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Therapeutic Potential of *Gymnema sylvestre* Leaves in Streptozotocin Induced Diabetic Rats

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Abstract

Diabetes mellitus; a metabolic disorder is associated with a large number of lipid abnormalities. *Gymnema sylvestre* leaf powder (GLP) was supplemented at a low dose (LD) of 1.5mg/kg b.w. and a high dose (HD) of 3mg/kg b.w. to male albino wistar strain for a period of six weeks. Hyperlipidemia and diabetes were induced in experimental animals through dietary and pharmacological means. Results showed Gymnemea sylvestre leaves are rich in phytochemicals attributing for its antioxidant potential. *In vivo* study showed a marked improvement in serum and hepatic lipid profile of treated animals as compared to untreated ones. Feeding of Gymnemea sylvestre reduced oxidative stress and strengthened the antioxidant defence system thereby improving insulin levels in rats. The study indicated that intake of Gymnemea sylvestre can be used as a promising functional food for diabetics.

Key-Words: Gymnema sylvestre, Diabetes Mellitus, Antioxidants, Hyperlipidemia, Streptozotocin

Introduction

Diabetes mellitus; a metabolic disorder of multiple aetiology characterized by chronic hyperglycaemia is associated with a large number of lipid abnormalities. According to WHO, 171 million people worldwide are suffering from diabetes mellitus and the number is likely to increase up to 366 million by 2030 (WHO 1999). Diabetes and hyperlipidemia are intertwined and accretion and alterations in lipid- lipoprotein fractions in diabetics lead to derangements in metabolic and regulatory processes leading to increased production of free radicals and oxidative stress. This results in detrimental effects at cellular and tissue level thereby rendering the diabetics to deleterious clinical manifestations (Godin et al. 1988; Niskanen et al. 1995). Treatment of diabetes mellitus through insulin, hypoglycaemic drugs and hyperlipidemia through hypolipidemic drugs were the main focus of research for many years. However, the pertinent approach of majority of these drugs remained constrained owing to their limited action and undesirable side effects. Therefore it led to a shift in focus to alternative therapies based on dietary supplementation of bioactive compounds present in herbal products (Apostolidis et al. 2011; Prangthip et al. 2013).

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Gymnema sylvestre R.Br (Asclepiadaceae); a climbing woody plant, is widely grown in central and western India, Malaysia, Srilanka, Australia, Indonesia, Japan and Vietnam (Prakash et al. 1986). The plant has been extensively used in Ayurvedic medicine (Suttisri et al. 1995) since times immemorial to cure various ailments viz. gastrointestinal, respiratory, liver disorders, cardiovascular, eye, renal and skin diseases, diabetes mellitus and even for snake bites. The plant is gaining importance in the Western world and several products are being developed with therapeutic claims (Nakamura et al. 1999).

Gymnema sylvestre leaves have a bitter taste and disrupts the ability of the taste buds to sweetness (Warren and Pfaffmann 1959). The active ingredient in *Gymnema sylvestre* is gymnemic acid which acts as a structural analogue to glucose, thus inhibiting glucose uptake by intestinal mucosal cells thereby reducing blood glucose levels and insulin secretion. Animals supplemented with Gymnemic acids have shown alterations in glucose utilization and enzymatic activities in experimentally induced diabetic animals (Nakamura et al. 1999). Few studies report that gymnemic acid exerts hypolipidemia (Nakamura et al. 1999) (Shigematsu et al. 2001). The objective of the study was *in vitro and in vivo* evaluation of antioxidant activity of *Gymnema sylvestre* leaves in Streptozotocin (STZ) induced diabetic rats.

Material and Methods

Chemicals

All the chemicals used in the study were of analytical grade, procured from the credible concerns e.g.: Sigma, Merck, BDH and Qualigens. Chemicals of higher purity and of scarce availability were obtained from M/S chemical Co; St Louis USA.

Collection of plant material, preparation of Gymnemea sylvestre extract and phytochemical screening

Gymnemea Sylvestre leaves were collected from Banasthali University, Rajasthan, India. The leaves were shade dried; ground to a fine powder with an auto-mix blender and stored in air tight containers until the time of use. The G. Sylvestre leaf powder was defatted with petroleum ether. Dried samples of defatted powders were then subjected to extraction with various organic solvents (acetone, chloroform, ethanol, petroleum ether) and water. The *Gymnemea Sylvestre* aqueous extract were prepared by steeping suitable volume of dried leaves powder (1.5%w/v and 3%w/v) with boiled distilled water at 100^0C for 30 min, with a stirrer bar and laboratory stirrer/hot plate (Model: PC420, Corning Inc., USA) for efficient extraction. The extracts were then filtered through whatmann No. 1 filter paper, concentrated in a rotary evaporator and stored at- 20^0C. Filtered leaf extracts were concentrated and were subjected to qualitative tests for phytochemical screening of constituent's viz. alkaloids, anthraquinone, glycosides, flavonoids, saponins, tannins, sterols and steroids using standard protocols (Lala 1993; Brindha et al. 1981). Total phenols were estimated by the method of Folin-Ciocalteu's using gallic acid as standard (Julkunen-Tiitto 1985). Total phenolic content was expressed as µg gallic acid equivalent (GAE)/g of extract. Flavanoid content was determined by the method of (Zhishen et al. 1999), using catechin as standard, expressed as µg catechin equivalent (CE)/ g of extract. Total condensed tannins were estimated using the method of (Julkunen-Tiitto 1985), expressed as µg catechin equivalent (CE)/ g of extract. Total antioxidant activity was assessed by the method of (Kumaran and Joel Karunakaran 2007), expressed as equivalents of ascorbic acid. Free radical scavenging activity was measured with DPPH by (Yen and Chen 1995) and was calculated by:

Scavenging activity (%) =

$$[(\underline{\text{A sample}–\text{A sample blank}})]\times 100$$
$$[(\text{A control})]$$

Experimental Animals

The study protocol was approved by Institutional Animal Ethics Committee (IAEC) of the University constituted as per the directions of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). Healthy male albino wistar rats of 6 weeks age (50g) were procured from the small animal house of Chaudhary Charan Singh Haryana Agriculture University Hissar (CCSHAU), India. Each animal was housed individually in the polypropylene cage with sterilized wood chip bedding in a specific pathogen free animal house room under the constant environmental condition with 12 hour light and dark cycle, 22±1 ^{0}C temperature and 50 ±10% relative humidity. Animals were given the standard pellet diet (Hindustan Liver Ltd.,India) and water *ad libitum* during acclimatization period of 1 week. The diet contained 20% protein, 5% protein, 5% fat and 5% fibre, 60% carbohydrates and 10% mixture of vitamins and minerals. The diet served as normal fat diet (NFD) for control group. High fat high cholesterol diet (HFHC) was formulated incorporating 84.5% NFD; 15% coconut oil (w/w) and 0.5% cholesterol (w/w).

