INTERNATIONAL JOURNAL OF ADVANCED BIOLOGICAL RESEARCH

www.scienceandnature.org

EFFICACY OF *GYMNEMA SYLVESTRE* IN ALLEVIATION OF STREPTOZOTOCIN INDUCED DIABETES IN RATS

[a]Mallikarjuna, G., [b]Suguna Rao, [c]Shridhar, N.B., [b]Sathyanarayana, M.L. [d]Byregowda, S.M. and [e]Ravikumar, P.
[a]Veterinary Dispensary, Tavarekaere, Kunigal Tq, Tumkur,Karnataka
[b]Department of Pathology, Veterinary College, Hebbal, Bangalore-560 024
[c]Department of Veterinary Pharmacology and Toxicology, Veterinary College, Hebbal, Bangalore-560 024
[d]Institute of Animal Health and Veterinary Biologicals, Hebbal, Bangalore-560 024
[e]Department of Pathology, Veterinary College, Hassan – 573 201

ABSTRACT
A hydroalcoholic extract of *Gymnema sylvestre* leaves (100 mg/kg b w / day) was tested for its biochemical and pathomorphological effects in streptozotocin induced diabetes in rats. Streptozotocin produced diabetes effectively in all the animals at 45 mg/kg in citrate buffer intraperitonially with a significant ($P \leq 0.001$) increase in the mean (± SE) serum levels of glucose, cholesterol, triglycerides, ALT and AST and a significant reduction in serum insulin in diabetic rats. *Gymnema sylvestre* extract supplement significantly ($P \leq 0.001$) reduced the serum levels glucose, cholesterol, triglycerides, ALT and AST from Day 3 post treatment till the end of the study with significant improvement in serum insulin levels ($P \leq 0.001$) in diabetic animals. *Gymnema sylvestre* also alleviated the damage caused by STZ morphologically in beta cells of islets of Langerhans and hepatocytes. However the effect was not totally comparable to that produced by glibenclamide in diabetic rats.

KEY WORDS: *Gymnema sylvestre,* streptozotocin, rats, glibenclamide, insulin, beta cells, diabetes mellitus.

INTRODUCTION

Diabetes is a common endocrine disorder that occurs either due to absolute or relative deficiency of insulin and/or insulin action affecting both humans and animals especially dogs and cats. *Gymnema sylvestre* is a medicinal plant belonging to the family of Asclepiadaeceae used by diabetics from the time immemorial (Alarcon-Aguilaria *et al.,* 1998) and the usage has gained further importance as the leaves of the plant contain certain active principles which can help in either repair or regeneration of islets of Langerhans (Shanmugasundaram *et al.,*1990; Baskaran *et al.,* 1990). Though there is scientific and medicinal evaluation of plant to support its antidiabetic effect, information on the pathomorphological effect of the plant on the pancreatic beta cells is lacking. The extract also has hypolipidaemic effect both in non-diabetic and high fat diet fed rats (Wang *et al.,* 1998). To establish a systematic scientific support over the effect of *Gymnema sylvestre* on the diabetic pathology, the study was designed and the results were compared with glibenclamide, a sulphonylurea antidiabetic drug.

MATERIAL AND METHODS:

Healthy male Wister albino rats weighing 180-260 g were procured from Central Animal Facility, Indian Institute of Science, Bangalore. They were maintained under standard laboratory condition and offered *ad lib* of standard commercial rat feed. The experiment was carried out for a period of 45 days upon permission from Institutional Animal Ethics Committee.

SOURCES

Streptozotocin

To induce diabetes in rats, streptozotocin (Sigma Chemicals, St.Louis, USA) was used intraperitonially in ice-cold citrate buffer (pH 3.5-4.5) at the dose of 45 mg/kg.

Gymnema sylvestre

The hydroalcoholic extract of *Gymnema sylvestre* leaves was procured from PLANTEX, Vijayawada, India (batch number P8121911). The plant was identified by HPTLC finger printing and assayed by Gravimetric method. The extract was administered at the dose rate of 100 mg/kg body weight as aqueous solution.

Glibenclamide solution

Glibenclamide (Daonil®, 5 mg) an oral hypoglycaemic drug was administered orally at a dose of 600 µg/ kg (Babu *et al*., 2003)

Administration of plant extract and glibenclamide

The plant extract and glibenclamide were administered orally to their respective groups by using clean rat gavaging needle attached to an appropriate disposable syringe every day for a period of 45 days.

