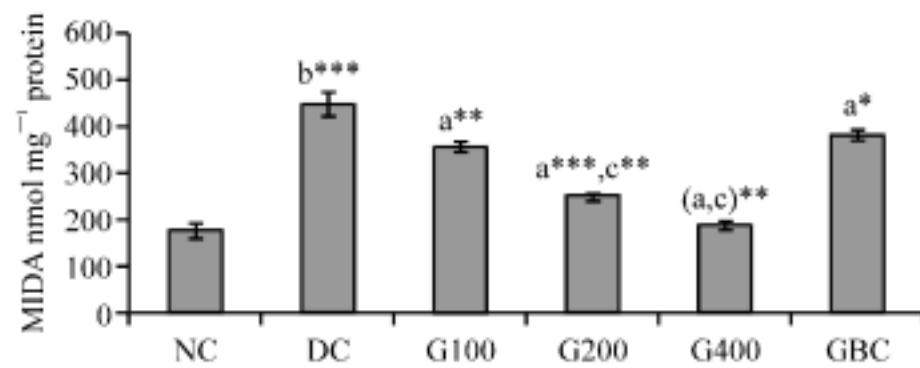

Fig. 6: Effect of Gly-13-C on the lipid peroxidation in liver tissue of STZ-induced diabetic animals. Values are expressed as Mean±SEM (n = 6). *p<0.05, **p<0.01 and ***p<0.001. (a) when compared with vehicle treated DC, (b) when compared with NC and (c) when compared with GBC treated group

diabetic animals. Gly-13-C 400 mg kg^{-1} treated diabetic animals showed a maximum improvement of liver vitamin C as well as vitamin E levels.

Lipid peroxidation: Figure 6 summarizes the effect of Gly-13-C on the lipid peroxidation of liver tissue in STZ-diabetic animals. The MDA levels in liver of diabetic animals were measured as an index of lipid peroxidation. All doses of Gly-13-C i.e., 100 mg kg^{-1}, 200 mg kg^{-1} and 400 mg kg^{-1} showed significant protection (p<0.01, p<0.001 and p<0.001, respectively) against lipid peroxidation in diabetic animals and was dose dependent. Moreover, Gly-13-C 200 and 400 mg kg^{-1} showed a significant reduction in liver MDA levels when compared with glibenclamide 600 μg kg^{-1} (p<0.001 for both doses). The decreased levels of liver MDA in glibenclamide 600 μg kg^{-1} (p<0.05) treated diabetic animals were comparable to that of Gly-13-C 100 mg kg^{-1} treated diabetic animals.

Total protein content: Table 4 summarizes the effect of Gly-13-C on the plasma and liver protein content in STZ-induced diabetic animals. There was a significant loss of plasma and liver protein (p<0.001) in vehicle treated diabetic animals when compared with vehicle treated diabetic animals. The diabetic animals administered with Gly-13-C 100 mg kg^{-1}, 200 mg kg^{-1}

and glibenclamide 600 μg kg^{-1} b.wt. significantly raised the plasma and liver protein levels (p<0.05, p<0.01 and p<0.05, respectively). The effect of Gly-13-C 100 mg kg^{-1} was comparable to that of glibenclamide in stimulating protein synthesis. The diabetic animals treated with Gly-13-C 400 mg kg^{-1} showed a significant increase in the plasma and liver protein when compared with vehicle treated (p<0.001) and glibenclamide 600 μg kg^{-1} (p<0.001) treated diabetic animals.

DISCUSSION

STZ has been widely used for the induction of diabetes mellitus in various experimental animals. Cytotoxic moiety of STZ, 1-methyl-1-nitrosourea has been shown to be responsible for its cytotoxic action on pancreatic β-cells (Szkudelski, 2001). This cytotoxic action of STZ causes DNA strands to break in pancreatic islets, stimulating nuclear poly (ADP-ribose) synthase and thus, depletes the intracellular NAD$^+$ and NADP$^+$ levels. This destroys pancreatic β-cells and inhibits proinsulin synthesis. The entire process results in diabetes mellitus (Szkudelski, 2001). Many reports have shown that the cytotoxic action of STZ is also mediated via generation of Reactive Oxygen Species (ROS). The role of ROS was also confirmed by Kakkar *et al.* (1998) who demonstrated gradually decreasing endogenous antioxidant defense against elevated free radical attack in pancreatic β-cells of STZ- induced diabetic rats. Thus, suggesting the role of free radicals in pathogenesis of diabetes mellitus.