Induction of Diabetes Mellitus to Experimental Animals

Rats were rendered diabetic by single intraperitoneal injection of freshly prepared streptozotocin (45mg kg^{-1}) in 0.1 M citrate buffer (pH 4.5) in a volume of 1ml kg-^{1} body weight (Siddiqui et al. 1987). The control group received 1 ml citrate buffer as vehicle. The animals had free access to food and water and were given 5% glucose solution to drink overnight to counter the hypoglycemic shock. After 48-hours of STZ administration, blood glucose was evaluated in overnight fasting rats. Rats having blood glucose levels ranging between 200-300 mg/dl were considered to be diabetic and were selected for the study. Experimental dietary regime was started on the third day after STZ injection and continued for a period of six weeks.

Acute Toxicity Study

Acute oral toxicity was performed as per Organisation for Economic Cooperation and Development (OECD 2001). After the dietary supplementation of dried leaves of G. Sylvestre, animals were observed individually for general behavioural at least once during the first 30 minutes and periodically during the first 24 hours with special attention given during first four hours and daily thereafter for a period of 14 days.

Experimental Diets

Animals were randomly divided into three groups. The groups were fed on the following diets for a period of six weeks: NC (n=6), fed NFD and served as control; HFHC (n=6), fed High fat- high cholesterol diet; Diabetic rats (n=18) were randomly divided into three groups of six animals each, as under: DC, fed normal fat diet to serve as diabetic control; HFHC-GLP(LD);

fed HFHC + *G Sylvestre* leaf powder- Low Dose (1.5g/kg); HFHC-GLP (HD); fed *G Sylvestre* leaf powder- High Dose(3.0g/kg). Basal and experimental diets were were isoenergic (~3600C) and were freshly prepared weekly in a pathogen free sterilized room (Reeves et al. 1993) and were stored at -20^{0}C.

Biochemical Assays

The blood sample was collected from retrorbital plexus with heparinized capillary tubes fortnightly until the end of feeding schedule. 1ml of the blood sample was added to prechilled heparin coated vial and kept at 4^{0}C. The remaining blood sample was collected into two prechilled heparin coated and heparin uncoated vials and centrifuged at 3000g at 4^{0}C for 10 minutes to obtain plasma and serum respectively. At the end of experimental period, the animals were sacrificed by cervical decapitation. The organs were removed and weighed. Liver was excised, washed with ice cold isotonic saline and small part of the hepatic tissue was minced and used for enzyme activity assay and other biochemical evaluation. The remaining tissues were stored at -80 ^{0}C for further biochemical analysis.

Biochemical analysis of serum

Serum glucose was measured by the O-toluidine method (Sasaki et al. 1972). Insulin level was assayed by Enzyme Linked Immunosorbant Assay (ELISA) kit (Andersen et al. 1993). Glycosylated haemoglobin (HbA_1C) estimation was carried out by a modified colorimetric method of (Karunanayake and Chandrasekharan 1985). Serum total cholesterol (TC), triglycerides (TG) and HDL-cholesterol were estimated by using diagnostic kits (Span Diagnostic). VLDL and LDL-cholesterol were calculated as per (Friedewald et al. 1972) as: VLDL-C = TG/5 and LDL-C =TC – (HDL-C + VLDL-C).Atherogenic index was calculated by the following formula: AI = (Total-C - HDL-C)/HDL-C.

Serum oxidative stress biomarkers involved, lipid peroxidation (TBARS) (Ohkawa et al. 1979), red cell reduced glutathione (GSH) (Sedlak and Lindsay 1968), glutathione reductase (GR) (Goldberg and Spooner 1983) . Oxygen radical absorbance capacity (ORAC) in plasma was measured as Trolox equivalents (µmolTE/g) by the method of (Prior et al. 2003).

Biochemical analysis of Hepatic Tissue

Hepatic lipids were extracted using the procedure of (Folch et al. 1957) in 2:1(v/v) ratio of chloroform and methanol. The extracts were subjected to drying under nitrogen and were resuspended in isopropanol through ultrasonic cleaner. Hepatic lipids were estimated using commercial kits (Span Diagnostic).

For enzyme activity assay, 0.8-1.0g of hepatic tissue was minced and homogenized in 10 times its volume of 0.2M/L tris HCl (pH=8.0) containing 0.5M/L $CaCl_2$ using Potter Elevehjem apparatus at 0-4^{0}C using motor driven Teflon pestle rotated at 3000rpm. The homogenate was centrifuged at 10000g for 30 minutes at 4^{0}C and 3/4th of the volume was carefully drawn using Pasteur's pipette. The supernatants were stored at -80^{0}C until analysis. Enzyme assay involved, lipid peroxidation (TBARS) (Ohkawa et al. 1979), reduced glutathione (GSH) (Sedlak and Lindsay 1968) and hepatic antioxidant enzymes viz. glutathione peroxidase (GSHPx) (Necheles et al.); catalase (CAT) (Luck 1971) and superoxide dismutase (SOD) (Kono 1978), glutathione -S-transferase(GST) (Habig et al. 1974) The liver supernatant was extracted and used for the estimation of liver glycogen (Montgomery 1957). Protein content was measured using Bio-Rad protein assay kit and BSA as standard.

Statistical Analysis

Data was analysed using SPSS 13.0 version. Results were expressed as mean ± SEM of 6 rats. Statistical analysis of data involved ANOVA- one way and Student's-'t' test. The values with $p \leq 0.05$ were considered as statistically significant.

Results and Discussion

Phytochemical screening and antioxidant activity in vitro

Gymnemea sylvestre leaf extracts revealed the presence of alkaloids, anthraquinones, catechins, flavonoids, glycosides, phenols, steroids and saponins and tannins (Table 1).

High phenol, flavanoid and tannin content contribute to high scavenging capacity of *Gymnemea sylvestre* leaves, thereby reducing oxidative stress. Free radical-mediated oxidative stress has been one of the prime factors in the pathogenesis of diabetes and other related diseases. Table 2 depicts high total antioxidant activity and DPPH radical scavenging capacity of *Gymnemea sylvestre* leaf extracts, which substantiates the presence of bioactive compounds (Rachh et al. 2009); (Sarkar et al. 2009).