Experimental design

The rats were divided into four different groups of ten animals each based on body weight. Care was taken to maintain the intra group weight variation to be less than 20 g and inter-group weight variation by 30 g. Group-I was normal control, group-II- diabetic control, group-III was diabetic animals treated with glibenclamide. The group-IV was diabetic rats supplemented with hydroalcoholic extract of *Gymnema sylvestre* leaves.

Experimental induction of diabetes

Freshly prepared streptozotocin at the dose of 45 mg/kg intraperitonially in 0.1M citrate buffer (pH 3.5- 4.5) was injected to the rats fasted for 16 hours (Babu *et al.,* 2004). The normal control animals received citrate buffer alone.

The diabetic state was confirmed by estimating the serum glucose level at 72 hours post STZ injection using Span Diagnostic kit with Semi-Automatic Biochemical Analyser (ARTOS, Bangalore). The animals that showed the serum glucose level above 200 mg/dl were considered diabetic and selected for the study.

Rats of all the groups were observed clinically for the feed and water intake, general behaviour, alertness, urine output, diarrhoea and for the development of clinical symptoms.

Collection of serum samples

About 2 ml of blood from the retro-orbital plexus of the rats of all the groups was collected under light ether anaesthesia separately in clean test tubes at different time intervals of the study such as 3rd, 15th, 30th and 45th day post STZ injection. The collected blood was allowed to clot for 30 min and then centrifuged at 3000 rpm for 10 min. The separated serum was subjected for glucose estimation, cholesterol, triglycerides, AST, ALT, creatinine biochemically and insulin level by RIA and the remaining serum was stored at -20^0 C.

Collection of tissue samples

To study the progressive effects of the treatments given to different groups, two rats from each group were sacrificed on 15th and 30th day using ether anaesthesia and the remaining rats on 45th day of experimentation. Sacrificed animals were subjected for detailed post mortem examination and gross change if any, were recorded. Further, representative tissue samples from pancreas, liver, kidney, lungs, heart, intestine, brain, stomach, skin and muscle were collected in 10 % neutral buffered formalin (NBF) for the pathomorphological evaluation.

Statistical analysis

Statistical analysis was performed using the statistical software Graph pad Prism, version 5. Mean values and standard error of mean were calculated and all values were expressed as Mean (± SE). The data were analysed by Two Way ANOVA.

RESULTS

Hyperglycaemic state was induced effectively by Streptozotocin in all the animals of groups II to IV by 72 hour post inoculation. Clinically the animals exhibited the signs of polyurea, polydypsia, polyphagia and weight loss. The diabetic animals (Group II) showed hyperglycaemia, hyperlipidemia, increased levels of serum ALT, AST and hypoinsulinism which persisted till the end of the experiment. The serum glucose levels ranged from 428.50±6.74 mg/dl on 3rd day to 440.83±4.68 mg/dl on 45th day post treatment against 103.83± 3.60 mg/dl to 107.13±3.21 mg/dl of control rats (Group I) on the respective days. (Table 1,Graph 1).

TABLE 1: Effect of *Gymnema sylvestre* on serum glucose (mg/dL) in STZ induced diabetic rats

Groups	Days of post-treatment			
	3	15	30	45
Group I	106.00± 5.18	103.83± 3.60	106.83±4.24	107.13±3.21
Group II	428.50±6.74^{a}	474.66±5.57^{a}	513.66±7.09^{a}	557.83±5.71^{a}
Group III	436.50±4.50 ^{a}	363.50±6.28^{ab}	306.16±5.82^{ab}	225.00±6.04 ^{ab}
Group IV	435.66±4.99 ^{a}	386.66±17.11^{ab}	313.00±6.46 ^{ab}	260.00±5.67^{abc}

TABLE 2: Effect of *Gymnema sylvestre* on serum cholesterol (mg/dL) in STZ induced diabetic rats

Groups	Days of post-treatment			
	3	15	30	45
Group I	43.03±1.82	42.71±1.86	42.16±1.93	41.48±1.78
Group II	74.98±4.25 ^{a}	91.90±5.53 ^{a}	105.60±5.24 ^{a}	119.28±4.19 ^{a}
Group III	74.81±1.92^{a}	54.26±2.81 ^{ab}	45.29±3.23^{b}	36.21±2.57 ^{b}
Group IV	73.54±2.19 ^{a}	63.10±1.78 ^{ab}	54.18±1.95 ^{ab}	50.87±1.72 ^{bc}