In our present study, a single i.p. injection of STZ- in a dose of 65 mg kg^{-1} b.wt. resulted in severe hyperglycemia and significantly elevated fasting plasma glucose in albino Wistar rats. The elevated plasma glucose level produced by STZ indicates its cytotoxicity on pancreatic β- cells in experimental animals.

In acute study, no significant decrease in blood plasma level was observed at Gly-13-C 400 mg kg^{-1} b.wt. in normal animals indicating that Gly-13-C is devoid of hypoglycemic action. However, a significant reduction in fasting plasma glucose was achieved after the treatment with Gly-13-C over a period of 21 days in a dose

dependent manner. Antihyperglycemic efficacy of Gly-13-C in a dose of 200 mg and 400 mg kg^{-1} b.wt. was superior to that of glibenclamide at a dose of 600 µg kg^{-1} b.wt. Gly-13-C in 400 mg kg^{-1} b.wt. lowered elevated fasting plasma glucose level which was comparable to that of normal animals. This antihyperglycemic effect offered by Gly-13-C in STZ- induced diabetic animals may probably due to the synergistic action of its individual components. The possible mechanism underlying the antihyperglycemic action of Gly-13-C may be by virtue of its substantially diverse active principles present in individual herb extracts such as *Pterocarpus marsupium*, *Azadirachta indica*, *Eugenia jambolona*, *Swertia chirata*, *Salacia reticulata*, *Gymnema sylvestre*, *emblica officinalis*, *Trigonella foenum graecum* etc. These active principles may modulate various biochemical mechanisms involved in the regulation of glucose homeostasis and pathogenesis of diabetes. Thus, contributing to total antihyperglymic potential of Gly-13-C.

STZ-induced experimental diabetes is characterized by severe loss of body weight in experimental animals. Body weight loss may result from relative or absolute deficiency of insulin due to defective β- cells functioning. Loss or destruction of various structural proteins is also considered in body weight reduction in diabetic animals (Rajkumar *et al.*, 1991). Insulin has anabolic action on protein metabolism that stimulates protein synthesis and retards protein degradation (Jorda *et al.*, 1988). The observed body weight loss in our investigation could be the result of absolute or relative deficiency of insulin thus, decreased ATP production and structural protein synthesis in vehicle treated STZ-induced diabetic animals when compared to normal animals. Our study data revealing a significant decline in body weight due to defective protein synthesis can be substantiated by the plasma and liver protein levels in vehicle treated STZ-induced diabetic animals. There was a significant loss of plasma and liver protein content in positive control diabetic animals when compared with normal animals. Earlier report suggests that there are some fundamental changes in the destruction of proteins in STZ-induced diabetes and average rate of protein catabolism are accelerated by 30-150% in liver of insulin deficient animals (Jorda *et al.*, 1988). Present results on body weight loss in STZ-induced diabetic animals are well in agreement with this report. A significant body weight gain and increased protein levels in plasma and liver observed in diabetic animals treated with Gly-13-C might be due to decreased protein catabolism as a result of improved glycemic control and availability of insulin levels in physiologically sufficient quantity. These events might reflect the capacity of Gly-13-C to restore β- cells function, probably due to regeneration of islets of Langerhans.

Glycogen contents in the liver and skeletal muscle of diabetic animals were reduced due to impaired glycogen synthesis during diabetes (Huang *et al.*, 2000). In our present study, there was a significant reduction of glycogen content in liver and skeletal muscle of vehicle treated diabetic animals as reported earlier (Huang *et al.*, 2000). The decreased glycogen may result from insulin deficiency in diabetic state that leads to low expression of glycogen synthase gene in liver and skeletal muscles. Gly-13-C treatment significantly increased the glycogen synthesis in both tissues, probably due to elevated insulin levels in diabetic animals which may result from regeneration or increased stimulation of insulin secretion from remnant pancreatic β-cells.