Body weights, Organ Weights, Relative food intake and Food Efficiency Ratio

The effect of Gymnemea sylvestre on growth parameters is shown in Table 3. Body weights of HFHC diet fed group increased and diabetic controls decreased significantly ($p \leq 0.05$) during the feeding period. Inclusion of G sylvestre leaf powder along with HFHC diets to diabetic rats showed a decreasing trend in body weights indicating that GLP can reduce body weight gain in the ones partaking, high fat diets. The weight loss in diabetic rats is associated with decreased appetite, muscle wasting and increased catabolism of tissue proteins (Ozsoy-Sacan et al. 2006). Relative

organ weights (heart, lung and kidney) (results not shown) of all the treatment groups showed no significant change, however, relative weight of liver increased significantly ($p \leq 0.05$) in HFHC group (31.2 ±0.58mg/g) as compared to the normal control group (23.4 ±0.91mg/g). G. Sylvestre treated rats ($p \leq 0.05$) showed a significant decrease in relative liver size (RLS). Dietary fat is one of the plausible causes of obesity and other related diseases. Several animal studies have shown that intake of high fat and high cholesterol diets induces hyperlipidemia and fatty infiltration of liver (Yang et al. 2010); (Chang et al. 2013) and antioxidant supplemented diets counteracts the effect (Sarvanan and Pari 2007). The relative food consumption (RFC) of animals during experimental period was not different ($p \geq 0.05$) among various treatments. However, the intake of food remarkably ($p \leq 0.05$) increased and decreased in HFHC group and diabetic controls respectively. The food efficiency in the HFHC fed group was significantly higher ($p \leq 0.05$) as compared to GLP supplemented diets.

Effect of Gymnemea sylvestre leaves on serum glucose, insulin levels, glycated haemoglobin and glycogen

The blood glucose level was monitored at two weeks interval. Fig.1 depicts an increasing trend in serum glucose levels of diabetic controls and HFHC fed group during feeding regime period and showed a marked increase at the end as compared to normal controls. Supplementation of G.Sylvestre leaves to HFHC diets apparently lowered blood glucose levels ($p \leq 0.05$). Hypoglycaemic effect of bioactive compounds in G. sylvestre has been reported earlier (Suttisri et al. 1995); (Thakur et al. 2012).

Serum insulin levels markedly increased (HFHC-GLP-HD,13.7±0.14g/dl; HFHC-GLP-LD, 14.6±0.27g/dl) with concomitant decrease in HbA1c (HFHC-GLP-HD, 9.1 ± 0.82%; HFHC-GLP-LD, 5.3±0.21%) levels by regulating blood glucose levels thereby restoring the glycogen content (HFHC-GLP-HD, 36.8±0.42mg/g; HFHC-GLP-LD, 44.5±0.23mg/g) in animals reared on HFHC-GLP diets as compared to the DC(3.7 ± 0.45g/dl; 11.8 ± 0.83% and 26.4±0.58 mg/g) and HFHC groups (21.4±0.21g/dl; 4.9 ±0.18% and 54.8±0.53mg/g), indicating that the leaves of GS could stimulate the production of insulin in vivo (Table 4). (Ahmed et al. 2010) concluded of G. sylvestre leaf and callus extracts can be used as strong herbal remedies and suggested that they may be capable of fully restoring pancreatic -cells function and thus curing type I diabetes. Similar results were obtained in another study by (Al-Romaiyan et al. 2010). The hypoglycaemic effect of G sylvestre probably may be due to the insulin release from intact β-cells or antidiabetic effect of the bioactive compounds present in the Gymnemea sylvestre leaves. The study conducted by (Baskaran et al. 1990), reported raised insulin levels in the serum of the patients suggesting that β cells may have regenerated or repaired. (Persaud et al. 1999) assessed the alcoholic extract of *G. sylvestre* on insulin secretion from the islets of langerhans and several pancreatic β-cell lines of rats and indicated that the extract stimulated insulin release from β-cells and islets was due to increased cell permeability.

Effect of Gymnemea sylvestre leaves on Serum Lipid-Lipoprotein Fractions and Hepatic lipids

HFHC diets showed a significant increase ($p \leq 0.05$) in serum and hepatic lipids (total cholesterol and triglycerides) as shown in Fig. 2 (A and B). Animals fed on supplemented diets showed a significant decrease ($p \leq 0.05$) in serum and hepatic lipid profile as compared to the rats reared on unsupplemented diets. High fat diet adversely affects the lipid profile (Lavie and Milani 2003) and induces hyperlipidemia. Diabetic rats have increased activity of HMG CoA reductase resulting in hypercholesterolemia (Young et al. 1982). Lowering of lipids and cholesterol in treatment groups supplemented by G. Sylvestre leaves can be attributed to inhibition of enzymes required for fatty acid and cholesterol biosynthesis (Chi et al. 1982); (Jangra and M. 2013). The low density lipoproteins (LDL-C) and very low density lipoproteins cholesterol (VLDL-C) apparently increased with a marked decrease in high density lipoprotein cholesterol (HDL-C) in HFHC fed and diabetic rats. Administration of G.Sylvestre leaf powders along with HFHC diets to diabetic rats showed a decrease ($p \leq 0.05$) in lipoprotein fraction (LDL-C and VLDL-C) with a concomitant increase in high density lipoprotein cholesterol (HDL-C) (Fig. 2 A). This result was in agreement with (Daisy et al. 2009) and (Aralelimath and Bhise 2012) who reported that increasing insulin secretion after administration of G. sylvestre extract led to a decrease of cholesterogenesis and fatty acid synthesis. This result was supported also by (Mall et al. 2009) who reported that G. sylvestre decreases total cholesterol, LDL-cholesterol, VLDL-cholesterol and triglyceride levels in diabetic rats and that could be due to the presence of hypolipidemic agent such as sitosterol in the aqueous leaf extract.

Atherogenic index (AI) indicates the risk for the deposition of foam cells, plaque, fatty infiltration or lipids in heart, coronaries, aorta, liver and kidney (Basu et al. 2007). The higher the AI, the higher is the risk of oxidative damage to these organs. Atherogenic index

significantly increased in HFHC and diabetic control rats. The atherogenic power of high fat intake in animals have been confirmed in earlier studies (Narasimhamurthy and Raina 1999). The effect was however, neutralized by supplementing HFHC diets with GLP thus, positively affecting the lipoprotein status of the experimental animals. Decreasing levels of triglyceride, cholesterol and LDL-cholesterol and increasing level of HDL-cholesterol might be due to an increase in insulin which caused an increased activity of lipoprotein lipase (Facilitated chylomicron transport through cell membranes) and a decreased activity of hormone-sensitive lipase (converted neutral fats into free fatty acids). This is in agreement with the findings reported by (El Shafey et al. 2013).