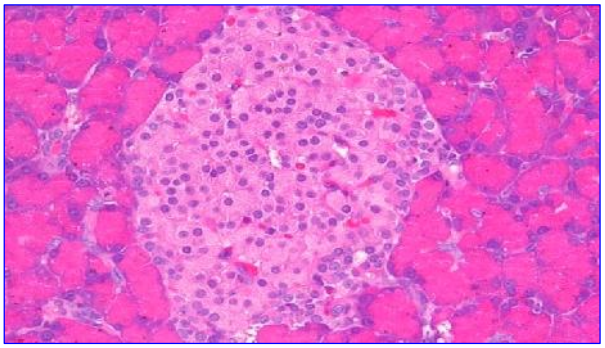

Plate 1 Section of pancreas showing normal islet of Langerhans with round to oval shape, compact arrangement of beta cells at the centre and alpha cells at the periphery H&E X 200

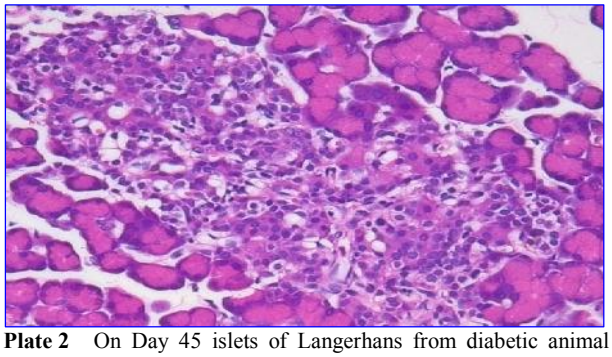

Plate 2 On Day 45 islets of Langerhans from diabetic animal showing loss of shape and presence of vacuolated and a few necrotic cells. Proliferation of fibroblasts with elongated nucleus H&E X 200

The serum cholesterol, triglyceride, ALT and AST values were significantly increased (P≤ 0.001) in diabetic rats from Day 3 onwards. In addition the serum insulin levels were significantly reduced compared to normal rats. However, serum creatinine values did not show significant variation compared to control rats throughout the experiment (Table 4,5 & 6,Graph 4,5&6). Microscopically the islets of Langerhans were reduced in number and size and showed loss of normal architecture with the altered shape. Beta cells were highly swollen with vacuolations and were reduced in number. By 45th day, most of the islets were depleted of beta cells with mild infiltration of mononuclear cells and fibrosis. The hepatocytes were swollen with granular and vacuolated cytoplasm. In addition, areas of centri lobular necrosis which became bridging type and more severe and diffused by the end of the study along with mild infiltration of inflammatory cells were also observed in liver.

TABLE 3: Effect of *Gymnema sylvestre* on serum triglyceride (mg/dL) in STZ induced diabetic rats

Groups	Days of post-treatment			
	3	15	30	45
Group I	98.85 ± 2.18	98.51±1.20	100.86±1.22	99.48±1.29
Group II	211.10±3.41 [a]	248.95±3.16 [a]	285.35±2.46 [a]	328.22±4.35[a]
Group III	209.17±3.01 [a]	180.67±1.58 [ab]	137.42±1.90[ab]	99.67±3.52 [b]
Group IV	209.49±2.94 [a]	182.75±3.17 [ab]	155.30±1.94[abc]	124.07±2.59[abc]

TABLE 4: Effect of *Gymnema sylvestre* on serum ALT (IU/L) in STZ induced diabetic rats

Groups	Days of post-treatment			
	3	15	30	45
Group I	52.47 ±1.35	51.92±0.36	52.87±0.49	51.96±0.90
Group II	142.96± 7.7[a]	206.98±1.87[a]	249.18±2.93[a]	345.34±1.62 [a]
Group III	135.47± 5.7[a]	120.97±5.13 [ab]	108.90±5.95[ab]	102.80±7.01 [ab]
Group IV	135.88±5.99[a]	118.21±4.27 [ab]	104.36±4.77[ab]	88.16 ± 0.69 [ab]

TABLE 5: Effect of *Gymnema sylvestre* on serum AST (IU/L) in STZ induced diabetic rats

Groups	Days of post-treatment			
	3	15	30	45
Group I	64.95 ± 1.08	64.86± 0.96	64.93 ± 0.99	64.68 ± 1.08
Group II	180.50± 0.74 [a]	215.03± 3.84 [a]	271.11 ± 1.97 [a]	355.68 ± 4.97[a]
Group III	185.36± 1.73 [a]	152.88±3.52[ab]	123.11±6.59 [ab]	114.65±7.25[ab]
Group IV	176.92± 3.94 [a]	144.34±3.90[ab]	106.53±8.11 [ab]	86.42± 5.54 [bc]