Various proteins such as albumin, globulin, hemoglobin, collagen, LDL, crystalline proteins may undergo non-enzymatic glycation when they are exposed to excess glucose in hyperglycemic state (Wu and Monnier, 2003). Carbonyl group of reducing sugars (e.g., glucose, fructose etc.) reacts covalently with amino groups on protein (such as lysine, arginine etc.) to form Amadori rearrangement products via Schiff's base. The best know Amadori products are glycated hemoglobin (HbA$_{1c}$) and fructosamine (fructolysine) (Jakus and Rietbrock, 2004). Amadori products gradually undergo chemical cleavage and conformational changes by the Maillard reaction to form advanced glycated end products (AGEs). These AGEs are highly reactive and induce chemical modifications and cross linking of tissue proteins, lipids and DNA, altering their structure and subsequently deteriorating the normal tissue function (Jakus and Rietbrock, 2004). Excessive accumulation of AGEs may play an important role in the development of diabetic complications (Hunt *et al.*, 1993). This indicates that the first step in the production of AGEs is non- enzymatic glycation of various proteins. Inhibition of glycation could prove beneficial in the prevention of diabetic complications. Glycated hemoglobin (HbA$_{1c}$) is generally considered to be a useful index of long- term glycemic control of diabetes mellitus because it reflects blood glucose levels over a period of previous 2-3 months (Phillipou *et al.*, 1988). However, measurement of glycated hemoglobin may not prove useful screening tool as this test does not reflect short-term glycemic changes in blood due to its longer life span (approximately 120 days). Thus, glycated hemoglobin may not depict the exact picture of blood glucose fluctuation in short duration studies. In contrast to this, measurement of glycated plasma proteins/albumin, known as fructosamine test could reveal the status of glycemic control over a period of 2-3 weeks due to their shorter life span (approximately 17-20 days). For this reason, this test is beneficial tool to

measure short-term glycemic changes more accurately. According to the opinion of Day *et al.* (1980) hemoglobin and albumin do not react in the same manner to variation in blood glucose and albumin is more sensitive than hemoglobin to change in plasma glucose. Johnson and Barker (1986) has reported that albumin incubated with glucose gained reducing activity more rapidly than did other plasma protein fractions. This behavior is likely to be reproduced *in vivo*, because albumin is present in plasma in high concentration, has ample of amino groups (58 lysine residues per molecule) and compared with other plasma proteins, albumin has a relatively long biological half life.

Albumin is a major constituent of blood proteins (Koplik *et al.*, 2003). In present investigation, fructosamine assay was carried out using extracted plasma albumin of diabetic animals to measure the degree of albumin glycation since, some studies have shown the presence of other low molecular weight substances such as uric acid, ascorbic acid etc., in the plasma which are also capable of reducing NBT and contribute to the colour development, thereby giving inaccurate results (Mashiba *et al.*, 1992). We observed a significant decrease in levels of plasma albumin in vehicle treated diabetic animals when compared with normal animals. Insulin has shown to regulate the synthesis of albumin by modulating the amount of albumin mRNA (Jefferson *et al.*, 1983). Thus, the observed loss of serum albumin could be due to insulin deficiency in diabetic animals. Another reason could be microalbuminuria, which is associated with increased endothelial microvascular permeability to macromolecules including albumin, thereby increasing urinary albumin of excretion (Afkhami-Ardekani *et al.*, 2008). There was a significant recovery of plasma albumin on treatment with Gly-13-C for 21 days when compared with vehicle treated diabetic animals. The mechanism of this action could be attributed to the increased abundance of insulin which may have induced elevated expressions of albumin mRNA in Gly-13-C treated diabetic animals. Reversal of microaluminuria could also be responsible for these increased levels of plasma albumin in treated animals. Microalbuminuria is considered as an early marker of diabetic nephropathy and independent risk factor for cardiovascular disorders (Afkhami-Ardekani *et al.*, 2008). This suggests the protective role of Gly-13-C in the prevention of diabetic complications such as nephropathy and associated cardiovascular disorders in DM.