Effect of Gymnemea sylvestre leaves on Oxidative stress and antioxidant enzymes

Several animal and clinical trials have shown that obesity induced by high dietary fat intake, is a predisposing factor for diabetes and is associated with weakening of antioxidant defence system (Liou et al. 1993); (Olusi 2002). The imbalance created between the oxidants and antioxidants disturbs the equilibrium leading to oxidative stress. Bioactive compounds from various fruits, vegetables and herbs are being utilized as antioxidants for preventing oxidative damage in living systems and to delay the onset of degenerative diseases (Kaur and Kapoor 2001). GSH constitutes the first line of defence against free radicals at the cellular and tissue levels to protect against the toxic effects of lipid peroxidation. It helps in the maintenance of thiols in proteins and also acts as a substrate for other glutathione dependent enzymes viz. glutathione peroxidase (GSHPx) and glutathione –S- transferase (GST). Serum TBARS, an index of lipid peroxidation, significantly increased with simultaneous decrease in red cell reduced glutathione in HFHC diet fed and DC animals ($p \leq 0.05$) as compared to the normal fat fed ones (Table 5). Inclusion of G. sylvestre leaves to HFHC diets reversed the effect and significantly decreased TBARS levels and increased GSH ($p \leq 0.05$) levels. Increased TBARS levels indicate increased susceptibility to lipid peroxidation. (Kang et al. 2012) reported that feeding G. sylvestre extract to diabetic rats decreased lipid peroxidation levels by 31.7% in serum. A high free radical activity as a result of increased fat and cholesterol intake initiates lipid peroxidation forming lipid hydroperoxides. The increase in lipid peroxidation as a consequence of increased dietary fat intake has also been reported in earlier studies (Chauhan et al. 2010); (Chauhan et al. 2012). The GSH-dependent antioxidant enzymes activities GSHPx, GR and GST showed marked reductions with a concomitant increase in TBARS levels. Results indicate that GSH content was depleted in the HFHC and DC rats to counteract the free radicals generated and were restored to near normal levels in Gymnemea sylvestre treated rats.

Furthermore, HFHC and DC groups showed a significant reduction in antioxidant enzymatic activity ($p \leq 0{\cdot}05$) in hepatic tissue as compared to normal control group. Superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSHPx) helps in scavenging of free radicals thus strengthening the defence system. A significant decrease ($p \leq 0.05$) was observed in superoxide dismutase and catalase in untreated rats. Treatment with GLP neutralized the effect of high fat and high cholesterol and STZ, thereby restoring the enzymic activities to normal. The results are consistent with previous studies whereby the TBARS level increases with simultaneous decrease in GSH and antioxidant enzymes in STZ induced diabetes (Chauhan et al. 2010; Chauhan et al. 2012).

Plasma ORAC significantly ($p \leq 0.05$) decreased in HFHC and DC groups. Supplementation of Gynemea sylvestre leaves markedly improved the levels. Similar findings have been observed in previous studies, resulting in significant decrease in the plasma ORAC status of the STZ control group when compared with the normal control group while it significantly increased in the diabetic rats treated with red palm oil and roobios tea extract singly and in blends (Ayeleso et al. 2014). A possible reason for this might be high content of phytochemicals in Gymnemea sylvestre leaves which attenuates the effect of high fat diet and STZ in diabetic rats and suggest their ability to boost antioxidant levels in diabetic conditions.

G. Sylvestre have an antioxidative, antihyperlipidemic and antidiabetic potential in STZ induced diabetes. The bioactive compounds present in the leaf of G.Sylvestre can quench free radicals, and protect the cellular and tissue damage by oxidative stress. G.Sylvestre can be used as a promising functional food for various chronic diseases.

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References

1. Ahmed, A. B., A. S. Rao, and M. V. Rao. 2010. In vitro callus and in vivo leaf extract of Gymnema sylvestre stimulate β-cells regeneration and anti-diabetic activity in Wistar rats. *Phytomedicine* 17 (13):1033-1039.

2. Al-Romaiyan, A., B. Liu, H. Asare-Anane, C. R. Maity, S. K. Chatterjee, N. Koley, T. Biswas, A. K. Chatterji, G. C. Huang, S. A. Amiel, S. J. Persaud, and P. M. Jones. 2010. A novel Gymnema sylvestre extract stimulates insulin secretion from human islets in vivo and in vitro. *Phytother Res* 24 (9):1370-1376.
3. Andersen, L., B. Dinesen, P. N. Jørgensen, F. Poulsen, and M. E. Røder. 1993. Enzyme immunoassay for intact human insulin in serum or plasma. *Clin Chem* 39 (4):578-582.
4. Apostolidis, E., L. Li, C. Lee, and N. P. Seeram. 2011. In vitro evaluation of phenolic-enriched maple syrup extracts for inhibition of carbohydrate hydrolyzing enzymes relevant to type 2 diabetes management. *Journal of Functional Foods* 3 (2):100-106.
5. Aralelimath, V. R., and S. B. Bhise. 2012. Anti-diabetic effects of Gymnema sylvestre extract on streptozotocin induced diabetic rats and possible β-cell protective and regenerative evaluations. *Digest Journal of Nanomaterials and Biostructures* 7.
6. Ayeleso, A., N. Brooks, and O. Oguntibeju. 2014. Modulation of antioxidant status in streptozotocin-induced diabetic male Wistar rats following intake of red palm oil and/or rooibos. *Asian Pac J Trop Med* 7 (7):536-544.
7. Baskaran, K., B. Kizar Ahamath, K. Radha Shanmugasundaram, and E. R. Shanmugasundaram. 1990. Antidiabetic effect of a leaf extract from Gymnema sylvestre in non-insulin-dependent diabetes mellitus patients. *J Ethnopharmacol* 30 (3):295-300.
8. Basu, M., R. Prasad, P. Jayamurthy, K. Pal, C. Arumughan, and R. C. Sawhney. 2007. Anti-atherogenic effects of seabuckthorn (Hippophaea rhamnoides) seed oil. *Phytomedicine* 14 (11):770-777.
9. Brindha, P., B. Sasikala, and K. K. Purusothaman. 1981. Pharmacognostic studies on merugan kizhangu. *Bulletin of Medico-Ethno-Botanical Research* 3 (1):84-96.
10. Chang, Y.-Y., D.-J. Yang, C.-H. Chiu, Y.-L. Lin, J.-W. Chen, and Y.-C. Chen. 2013. Antioxidative and anti-inflammatory effects of polyphenol-rich litchi (Litchi chinensis Sonn.)-flower-water-extract on livers of high-fat-diet fed hamsters. *Journal of Functional Foods* 5 (1):44-52.
11. Chauhan, K., S. Sharma, K. Rohatagi, and B. Chauhan. 2012. Antihyperlipidemic and antioxidative efficacy of Catharanthus roseus Linn (Sadabahar) in Streptozotocin induced diabetic rats. *Asian Journal of Pharmaceutical and Health Sciences* 2 (1):235-243.
12. Chauhan, K., Sharma S., B. Chauhan, and G. Bajaj. 2010. Biochemical evaluation of lipid and oxidative stress modulating effects of neutraceuticals. *Inventi Impact Neutraceuticals* 1 (2):44-50.
13. Chi, M. S., E. T. Koh, and T. J. Stewart. 1982. Effects of garlic on lipid metabolism in rats fed cholesterol or lard. *J Nutr* 112 (2):241-248.
14. Daisy, P., J. Eliza, and K. A. Mohamed Farook. 2009. A novel dihydroxy gymnemic triacetate isolated from Gymnema sylvestre possessing normoglycemic and hypolipidemic activity on STZ-induced diabetic rats. *J Ethnopharmacol* 126 (2):339-344.
15. El Shafey, A. A. M., M. M. El-Ezabi, M. M. E. Seliem, H. H. M. Ouda, and D. S. Ibrahim. 2013. Effect of Gymnema sylvestre R. Br. leaves extract on certain physiological parameters of diabetic rats. *Journal of King Saud University - Science* 25 (2):135-141.
16. Folch, J., M. Lees, and G. H. Sloane Stanley. 1957. A simple method for the isolation and purification of total lipides from animal tissues. *J Biol Chem* 226 (1):497-509.
17. Friedewald, W. T., R. I. Levy, and D. S. Fredrickson. 1972. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem* 18 (6):499-502.
18. Godin, D. V., S. A. Wohaieb, M. E. Garnett, and A. D. Goumeniouk. 1988. Antioxidant enzyme alterations in experimental and clinical diabetes. *Mol Cell Biochem* 84 (2):223-231.
19. Goldberg, D. M., and R. J. Spooner. 1983. *Glutathione Reductase, In: Methods in Enzymatic Analysis. :* . Germany: VCH Weinhem,.
20. Habig, W. H., M. J. Pabst, and W. B. Jakoby. 1974. Glutathione S-transferases. The first enzymatic step in mercapturic acid formation. *J Biol Chem* 249 (22):7130-7139.
21. Jangra, M., Shama S.,, and K. M. 2013. Ameliorative potential of Vigna mungo seeds on hyperglycaemia mediated oxidative stress and hyperlipidemia in STZ diabetic rats. *International Journal of Green Pharmacy*. :266-272.