TABLE 6: Effect of *Gymnema sylvestre* on serum insulin (µU/L) in STZ induced diabetic rats

Groups	Days of post treatment		
	15	30	45
Group I	56.86±1.73	56.51±1.80	56.75±1.78
Group II	16.03±0.39 [a]	12.29±0.33 [a]	11.22±0.44 [a]
Group III	25.75±1.13 [ab]	35.20±0.71 [ab]	39.16±0.73 [ab]
Group IV	19.95±0.50 [ac]	27.65±2.13 [abc]	37.71±3.13 [ab]

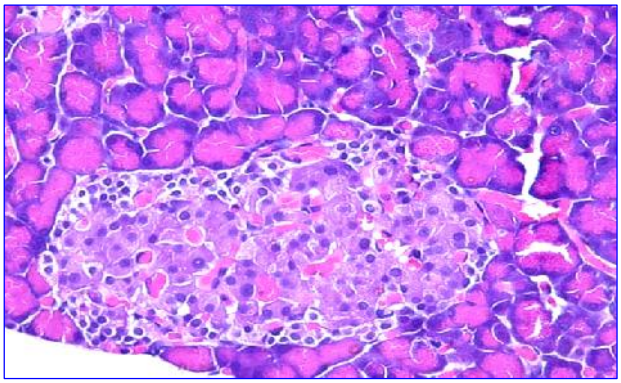

Plate 3 Pancreas from a diabetic rat treated with glibenclamide showing well formed islets with normal architecture on Day 45of the study, H&E X 200

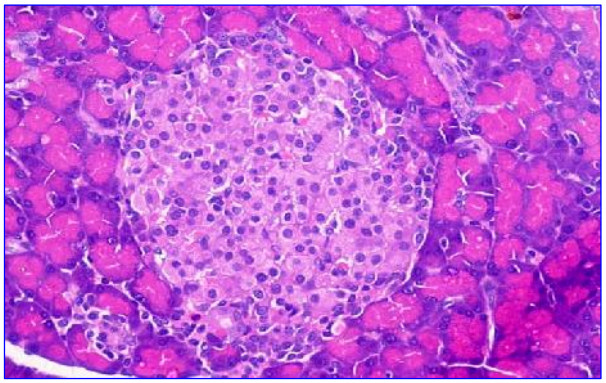

Plate 4 Section of pancreas from a diabetic rat treated with *Gymnema sylvestre* on Day 45 showing round, hypercellular islet of Langerhans. Note the improved cell size and granularity. H&E X 200

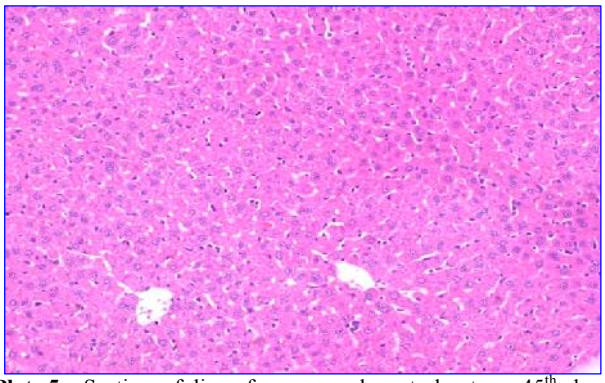

Plate 5 Section of liver from normal control rat on 45th day showing central vein and well formed hepatic cords with normal appearing hepatocytes H&E X 100

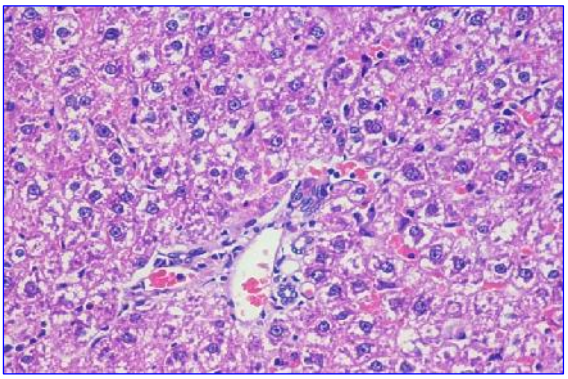

Plate 6 Section liver from diabetic rat on day 45 showing highly swollen hepatocyte with increased cytoplasmic granularity, vacuolations and necrosis of hepatocytes H&E X 200

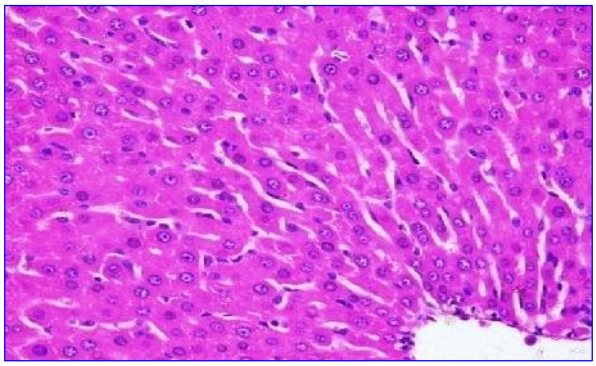

Plate 7 Section of liver from a diabetic rat treated with Glibenclamide on Day30 of the study showing improved architecture from STZ effects. H&E X 200.

