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ANTIOXIDANT AND ANTIGLYCATION PROPERTIES OF *GYMNEMA SYLVESTRE* IN STREPTOZOTOCIN-INDUCED DIABETIC RATS

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ABSTRACT

Objectives: The present study was undertaken to evaluate antiglycation and antioxidant activity of *Gymnema sylvestre* (Gs) aqueous leaves extract at a dose(200mg/kg body weight) against STZ- induced diabetes in Albino rats (45 mg/kg body weight) and was compared with standard hypoglycemic drug, glibenclamide (0.5 mg/kg/ body weight) for its anti hyperglycemic effect. **Methods:** Animals were divided into five groups; namely, healthy control, healthy Gs, STZ- diabetic control, Diabetic + Gs and Diabetic + GLB. **Results:** The oral administration of both Gs aqueous extract and glibenclamide to STZ diabetic rats significantly reduced advanced glycation end products (AGEs) formation. Similarly, low levels of SOD, GSPx and GSH in diabetic rats were reverted to near normal values after treatment with *Gymnema sylvestre* and glibenclamide. These findings indicate that oxidative stress is increased in the diabetic rat liver and kidney and that Gs can prevent hepatic and renal damage associated with diabetes by attenuating the oxidative stress. Our data confirmed with the inhibitory effect of Gs aqueous extract on glycation haemoglobin. Also, There was improvement in various parameters such as body weight, serum glucose, insulin levels, glycogen content, lipid profile and kidney function in both of diabetic groups administrated with Gs leaves extract and glibenclamide. The findings were clearly substantiated by histopathology of pancreatic tissues. **In conclusion:** G. sylvestre has significant antidiabetic, antiglycation, antioxidant and hypolipidemic activity. Also, *Gymnema sylvestre* was found to be more effective than glibenclamide in alleviation of STZ induced diabetes in rats.

KEY WORDS: antiglycation, antioxidant, *Gymnema sylvestre*, antidiabetic, hypolipidemic.

INTRODUCTION

Diabetes mellitus(DM) is a group of metabolic diseases characterized by hyperglycemia and dyslipidemia which results from defects in both insulin secretion and/or insulin action[1]. The disease is associated with reduced quality of life and increased risk factors for mortality and morbidity. Long-term hyperglycemia is an important factor in the development and progression of micro- and macro-vascular complications, which include neuropathy, nephropathy, cardiovascular and cerebro vascular diseases[2].

During diabetes, persistent hyperglycemia causes increased production of free radicals especially reactive oxygen species (ROS), for all tissues from glucose auto-oxidation and protein glycosylation. Free radicals are generated as by-products of normal cellular metabolism; however, uncontrolled DM is known the best to disturb the balance between ROS production and cellular defense mechanisms[3]. This imbalance can result in cell dysfunction and destruction resulting in tissue injury[4]. The increase in the level of ROS in diabetes could be due to their increased production and/ or decreased destruction by nonenzymatic antioxidants e.g. reduced glutathione (GSH) and enzymatic antioxidants which may include glutathione peroxidase (GSPx), and superoxide dismutase (SOD). Therefore, the level of these antioxidant enzymes critically influences the susceptibility of various tissues to oxidative stress and is associated with the development of complications in diabetes[5]. In addition, this is particularly relevant and dangerous for the beta islet, which is among those tissues that have the lowest levels of intrinsic antioxidant defenses[6]. Free radicals may play an important role in the causation and complications of diabetes mellitus. During the course of the disorder, alterations in the endogenous free radical scavenging defense mechanisms may lead to ineffective scavenging of reactive oxygen species, resulting in oxidative damage and tissue injury. Oxidative stress is currently suggested as mechanism underlying diabetes and diabetic complications[7].

In diabetes, increased free radical generation leads to protein cross-linking, protein glycosylation and generation of AGEs[8]. Increased ROS and reactive carbonyl species(RCS) such as 3-deoxyglucosone, glyoxal, and methyl glyoxal (MGO), are critical intermediates formed during protein glycation. The end product of Amadori rearrangement in aldemine linkage between glucose and protein is called fructosamine (1-amino-1-deoxyfructose/isoglucosamine). All the above mentioned intermediates are identified as the

important precursors of AGEs in diabetes[9]. Further, AGEs can bind to cell membrane receptor called RAGE (receptor for AGEs) and mediate the release of a series of inflammatory mediators and growth factors. Reports suggest that AGEs-RAGE is the predominant mechanism in pathogenesis of nephropathy and retinopathy[10].