22. Julkunen-Tiitto, R. 1985. Phenolic constituents in the leaves of northern willows: methods for the analysis of certain phenolics. *Journal of Agricultural and Food Chemistry* 33 (2):213-217.
23. Kang, M. H., M. S. Lee, M. K. Choi, K. S. Min, and T. Shibamoto. 2012. Hypoglycemic activity of Gymnema sylvestre extracts on oxidative stress and antioxidant status in diabetic rats. *J Agric Food Chem* 60 (10):2517-2524.
24. Karunanayake, E. H., and N. V. Chandrasekharan. 1985. . An evaluation of a colorimetric procedure for the estimation of glycosylated haemoglobin and establishment of reference values for Sri lanka. . *Journal National Science Council Sri Lanka* 13:235-258.
25. Kaur, C., and H. C. Kapoor. 2001. Antioxidants in fruits and vegetables – the millennium's health. *International Journal of Food Science & Technology* 36 (7):703-725.
26. Kono, Y. 1978. Generation of superoxide radical during autoxidation of hydroxylamine and an assay for superoxide dismutase. *Arch Biochem Biophys* 186 (1):189-195.
27. Kumaran, A., and R. Joel Karunakaran. 2007. In vitro antioxidant activities of methanol extracts of five Phyllanthus species from India. *LWT - Food Science and Technology* 40 (2):344-352.
28. Lala, P. K. 1993. *Lab Manuals of Pharmacognosy* edited by t. Edition. Calcutta, India CSI Publishers. .
29. Lavie, C. J., and R. V. Milani. 2003. Obesity and cardiovascular disease: the hippocrates paradox? *J Am Coll Cardiol* 42 (4):677-679.
30. Liou, W., L. Y. Chang, H. J. Geuze, G. J. Strous, J. D. Crapo, and J. W. Slot. 1993. Distribution of CuZn superoxide dismutase in rat liver. *Free Radic Biol Med* 14 (2):201-207.
31. Luck, H. 1971. *Catalase, In: Methods of enzymatic analysis.* . edited by B. H. (eds). New York: London: Academic Press.
32. Mall, G. K., P. K. Mishra, and V. Prakash. 2009. Antidiabetic and hypolipidemic activity of Gymnema Sylvestre in alloxan induced rats. . *Global Journal Biotechnology Biochemistry* 4 (1):37-42.
33. Montgomery, R. 1957. Determination of glycogen. . *Archives Biochemistry Biophysics* 67:378-386.
34. Nakamura, Y., Y. Tsumura, Y. Tonogai, and T. Shibata. 1999. Fecal steroid excretion is increased in rats by oral administration of gymnemic acids contained in Gymnema sylvestre leaves. *J Nutr* 129 (6):1214-1222.
35. Narasimhamurthy, K., and P. L. Raina. 1999. Long term feeding effects of heated and fried oils on lipids and lipoproteins in rats. *Mol Cell Biochem* 195 (1-2):143-153.
36. Necheles, T. F., T. A. Boles, and D. M. Allen. Erythrocyte glutathione-peroxidase deficiencyand hemolytic disease of the newborn infant. *The Journal of Pediatrics* 72 (3):319-324.
37. Niskanen, L. K., J. T. Salonen, K. Nyyssönen, and M. I. Uusitupa. 1995. Plasma lipid peroxidation and hyperglycaemia: a connection through hyperinsulinaemia? *Diabet Med* 12 (9):802-808.
38. OECD. 2001. Guidelines of testing of chemicals, 423 acute oral toxicity (acute toxic class method). .
39. Ohkawa, H., N. Ohishi, and K. Yagi. 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal Biochem* 95 (2):351-358.
40. Olusi, S. O. 2002. Obesity is an independent risk factor for plasma lipid peroxidation and depletion of erythrocyte cytoprotectic enzymes in humans. *Int J Obes Relat Metab Disord* 26 (9):1159-1164.
41. Ozsoy-Sacan, O., R. Yanardag, H. Orak, Y. Ozgey, A. Yarat, and T. Tunali. 2006. Effects of parsley (Petroselinum crispum) extract versus glibornuride on the liver of streptozotocin-induced diabetic rats. *J Ethnopharmacol* 104 (1-2):175-181.
42. Persaud, S. J., H. Al-Majed, A. Raman, and P. M. Jones. 1999. Gymnema sylvestre stimulates insulin release in vitro by increased membrane permeability. *Journal of Endocrinology* 163 (2):207-212.
43. Prakash, A. O., S. Mathur, and R. Mathur. 1986. Effect of feeding Gymnema sylvestre leaves on blood glucose in beryllium nitrate treated rats. *J Ethnopharmacol* 18 (2):143-146.
44. Prangthip, P., R. Surasiang, R. Charoensiri, V. Leardkamolkarn, S. Komindr, U. Yamborisut, A. Vanavichit, and R. Kongkachuichai. 2013. Amelioration of hyperglycemia, hyperlipidemia, oxidative stress and inflammation in steptozotocin-induced

diabetic rats fed a high fat diet by riceberry supplement. *Journal of Functional Foods* 5 (1):195-203.