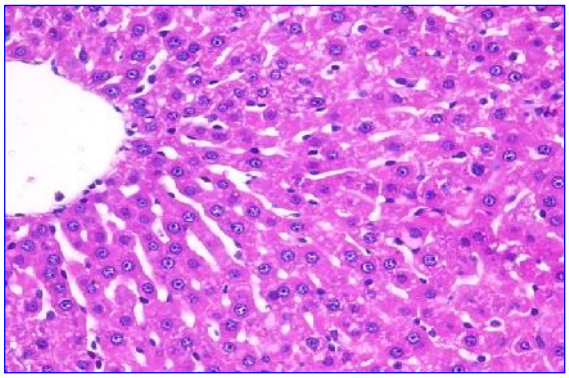

Plate 8 On Day 30 post-treatment liver from diabetic rat treated with *Gymnema sylvestre* showing improvement in architecture. However, note some cells still showing mild degree of granulations H&E X 200

Administration of hydroalcoholic extract of *Gymnema sylvestre* (Group III) at 100 mg/kg b w significantly reduced ($P \leq 0.001$) serum glucose from 435.66±4.99 mg/dl on 3rd day to 260.00±5.67 mg/dl on 45th day post-treatment (Table 1,Graph 1). In addition both cholesterol and triglyceride levels were significantly reduced ($P \leq 0.001$). The reduction was significant from Day 15 post-treatment compared to diabetic control in both cases (Table 2 Graph 2).

The *Gymnema sylvestre* extract supplement also produced a significant reduction in the serum levels of ALT and AST compared to diabetic control. The mean (±SE) insulin values were improved in plant extract treated group and the significant difference was observed from Day 15 post-treatment compared to diabetic group (Table 1,Graph 1).

There was a progressive improvement in the architecture of islets of Langerhans from 15th day to 45th day post treatment. Overall there was an increase in number, improvement in shape and size of islets. The number and size of beta cells was increased progressively. Throughout the study glibenclamide treatment (Group IV) produced a significant ($P \leq 0.001$) antihyperglycemic, hypolipidaemic and hepatoprotective effect in diabetic rats. The hypolipidaemic effect of glibenclamide was comparable to normal control rats. The hepatoprotective effect was also supported by the histopathology of liver. There was a progressive improvement in the condition of animals as evidenced by decreased diabetic signs. However, polyurea was observed till the end of the study. Islets of Langerhans showed improvement in the microscopic pathology, the number of islets increased, which were either round or oval shaped with compact arrangement of beta cells at the centre and alpha cells at the periphery. The beta cells appeared round with increased cytoplasmic granularity. There was an improvement in the liver morphology especially in the form of reduction in the hepatic vacuolar degenerative changes and attainment of almost normal architecture.

DISCUSSION

In the present study the hydroalcoholic extract of *Gymnema sylvestre* was administered to diabetic rats and the efficacy of the same was evaluated in comparison to glibenclamide. Diabetes mellitus, a most common endocrine disorder, is a group of metabolic disorders sharing the common underlying features of hyperglycaemia. Hyperglycaemia in diabetes results from defects in either insulin secretion, insulin action, or most commonly, both. The chronic hyperglycaemia and metabolic dysregulation may be responsible for the secondary damage in multiple organ system.

STZ has been extensively used to induce diabetes for various diabetes studies in laboratory animal models by

many workers (Ganda *et al.,* 1976 and Akbrazadeh *et al.,* 2007). STZ as a selective beta cell cytotoxic agent enters the cells through glucose transporter causing alkylation of DNA leading to their damage. Such damage has been reported to induce activation of poly ADP ribosylation causing depletion of cellular NAD, ATP and formation of free radicles which finally brings about further DNA damage and beta cells loss (Szkudelski, 2001 and Mir *et al.,* 2008).