There was a significant increase in glycated albumin in terms of fructosamine value in positive control diabetic animals when compared with normal control animals, indicating the poor glycemic control in these animals. Many evidences have outlined the role of vitamin E in reducing protein glycation in diabetic subjects that was independent of changes in plasma glucose concentration (Ceriello *et al.*, 1991). While vitamin C has shown to reduce protein glycation by interacting with vitamin E *in vivo* and *in vitro* (Vinson and Howard, 1996) thereby, reducing AGEs fructosamine and also act as scavenger of free radicals generated by glycated proteins or AGEs. The fructosamine values were significantly reduced and brought to normal range in Gly-13-C treated STZ-induced diabetic animals and these values were comparable to that of normal animals. These findings strongly support the inhibition of plasma albumin glycation, as a result of reduced fasting blood glucose and increased levels of vitamin E and vitamin C in Gly-13-C treated diabetic animals. Polyphenolic substances have shown to be capable of inhibiting protein glycation and AGE formation (Dearlove *et al.*, 2008). Polyphenol constituents-Mangiferin, epigallocatechin etc., isolated from *Salacia reticulata* root extract, one of the herbs of Gly-13-C formulation has demonstrated preventive effect on protein glycation in diabetic patients. This suggests that polyphenol-rich constituents of Gly-13-C may inhibit protein glycation in diabetes and contribute for the prevention of secondary complications in DM.

It is a well documented fact that excessive blood glucose leads to the increased generation of free radical species such as superoxide anion ($^{\bullet}O_2^{-}$), Hydroxyl ($^{\bullet}OH^{-}$) and peroxyl radicals as prooxidants which in turn cause oxidative stress in diabetes. All major organs have their own endogenous defense network which protects the tissue against detrimental effect of free radical attack (Firoozrai *et al.*, 2007). The cellular defense network includes both enzymatic antioxidants such as SOD, CAT, GPx etc and non- enzymatic antioxidant such vitamin C, vitamin E, β- carotene, ceruloplasm etc., (Maritim *et al.*, 2003) which together detoxifies hazardous oxygen radicals from the system. The disproportionate production of deleterious oxygen species and diminished ability of endogenous antioxidant defense to scavenge them efficiently may induce chemical alteration of all major classes of cellular biomolecules including nucleic acids, lipids, carbohydrates and proteins, changing their structural and functional properties that may accelerate the pathogenesis of diabetes and its complications (Maritim *et al.*, 2003). In the present investigation, low expression of liver antioxidant enzymes namely -SOD and CAT of vehicle treated diabetic animals were observed when compared with normal animals. Decreased activity of these enzymes could primarily due to increased free radicals generation resulting from elevated blood glucose metabolism via various biochemical pathways in hyperglycemic state (Loven *et al.*, 1982). The SOD has been postulated as one of the most important enzymes in

the enzymatic defense system which catalyzes the dismutation reaction of superoxide anions to produce H_2O_2 and molecular oxygen, thereby blocking the toxic effect of superoxide anion (Maritim *et al.*, 2003). Resultant H_2O_2 in the dismutation reaction itself is not reactive enough to impart damaging effect to the macromolecules. It is, however, a very potent prooxidant since, it can diffuse across biomembranes and form highly reactive $^{\bullet}OH^-$ radicals in the presence of transition metal ions such as Fe^+ or Cu^+. The CAT is a hemoprotein which catalyzes the reduction of H_2O_2 and protects tissue against hydroxyl radicals (Maritim *et al.*, 2003). The biological activity of these two enzymes is interdependent and seems to act in synergistic manner. The decrease in SOD activity may result from their over utilization during superoxide anion scavenging process or glycation induced enzyme inactivation (Yan and Harding, 1997). Horie *et al.* (1981) have demonstrated that hypoinsulinaemic state induces activation of enzyme fatty acyl -CoA oxidase that initiates β- oxidation of fatty acids resulting in increased production of H_2O_2 Accumulation of H_2O_2 in diabetic tissues has shown to inhibit/inactivate SOD enzyme, probably due to decreased CAT activity in oxidative stress conditions (Bray *et al.*, 1974). In our present study, diabetic animals receiving Gly-13-C have shown significant increase in liver SOD enzyme activity when compared with vehicle treated diabetic animals and these beneficial effects of Gly-13-C could be the result of restored levels of SOD or increased CAT activity that might have prevented H_2O_2 mediated SOD enzyme inactivation. The other reasons include reduced fasting plasma glucose that may prevent glycation induced enzyme inactivation or inhibition of H_2O_2 production through β -oxidation of FFAs, possibly due to the restored insulin levels by Gly-13-C treated diabetic animals.