45. Prior, R. L., H. Hoang, L. Gu, X. Wu, M. Bacchiocca, L. Howard, M. Hampsch-Woodill, D. Huang, B. Ou, and R. Jacob. 2003. Assays for hydrophilic and lipophilic antioxidant capacity (oxygen radical absorbance capacity (ORAC(FL))) of plasma and other biological and food samples. *J Agric Food Chem* 51 (11):3273-3279.
46. Rachh, P. R., S. R. Patel, H. V. Hirpara, M. R. Rupareliya, M. R. Rachh, A. S. Bhargava, Patel, N.M.,, and D. C. Modi. 2009. In vitro evaluation of antioxidant activity of Gymnemea Sylvestre leaf extract. . *Romanian Journal of Biology - Plant Biology* 54:141-148.
47. Reeves, P. G., F. H. Nielsen, and G. C. Fahey. 1993. AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet. *J Nutr* 123 (11):1939-1951.
48. Sarkar, R., B. Hazra, S. Biswas, and N. Mandal. 2009. Evaluation of the in vitro antioxidant and iron chelating activity of Gymnemea sylvestre. . *Pharmacologyonline* 3:851-865.
49. Sarvanan, G., and L. Pari. 2007. Effect of Syzygium cumini bark extract on plasma and tissue glycoproteins in STZ induced diabetic rats. *Journal of Cell and Tissue Research.* 7 (1):881-887.
50. Sasaki, T., S. Masty, and A. Sonae. 1972. Effect of acetic acid concentration on the color reaction in the O-Toludine – boric acid method of blood glucose determination. . *Rinsho. Kagaku* 1:346-353.
51. Sedlak, J., and R. H. Lindsay. 1968. Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent. *Anal Biochem* 25 (1):192-205.
52. Shigematsu, N., R. Asano, M. Shimosaka, and M. Okazaki. 2001. Effect of administration with the extract of Gymnema sylvestre R. Br leaves on lipid metabolism in rats. *Biol Pharm Bull* 24 (6):713-717.
53. Siddiqui, O., Y. Sun, J. C. Liu, and Y. W. Chien. 1987. Facilitated transdermal transport of insulin. *J Pharm Sci* 76 (4):341-345.
54. Suttisri, R., I. S. Lee, and A. D. Kinghorn. 1995. Plant-derived triterpenoid sweetness inhibitors. *J Ethnopharmacol* 47 (1):9-26.
55. Thakur, G. S., R. Sharma, B. S. Sanodiya, Pandey M., G. B. K. S. Prasad, and P. S. B. 2012. Gymnema sylvestre: An Alternative Therapeutic Agent for Management of Diabetes. *Journal of Applied Pharmaceutical Science* 2 (12):001-006.
56. Warren, R. M., and C. Pfaffmann. 1959. Suppression of sweet sensitivity by potassium gymnemate. *J Appl Physiol* 14 (1):40-42.
57. WHO. 1999. Report of WHO/IDF Consultation: Definition, Diagnosis and classification of Diabetes mellitus and its complications. Retrieved 2010 August from WHO. http://www.staff.ncl.ac.uk/philip.home/who_dmg.pdf. .
58. Yang, D.-J., Y.-Y. Chang, C.-L. Hsu, C.-W. Liu, Y. Wang, and Y.-C. Chen. 2010. Protective effect of a litchi (Litchi chinensis Sonn.)-flower-water-extract on cardiovascular health in a high-fat/cholesterol-dietary hamsters. *Food Chemistry* 119 (4):1457-1464.
59. Yen, G.-C., and H.-Y. Chen. 1995. Antioxidant Activity of Various Tea Extracts in Relation to Their Antimutagenicity. *Journal of Agricultural and Food Chemistry* 43 (1):27-32.
60. Young, N. L., C. D. Saudek, and S. A. Crawford. 1982. Total hydroxymethylglutaryl CoA reductase activity in the small intestine and liver of insulin-deficient rats. *J Lipid Res* 23 (2):266-275.
61. Zhishen, J., T. Mengcheng, and W. Jianming. 1999. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chemistry* 64 (4):555-559.

Table 1: Phytochemical screening of Gymnema sylvestre leaf extracts

Bioactive compounds	Extracts				
	Acetone	Chloroform	Ethanol	Petroleum Ether	Water
	Leaf	Leaf	Leaf	Leaf	Leaf
Alkaloids	-	+	+	-	+
Anthraquinones	+	-	+	-	-
Catechin	+	-	+	-	-
Coumarin	+	+	+	-	-
Flavonoids	-	-	+	-	-
Phenols	+	+	+	-	-
Quinones	-	-	-	-	-
Saponins	+	-	-	+	+
Steroids	-	+	+	+	-
Tannins	-	-	+	+	-
Terpenoids	-	-	+	-	-
Xanthoprotein	+	-	+	+	+
Carbohydrates	+	+	+	-	+
Glycosides	-	-	+	-	-

+ Presence, - Absence

Table 2: Phytochemical content and antioxidant activity of Gymnemea sylvestre leaves *in vitro*

Extracts (w/v)(%)	Phytochemical content (µg/g)			DPPH %	TEAC (µg/g)
	Total Phenols (µgGAE/g extract)	Total Flavanoids (µg CE/g extract)	Total Condensed Tannins (µg C E/ g extract)		
GLP-LD (1.5)	274 ±2.17	132.4 ±8.41	115.4 ±3.21	48.4 ±1.01	10.2 ±0.07
GLP-HD (3)	441±3.24	245±2.35	137±3.14	52.3±2.23	12.1±0.84

Table 3: Effect of Gymnemea sylvestre on growth parameters in STZ –Induced Diabetic Rats

Treatment	NC	HFHC	DC	HFHC- GLP (LD)	HFHC-GLP (HD)
Initial Body weight (g)	50.6±4.08	50.2±2.73	50.5±2.52	50.8±3.76	50.6±4.47
Final body Weight(g)	173.3±3.29	206.8±2.57[a]	123.6±6.32[a]	157.3±3.68[bc]	158.1±3.84[bc]
RLS (mg/g rat)	23.4±1.21	31.2±2.01 [a]	21.7±2.14[NS]	25.1±1.87 [bc]	22.7±1.53 [b]
RFC (g/100g body wt)	6.2± 0.61	7.1± 0.50[a]	5.9±0.24[a]	6.2± 0.61[bc]	6.4±0.54[bc]
FER	0.14±0.001	0.21±0.004 [a]	0.12±0.003[NS]	0.16±0.001 [NS]	0.15±0.002 [NS]

Values are Mean±SEMof 6 rats in each group
RLS-Relative Liver Size
RFC-Relative food consumption
FER-Food Efficiency Ratio Body Weight Gain (g/day)/ Food Intake (g/day)
Group NC is compared with Groups HFHC and DC
Other Treatment groups are compared with HFHC and DC
[a]P≤0.05 : Significantly different from NC
[b]P≤0.05 : Significantly different from HFHC
[c]P≤0.05: Significantly different from DC
NS: Non Significant

Table 4: Effect of Gymnemea sylvestre on glycogen, insulin and glycated haemoglobin in STZ –Induced Diabetic Rats