Hyperglycaemia in diabetic individuals is attributed to deficiency or resistance to insulin which leads to decreased glucose transport in muscles, elevated hepatic glucose production and increased breakdown of fat contributing to hyperglycaemia (Ganda *et al.,* 1976; Ali *et al.,* 1993 and Mahdi *et al.,* 2003). Further, insulin deficiency increases excessive breakdown of adipose store by stimulation of lipoprotein lipase leading to increased mobilisation of fatty acids for energy purpose and their excessive accumulation in liver which are converted to triglycerides (Ahmed *et al.,* 2000 and Nafisa *et al.,* 2007) contributing to hyperlipidaemia.

The serum ALT and AST mean (±SE) values were significantly higher in diabetic rats throughout the study. STZ has been reported to induce both plasma membrane and organellar membrane damage especially that of RER and mitochondria (Laguens *et al.,* 1980) in hepatocytes as a result of which the enzymes are leaked into circulation, a factor well supported by the microscopic evidence of hepatic damage (Rajesh and Latha, 2004 and Muhammad *et al.,* 2008).

Insulin is a major hormone secreted by the beta cells of islets of Langerhans. STZ causes selective destruction of beta cells, accounting for hypoinsulinemia and the finding was well supported by histopathology (Sarkar *et al.,* 1996 and Ahmed *et al.,* 2000). Shigematsu *et al.* (2001) reported that the *Gymnema sylvestre* suppresses the glucose absorption in the small intestine of rats, reduces plasma glucose increment in the oral sucrose tolerance test, lowers blood glucose level and alleviates diabetic symptoms in type-2 diabetes. However, Kanetker *et al.* (2007) reported that *Gymnema sylvestre* exerts its hypoglycaemic effects through increasing secretion of insulin, promoting regeneration of islets cells, increasing utilization of glucose through increased activities of enzymes responsible for utilization of glucose by insulin dependent pathways and by inhibition of glucose absorption from intestine. The present study also supports the foresaid mechanisms. However, Baskeran *et al.* (1990) and Shanmugasunderan *et al.* (1990) attributed the hypoglycaemic effect to the gymnemic acids found in the extract. The decrease in the cholesterol and triglyceride levels in serum has been attributed to the gymnemic acid which influenced faecal excretion of lipids (Shigematsu *et al.,* 2001 and Kanetkar *et al.,* 2007). Wang *et al.* (1998) attributed the same to inhibition of intestinal absorption of oleic acid whereas Shigimatsu *et al.* (2001) attributed decreased synthesis of VLDL in liver. The partial hepatoprotective effect of the plant extract could possibly due to the presence of antioxidant principles. The perusal of literature did not provide much information on the effect of *Gymnema sylvestre* in reversing the liver damage. However *Gymnema montanum* leaves, a plant related to *Gymnema sylvestre* has been reported to revert alloxan induced liver damage to the normal homeostasis by its hypoglycaemic action and stimulatory insulin release by the β-cells (Ananthan *et al.,* 2003).

The gymnemic acids of *Gymnema sylvestre* have been reported to have direct action on pancreatic β-cells and may bring about the regeneration or repair of beta cells leading to increase in serum insulin levels (Shanmugasundaram *et al.,* 1990; Persaud *et al.,* 1999 and Kanetkar *et al.,* 2007). This was also supported by the histopathological examination of the present study. Insulin secretion by the beta cells in response to glibenclamide treatment could be responsible for the reduction in serum glucose levels which further improves sensitivity of beta cells to glucose and potentiates insulin secretion (Luzi and Possa, 1997; Ling *et al.,* 2006). However, it was noticed that the serum glucose levels failed to reach the normal levels inspite of treatment with glibenclamide. This could probably be due to failure of complete recovery of beta cells population in response to the glibenclamide treatment. The significant reduction in serum cholesterol and triglyceride levels clearly indicates hypolipidaemic effect of glibenclamide. Skrapari *et al.* (2001) reported that alleviation of hypertriglyceridemia was due to acute reduction in triglycerides of intestinal origin and not with the VLD lipoprotein sub fraction levels. However, Juhavekkilainen *et al.* (2002) failed to notice any effect of glibenclamide on hyperlipemia in diabetic patients. It was observed that the serum ALT and AST values were significantly higher in glibenclamide treated group compared to normal control indicating that glibenclamide did not completely reverse the hepatic damage caused by STZ. This was substantially supported by the persistence of mild to moderate degree of hepatic damage till the end of the study, histopathologically.