In vivo antioxidant study of liver tissue of vehicle treated diabetic animals in present study has shown a significant reduction in CAT enzyme activity which may be due to their increased utilization during H_2O_2 elimination or glycation induced enzyme inactivation (Yan and Harding, 1997). Elevated levels of superoxide anion in diabetes could also result in decreased CAT activity (Kono and Fridovich, 1982). The Gly-13-C treatment in diabetic animals resulted in significant elevation of liver CAT enzyme activity. The inhibition of enzyme glycation due to reduced fasting plasma glucose, protection against CAT inactivation by superoxide anion due to increased expression of SOD enzyme might contribute to the increased liver CAT activity. The elevated levels of liver CAT and SOD enzymes in Gly-13-C treated diabetic animals in a dose of 400 mg kg^{-1} b.wt.

were almost two-fold higher than standard glibenclamide (600 µg kg^{-1} b.wt.) and comparable to normal animals.

In present investigation, the decreased levels of liver vitamin C and vitamin E in vehicle treated diabetic animals were in accordance with earlier reports (Garg *et al.*, 2005). These non-enzymatic antioxidants also play a significant role in scavenging free radicals along with enzymatic antioxidants, ensuring efficient removal of toxic free radicals to protect tissues against oxidative damage. Vitamin C is water soluble and active in hydrophilic compartment of cells. Vitamin C has been reported to be capable of directly reacting with H_2O_2, $^{\bullet}O_2^-$, $^{\bullet}OH^-$, peroxyl radicals and singlet oxygen and prevents lipid hydroperoxide formation (Padh, 1991) and vitamin C also found to preserve other antioxidants (Naziroglu and Butterworth, 2005). According to Kashiba *et al.* (2002) hepatic ascorbic acid regeneration is reduced in the STZ- induced diabetic animals in spite of increased gene expression of ascorbic acid generating enzymes because of decreased activity of L-gulono-gamma-lactone oxidase, a terminal enzyme of hepatic ascorbic acid biosynthesis. Fay *et al.* (1990) have shown that in hypoinsulinaemia or hyperglycemia, glucose inhibits cellular transport of ascorbic acid due to resemblance in their chemical structures. Therefore, vitamin C shares the membrane transport system with glucose and competes with glucose for its transport. Thus, decreased levels of liver ascorbic acid in vehicle treated diabetic animals in present study may be attributed to its overconsumption against elevated free radicals, hyperglycemic or insulin deficient state that in turn inhibits its membrane transport in preference to glucose, defective hepatic regeneration or increased urinary excretion of vitamin C and decreased levels of α- tocopherol (Hensely *et al.*, 2000). A significant increase in liver vitamin C was achieved after Gly-13-C treatment in STZ-induced diabetic animals. The observed increase in vitamin C might be due to reduced fasting plasma glucose levels or improved insulin levels, probably due to improved pancreatic β-cells functioning or β-cells regenerating capacity of Gly-13-C. Increased activity of L-gulono-gamma-lactone oxidase enzyme activity in liver could also be responsible for elevated hepatic ascorbic acid biosynthesis. In addition, *Emblica officinalis*, one of the herbs present in Gly-13-C is found to be rich in ascorbic acid (Rajeshkumar *et al.*, 2001). All these factors might be responsible for the elevated vitamin C levels observed in Gly-13-C treated diabetic animals compared to normal animals.

The lipid-soluble antioxidant, vitamin E is considered as an important defense against lipid peroxidation of polyunsaturated fatty acids of biomembranes by donating electron and thereby quenching free radicals, especially

•OH⁻ radicals. Vitamin E is also recognized as chain breaking antioxidant since, vitamin E prevents further generation of free radicals from oxidized products of lipid peroxidation (Kashif *et al.*, 2004). Hydroxyl radicals were found to react with α- tocopherol forming a stabilized phenolic radical (tocopheryl radical) which is reduced back to the phenol (tocopherol) by vitamin C and NAD(P)H- dependent reductase enzyme (Hensely *et al.*, 2000). The overconsumption of vitamin E as an antioxidant defense during oxidative stress elevated fasting plasma glucose or decreased hepatic regeneration of vitamin C may be responsible for the reduced liver vitamin E levels of vehicle treated diabetic animals as supported by the present study. There was a significant increase in the vitamin E levels in Gly-13-C treated diabetic animals and this observed elevated may be achieved due to decreased blood glucose levels, accelerated removal of damaging free radicals, restoration of vitamin C regenerating capacity of liver or its decreased urinary excretion.