Treatment	NC	HFHC	DC	HFHC- GLP (LD)	HFHC-GLP (HD)
Glycogen (mg/g wet tissue)	43.4 ± 0.24	54.8±0.53 [a]	26.4±0.58 [a]	36.8±0.42 [bc]	44.5±0.23 [bc]
Insulin (g/dl)	18.2± 0.27	21.4±0.21[a]	3.7 ± 0.45[a]	14.6±0.27 [bc]	13.7±0.14[bc]
HBA_1C (%)	4.1 ± 0.27	4.9 ±0.18[NS]	11.8 ± 0.83 [a]	9.1 ± 0.82 [bc]	5.3±0.21[c]

Values are Mean±SEMof 6 rats in each group
HBA_1C-Glycated Haemoglobin
Group NC is compared with Groups HFHC and DC
Other Treatment groups are compared with HFHC and DC
[a]P≤0.05 : Significantly different from NC
[b]P≤0.05 : Significantly different from HFHC
[c]P≤0.05: Significantly different from DC
NS: Non Significant

Table 5: Effect of Gymnemea sylvestre on oxidative stress, antioxidant enzymes and oxygen radical absorbance capacity (ORAC) of STZ induced diabetic rats

Treatment	NC	HFHC	DC	HFHC- GLP (LD)	HFHC-GLP (HD)
Serum					
TBARS (nM/100ml)	22.3±0.30	44.3±0.52 [a]	52.3±0.52 [a]	35.8±0.10 [bc]	31.1±0.57 [bc]
GSH (mM/100ml)	43.2±0.17	28.8±0.11 [a]	24.7± 0.44 [a]	35.6±0.21 [bc]	38.4±0.41 [bc]
GR (nM/100ml)	16.2±0.29	11.9±0.78 [a]	11.3±0.56 [a]	13.9±012 [bc]	13.9 ±0.87 [bc]
Total ORAC (µmolTE/100ml)	423.2±7.21	322.4±7.49[a]	331.4±8.43[a]	341.4±6.48[bc]	397.6±5.84[bc]
Liver					
TBARS (nm of TBARS/mg protein)	0.31±0.80	0.91±0.74 [a]	0.98±0.31[a]	0.78±0.14 [bc]	0.70±0.32 [bc]
GSH (mM/100g)	377.5±0.74	257±1.58 [a]	239.7±3.02 [a]	348.6±2.87 [bc]	353.2±1.31 [bc]
SOD (Units/ mg protein)	4.2±0.21	2.9±0.25 [a]	1.7±0.23 [a]	3.1±0.84 [c]	4.0±0.35 [bc]
CAT (value x10-3 unit/mg protein)	98.3±1.90	56.0±1.18 [a]	49.9±2.32 [a]	79.8.5±2.62[bc]	91.2±1.24 [bc]
GSHPx (GSH utilized per minute/ mg protein)	7.8±0.85	5.8±0.38 [a]	5.2±0.20 [a]	6.8±0.43 [bc]	7.1±0.81 [bc]
GST (nM/minute/mg protein	510±4.84	292±5.23[a]	278.1±4.91[a]	412±3.75[bc]	448.2±5.21[bc]

Values are Mean±SEMof 6 rats in each group
Group NC is compared with Groups HFHC and DC
Other Treatment groups are compared with HFHC and DC
[a]P≤0.05 : Significantly different from NC
[b]P≤0.05 : Significantly different from HFHC
[c]P≤0.05: Significantly different from DC
NS: Non Significant