Glibenclamide has been shown to activate protein translation in pancreatic beta cells through the calcium regulated mTOR, PKA and MEK signalling pathways resulting in insulin synthesis (Luzi and Pozza, 1997 and Wang *et al.,* 2008) on prolonged treatment. It was also reported that glibenclamide recruits beta cells subpopulation and causes elevated and sustained basal insulin synthetic activity (Ling *et al*., 2006). These may be responsible for the significant increase in the serum insulin levels. However, the values failed to reach the normal range at any period of observations of the present study. Gross pathological changes observed in the present group reduced progressively with advancement in time on treatment with glibenclamide. The results were in agreement with the findings of Ananthan *et al.* (2003) where they attributed the improvement to the alleviation of diabetes by the increased levels of insulin. Studies using [3H]-glibenclamide boluses have suggested that hepatocytes possess specific binding sites that may be relevant in mediating the action of the drug on the liver. Additional studies have shown that the drug has a positive action on glycogen deposition with direct action on the synthesis of GLUT2 rather than GLUT4 proteins and at the glycogen phosphorylase level. The effect of glibenclamide on the insulin levels and on the altered metabolism of various macromolecules may improve the liver's microscopic architecture (Luzi and Possa , 1997).

The progressive improvement in the microscopic pathology of islets of Langerhans attributed to increased proliferation as well as recruitment of subpopulation of beta cells and thereby increases in the beta cell mass upon treatment with glibenclamide (Guiot *et al.,* 1994 and Wang *et al.,* 2008). This was also supported by the histopathological observations of islets of Langerhans.

In the present study the hypoglycaemic effect of *Gymnema sylvestre* was almost similar to that of glibenclamide, a cardinal drug for diabetes and incites the possibility of replacing it by herbal preparation of *G. sylvestre* for diabetes treatment. However glibenclamide was found to be superior with respect to hypolipidaemic effect compared to *Gymnema sylvestre.* Both the agents were found to be partially hepatoprotective, although *Gymnema sylvestre* was slightly superior. Both *Gymnema sylvestre* and glibenclamide significantly improved serum insulin levels but the values failed to reach that of normal control rats. This clearly indicated that they could partially overcome the effects of diabetes. It was concluded that *Gymnema sylvestre* could be considered as an effective herbal alternative for the treatment of Diabetes mellitus.

REFERENCES

Ahmed, I., Lakhani, M.S., Gillett, M., John, A., Raza, H. (2000) Hypotriglyceridemic and hypocholesterolemic effects of anti-diabetic *Momordica charantia* (karela) fruit extract in streptozotocin-induced diabetic rats. *Diabetes Research and Clinical Practice*, 51:155–161.

Alarcon-Aguilara, F. J., Roman-Ramos, R., Perez-Gutierrez, S., Aguilar- Contreras (1998) Study of the anti-hyperglycemic effect of plants used as antidiabetics. *Journal of Ethnopharmacology*. 61 (2): 101-110.

Ali, L., Khan, A.K., Mamun, M.I., Mosihuzzaman, M., Nahar, N., Nur-E-alam, M. and Rokeya. B (1993). Studies on hypoglycemic effects of fruit pulp, seed, and whole plant of *Momordica charantia* on normal and diabetic model rats. *Planta Med*, 59: 408-412.

Akbarzadeh, D., Norouzian, Mehrabi, M., R., Jamshidi, A., Farhangi, A., Verdi, A., Mofidian and Lame, R. (2007) Induction of diabetes in streptozotocin in rats. *Ind J of clinical biochem,* 22(2): 60-64.

Ananthan, R., Muniappan, L., Leelavinothan, P., Chokkanna, G. B. and Vengatesh, N. B. (2003) Effect of *Gymnema montanum* on Blood Glucose, Plasma Insulin, and Carbohydrate Metabolic Enzymes in Alloxan-Induced Diabetic Rats. *J of MEDICINAL FOOD.* 6(1):43-49.

Babu, I.V., Gangadevi, A. and Subramoniam (2003) Antidiabetic activity of ethanol extract of *Cassia kleinii* leaf in streptozotocin-induced diabetic rats. *Ind. J. Pharmacol*, 35: 290-296.

Babu, P.S., Stanely, Mainzen and Prince, P. (2004) Antihyperglycaemic and antioxidant effect of hyponidd, an ayurvedic herbomineral formulation in streptozotocin-induced diabetic rats. *J. Pharm. Pharmacol*, 56(11):1435-1442.

Baskaran, K., Kizar, A.B., Radha, S. K., Shanmugasundaram, E. R. (1990) Antidiabetic effect of a leaf extract from *Gymnema sylvestre* in non-insulin-dependent diabetes mellitus patients. *J. Ethnopharmacol*, 30:295-300.