Lipid peroxidation is an index of oxidative stress and is generally measured as malondialdehyde (MDA), which is a lipid peroxidation end product. •OH⁻ is highly reactive and more damaging among other free radicals. Almost all major classes of biomolecules including nucleic acid, proteins and lipids are susceptible to the •OH⁻ attack but, more susceptible targets are Polyunsaturated Fatty Acids (PUFAs) of cellular membranes. Products of lipid peroxidation are found to be capable of interacting with DNA. In addition, a hydrogen atom subtracted from the lipid peroxidation end products result in increased free radical generation and contributes to the oxidative damage to the target tissues (Halliwell and Gutteridge, 1994). STZ is reported to induce increased •OH⁻ radicals that participates in the formation of lipid peroxidation products (Ohkuwa *et al.*, 1995). Elevated levels of MDA in diabetes are indicative of oxidative stress-induced peroxidative damage. Vitamin E has shown protection against hepatic lipid peroxidation without depressing other endogenous antioxidant defense (Cadenas *et al.*, 1995). The significant increase in liver MDA of vehicle treated STZ-induced diabetic animals was observed in present study, which matches with the earlier report (Garg *et al.*, 2005) indicating the deficient antioxidant status in these animals. Gly-13-C treated diabetic animals have shown a significant decrease of MDA levels in the liver, possibly due to elevated liver vitamin C and vitamin E levels.

Moreover, many plant polyphenols such as flavonoids, condensed tannins, saponins and coumarins have shown antioxidant and lipid peroxidation inhibitory activity. Gayathri Devi *et al.* (2006) have reported that