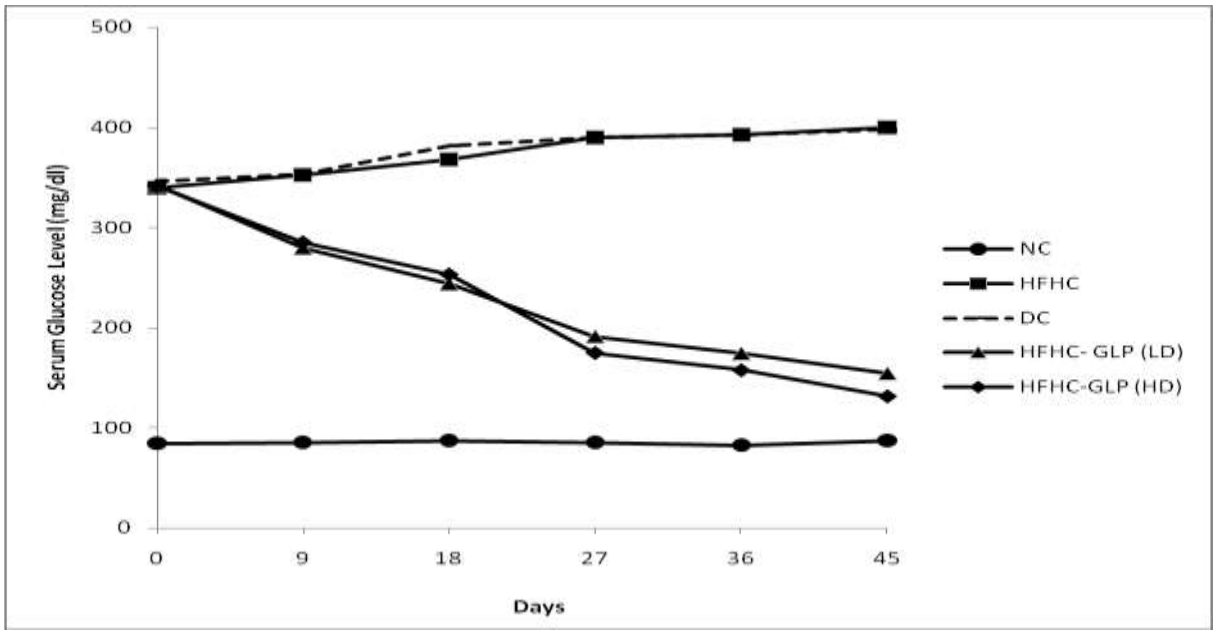
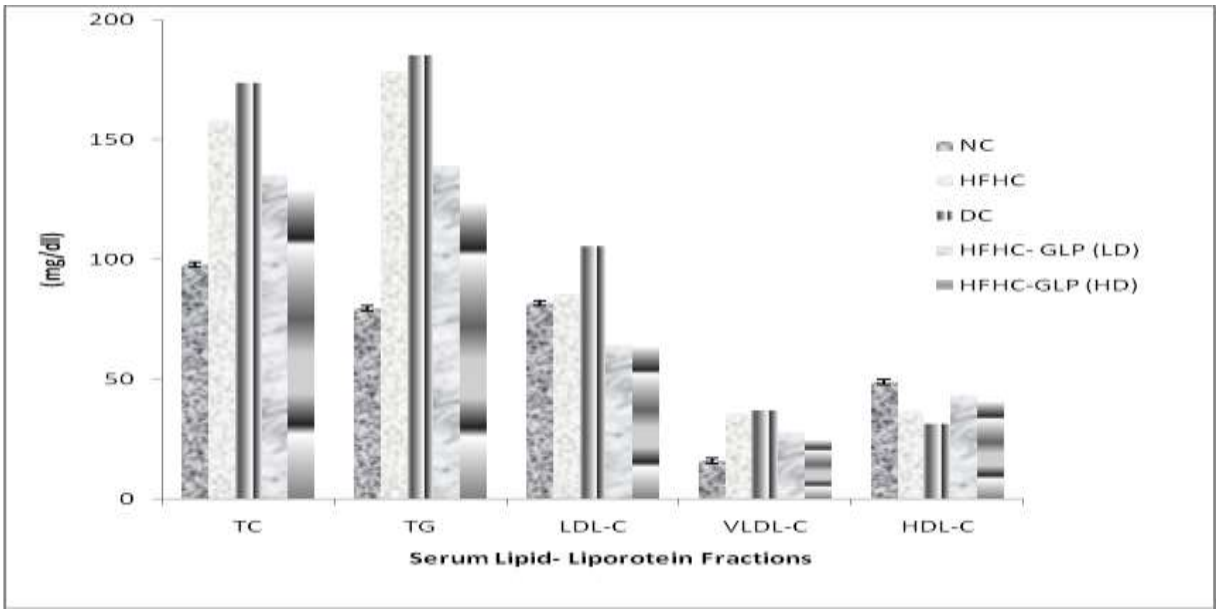
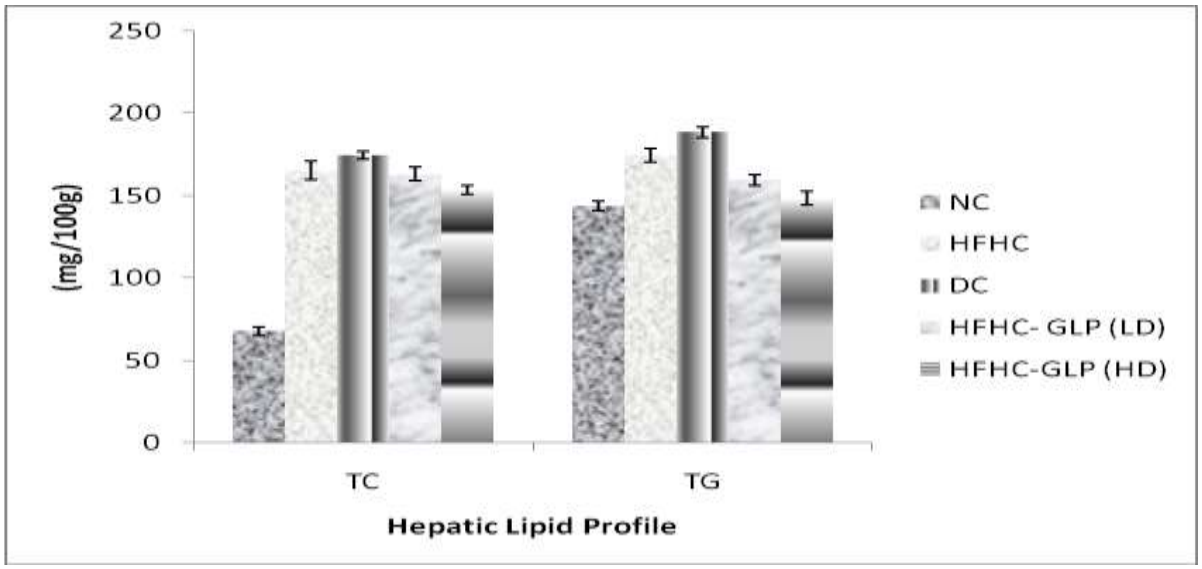


Fig. 1: Effect of Gymnemea sylvestre on Serum Glucose in STZ –Induced Diabetic Rats
(Values are Mean±SEMof 6 rats in each group)



(A)



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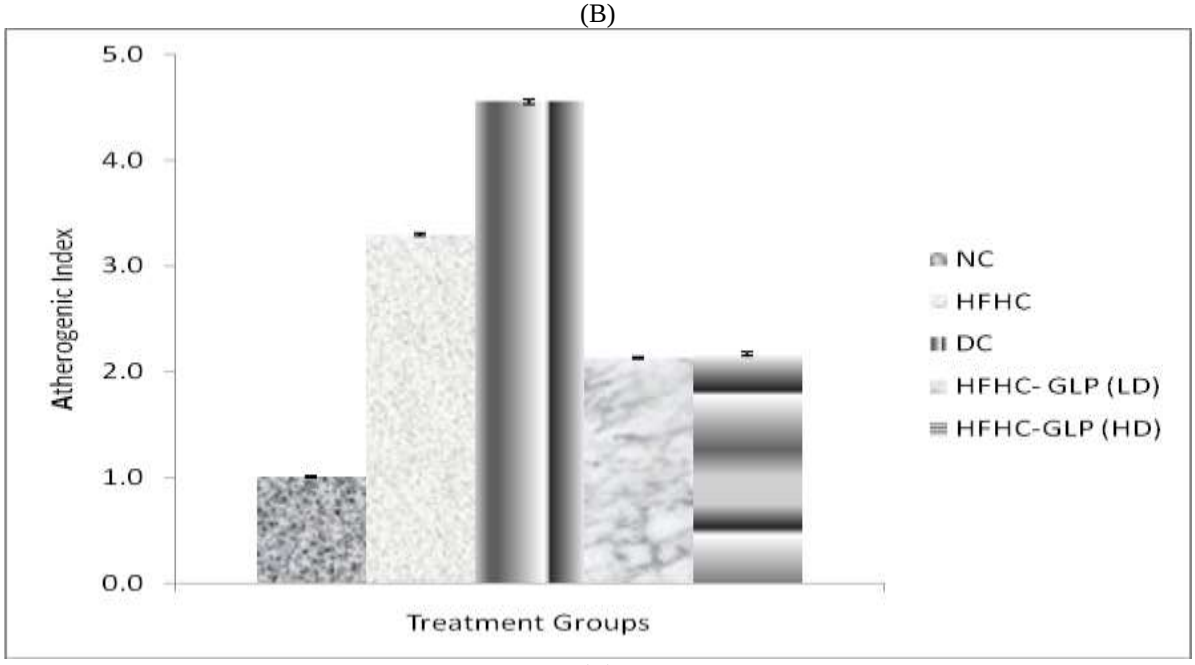

(C)

Fig. 2: Effect of Gymnemea sylvestre on (A) Serum Lipid-Lipoprotein Fractions; (B) Hepatic Lipid Profile; (C) Atherogenic Index of STZ –Induced Diabetic Rats

(Values are Mean±SEMof 6 rats in each group)

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