Ganda, O.P., Rossi, A.A., Like, A.A. (1976) Studies on streptozotocin diabetes. *Diabetes,* 25: 595-603.

Guiot, Y., Henquin, J.C., Rahier, J. (1994) Effects of glibenclamide on pancreatic beta-cell proliferation in vivo. *Eur J Pharmacol*, 11, 261(1-2):157-161.

Laguens, R.P., Candela, S., Hernandez, R.E., Gagliardino, J.J. (1980) Streptozotocin induced liver damage in mice. *Horm. Meteb. Res,* 12: 197-201.

Ling, Z., Wang, Q., Stange, G., Veld, P. I. and Pipeleers, D. (2006) Glibenclamide Treatment Recruits β-Cell Subpopulation Into Elevated and Sustained Basal Insulin Synthetic Activity. *DIABETES,* 55:78-85.

Luzi, L., Pozza, G. (1997) Glibenclamide: an old drug with a novel mechanism of action?. *Acta Diabetol,* 34: 239–244.

Juhavekkilainen, Mero, N., Anjaschweizer, Foly, K. and Rittajasinen, M. (2002) Effects of naleglinide and glibenclamide on the post prandial lipid and glucose metabolism intype-2 diabetes. *Diabetes/metabolism research and review*, 18(6): 484-490.

Kanetkar, P., Singhal, R. and Kamat, M. (2007) Gymnema sylvestre: A Memoir. *J. Clin. Biochem. Nut,* 41: 77–81.

Mahdi, A. A., Anu, C., Rajkumar, S., Shukla, S.L.C., Mishra and Sohail, A. (2003) Effect of herbal hypoglycemic agents on oxidative stress and antioxidant status in diabetic rats. *Ind J. Clini. Biochem,* 18 (2): 8-15.

Mir, S. H., Baqui, A., Bhagat, R.C. and Shah, A.W. (2008) Biochemical and histomorphological study of streptozotocin-induced diabetes mellitus in rabbits. *Pak. J. Nutr,* 7:359- 364.

Muhammad, K.S., Yulin, D. and Rongji, D. (2008) Attenuation of biochemical parameters in streptozotocin induced diabetic rats by oral administration of extracts and fractions of cephalotoxus sinensis, *J. Clin. Biochem. Nutr,* 42(1):21-28.

Nafisa, P.C. F., Chakradhar, V. L., Vandana, S. P. and Suresh, R.N. (2007) An experimental evaluation of the antidiabetic and antilipidemic properties of a standardized Momordica charantia fruit extract. *BMC Complementary and Alternative Medicine,* 7(29): 1-8.

Persaud, S.J., Al-Mhjed, A., Rahman, A. and Jones, P.M. (1999) *Gymnema sylvestre* stimulate insulin release in vitro by increased membrane permeability. *J. Endocri*, 163: 207-212.

Rajesh, M.G. and Latha, M.S. (2004) Preliminary evaluation of the antihepatotoxic effect of kamilari, a polyherbal formulation. *J. Ethnopharmacol,* 91: 99-104.

Sarkar, S., Pranava, M. and Marita, A. R. (1996) Demonstration of the hypoglycemic action of *Momordica charantia* in a validated animal model of diabetes. *Pharmacol. Res*, 33:1-4.

Shanmugasundaram, E.R., Gopinath, K.L., Radha, S.K., Rajendran, V.M. (1990) Possible regeneration of the islets of Langerhans in streptozotocin-diabetic rats given *Gymnema sylvestre* leaf extracts. *J. Ethnopharmacol*, 30:265-279.

Shigematsu, N., Asano, R., Shimosaka, M. and Okazaki, M. (2001) Effect of long term administration with *Gymnema sylvestre* R. BR on plasma and liver lipid in rats. *Biol. Pharm. Bull*, 24:643-64.

Skrapari, I., Perrea, D., Ivannidia, I. and Karabinas, A.P. (2001) Glibenclamide improves postprandial hypertriglyceridemia in type-2 diabetic patients by reducing chyloicrones but not the very low density lipoproteins subfration levels. *Diabetic medicine*, 18(10): 781-785.

Szkudelski, T. (2001) The Mechanism of Alloxan and Streptozotocin Action in B Cells of the Rat Pancreas. *Physiol. Res,* 50: 536-546.

Wang, L., Luo, H., Miyoshi, M. and Imoto, T. (1998) Inhibitory effect of gymnemic acid on intestinal absorption of oleic acid in rats. *Can. J. Physiol. Pharmacol*, 76: 1017-1023.