these phenols act as chain breaking antioxidants by donating a hydrogen atom to a peroxy or alkoxy radical thereby interferes with propagation of lipid peroxidation. Polyphenolic constituents like condensed tannins from *Emblica officinalis* (Bhattacharya *et al.*, 2000) and flavonoids from *Azadirachta indica* (Chattopadhyay *et al.*, 1992) act by scavenging •OH⁻ and •O₂⁻ radicals and prevent lipid peroxidation of target tissues. A phenolic coumarin, umbelliferone present in *Aegle marmelos* (Ramesh and Pugalendi, 2007) and *Emblice officinalis* (Ramesh and Pugalendi, 2006) has one hydroxyl and one acetoxy group in the benzoid ring has been reported to be responsible for antioxidant and lipid peroxidation inhibitory activities of these plants. Saponins from *Trigonella foenum graecum* has shown to inhibit lipid peroxidation (Anuradha and Ravikumar, 2001). Curcuminods present in *curcuma longa* has been found to inhibit lipid peroxidation in various experimental settings (Sreejayan, 1994). Thus, significant decrease in liver MDA in Gly-13-C treated animals could be due to these herbs present in polyherbomineral formulation, Gly-13-C. Mukherjee *et al.* (2006) have reported that several phytochemicals including flavonoids, alkaloids, glycosides, tannins, saponins, dietary fibers and amino acids have a potent hypoglycemic activity. Individual herbs of Gly-13-C are rich in such active constituents. A flavonoid (-)- epicatechin, is one of the active constituents of *Pterocarpus marsupium* (Sheehan *et al.*, 1983; Chakravarthy *et al.*, 1981; Ahmad *et al.*, 1989) and *Azardirachta indica* (Bajaj and Srinivasan, 1998) has shown to have insulin-like action, regenerate β cells, stimulate insulin release from remnant pancreatic β cells, increase the sensitivity of peripheral insulin receptors and glucose uptake in these tissues *in vivo* and in vitro. Saponins isolated from *Gymnema sylvestre* (gymnamic acid IV) (Shimizu *et al.*, 1997) and *Trigonella foenum graecum* (diosgenin, protodioscin) (Madar, 1984) has been reported to inhibit intestinal glucose absorption, stimulate regeneration of pancreatic β cells and insulin secretion. Alkaloids isolated from *Aegle marmelos* (aegelin) (Narendra and Sweta, 2007), *Trigonella foenum gracecum* (trigonelline) (Madar, 1984), *vinca rosea* (catharantus, vindoline, vindolidine) (Chattopadhyay *et al.*, 1991) and *Eugenia jambolana* (jambosine) (Grover *et al.*, 2000) have shown antidiabetic activity by various mechanisms including α-glucosidase inhibitory activity and β cells regeneration. Curcumin, a phenolic yellow curcuminoid from *Curcuma longa* has been found to modulate nuclear PPARγ receptors, thus increasing the peripheral insulin sensitivity (Kuroda *et al.*, 2005). Saponins from *Trigonella foenum graecum* (Vijayakumar *et al.*, 2005) and a new constituent
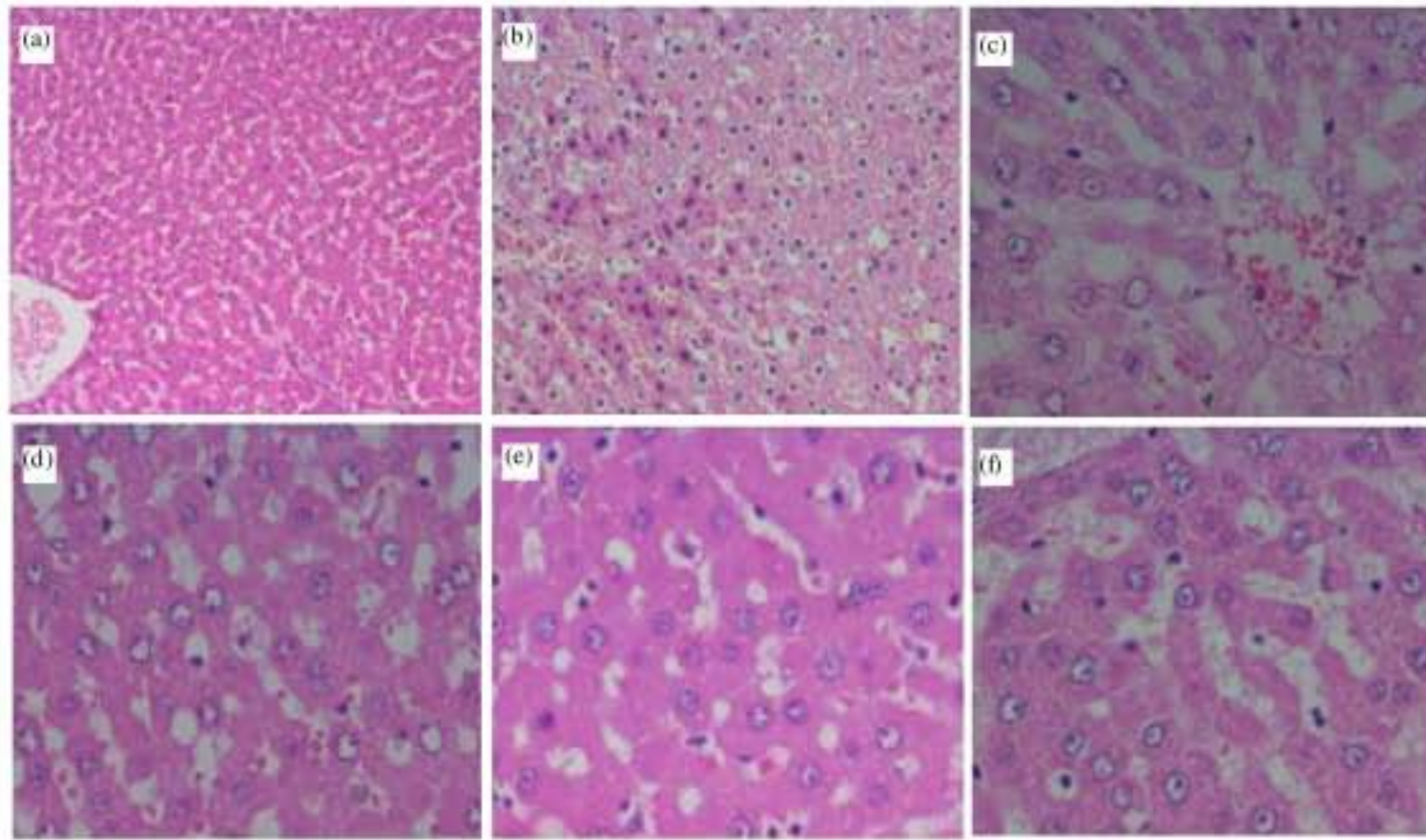
Fig. 7: Histopathological study of liver of diabetic animals. (a) Liver section of normal animal showing normal hepatic architecture (H and E X 200). (b) Showing liver section of vehicle treated diabetic animal with severe vacuolar degeneration and necrosis (H and E X 300). (c) Showing liver section of Gly-13-C 100 mg^{-1} kg treated diabetic animal (H and E X 400) with mild vacuolar degeneration and necrosis. (d) Showing liver section of Gly-13-C 200 mg^{-1} kg treated diabetic animal (H and E X 400) with increased eosinophilic granularity. (e) Showing liver section of Gly-13-C 400 mg^{-1} kg treated diabetic animal (H and E X 400) with normal hepatic lobules and homogenous cytoplasm. (f) Showing liver section of glibenclamide 600 μg^{-1} kg treated diabetic animal (H and E X 400) with mild degree of necrosis and vacuolar degeneration

7-O-α-L-rhamnopyranosyloxy-4'-methoxy-5-hydroxy isolated from *pterocarpus marsupium* (Dhanabal *et al.*, 2006) have been reported to stimulate glucose uptake by activating signal transduction that leads to increased PPARγ expression and GLUT4 translocation to the plasma membrane leading to the increased peripheral glucose utilization. Condensed tannins present in *Salacia reticulata* (mangiferin, epigallocatechin, ketolanol) (Yoshikawa *et al.*, 1998) have shown to inhibit α-glucosidase enzyme in the intestine, thus, halting the intestinal glucose absorption. Many researchers have concluded that polyphenolic constituents of *Trigonella foenum graecum* (Vats *et al.*, 2003) and *Eugenia jambolana* (Grover *et al.*, 2000) have capacity to modulate carbohydrate metabolizing enzymes such as glucokinase, hexokinase and phosphofructokinase in liver and skeletal muscle by improving pancreatic β cell function as well as exerting insulinomimetic action. Benzocoumarins from shilajit have demonstrated pancreatotrophic action (Gupta, 1966). Xanthones of *Swertia chirata* (swerchirin) was found to increase hepatic glycogen synthesis by stimulating insulin release from β cells (Sekar *et al.*, 1987). Thus, the potent antihyperglycemic action of Gly-13-C could be due to the ability of its individual constituents to modulate/act on various glucose metabolism-related

pathways. In present investigation, histopathological studies of liver tissue depicted in Fig. 7a-f have clearly demonstrated a significant protection against oxidative stress in Gly-13-C treated diabetic animals. Gly-13-C (400 mg^{-1} kg b.wt.) has shown maximum inhibition of degeneration and necrosis of hepatocytes indicating liver protection by Gly-13-C against free radical damage. The normalization of hepatic cellular architecture and reduced necrotic changes of hepatocytes can be attributed to the decreased lipid peroxidation in Gly-13-C treated diabetic animals. Eosinophilic cytoplasm was found to be elevated in dose dependent manner in Gly-13-C treated diabetic animals which may suggest the increased glycogen content in liver tissue when compared with vehicle treated diabetic animals. The hepatocytes protection offered by Gly-13-C 400 mg^{-1} kg treated diabetic animals was comparable to that of normal animals.

CONCLUSION

In conclusion, the significant antidiabetic and antioxidant activity of Gly-13-C observed in the present investigation could be the result of synergistic/potentiative action of its individual medicinal herbs and minerals since, they contain a diverse array of

active principles, which are able to target multiple mechanisms involved in the pathophysiology of diabetes. Gly-13-C has shown significant liver protection against ROS challenge. This indicates its protective role against oxidative damage to the vital organs such as liver. Therefore, it can be concluded that Gly-13-C has apparent supremacy in terms of multiple therapeutic approaches over standard drug glibenclamide in STZ induced diabetic experimental animals.

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