In the light of these facts a significant correlation, between total antioxidant capacity and clinical characteristics of diabetic patients including their blood levels of glucose and glycated hemoglobin, suggests that the measurement of total antioxidant capacity in diabetic patients can be a marker of glycaemic control[11]. Recent evidence suggests that impaired antioxidant status is involved in oxidative stress associated with diabetes[12].

The treatment of diabetes mellitus is based on insulin and/or oral hypoglycemic drugs. These drugs act by various mechanisms to control the blood glucose level, but many side-effects have been reported. Therefore, there is considerable interest in the field of medicinal plants due to their natural origin and less side effects[13,14].

One of these medicinal plants is *Gymnema Sylvester* (Gurmar), which means sugar killer from the Asclepiadaceae family. It is a wild plant that grows in the open forest in India, China, Indonesia, Japan, Malaysia, SriLanka, Vietnam, and South Africa[15]. previous studies have indicated that the leaves of *Gymnema sylvestre* (Gs. sylvestre)contain gymnemic acids, which are a group of triterpenoid saponins , alkaloids, acidic glycosides and anthroquinones[16].

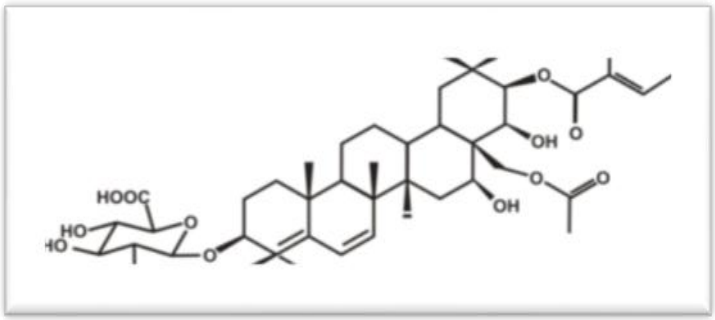

Fig. 1: Structure of gymnemic acid.

Prior studies demonstrated that an extract from Gs. sylvestre leaves exhibited antioxidant and lipid peroxidation inhibiting activity in an experimental model of gastric ulceration and colitis[17,18].

Though, recent studies had been conducted on the possible antidiabetic potential of Gs. sylvestre leaves[19,20,21], none has been done on the antiglycation potential of its in diabetic rats. It is based on this premise that the antidiabetic, antiglycation, antioxidation and hypolipidemic potential of Gs. sylvestre leaves extract was performed in streptozotocin induced diabetic rats.

2. MATERIALS AND METHODS

1- Chemicals

- Streptozotocin (STZ) was purchased from Sigma Company(U.S.A) and given as a single intraperitoneal dose (45 mg/ kg body weight) dissolved in citrate buffer (pH4.5).
- Kits used for AGEs assay were obtained from Biovision company, USA.
- Kits used for the determination of other biochemical measurements were obtained from Bio diagnostic company, Egypt.
- Glibenclamide: Each tablet contains 5 mg of glibenclamide - (Marcyrl pharmaceutical industries el obour city –Egypt). Glibenclamide drug was dissolved in distilled water at a dose 0. 5 mg/kg body weight of rat using a stomach tube[14].

2- Plant materials

Gymnema sylvestre (Gs) leaves extract is used as drug called (Diaglu), that is produced by Arab Company for Pharmaceuticals and Medicinal Plants - (MEPACO) Enshas El Raml, Sharkeiya, Egypt. Diaglu contains Gs. sylvestre leaves extract only. Gs leaves extract was given at a dose of (200 mg/kg body weight) dissolved in 1 ml distilled water according to Al-Rejaie et al.[17]

3-Animals

Thirty healthy male adult albino rats Spargue- Dawley strain were obtained from Laboratory Animal Colonies, Helwan, Egypt. The average weight was 150±10g. They were maintained under standard conditions of temperature, humidity and light (12 h dark, 12 h light) and provided with standard commercial pellets diet and having free access to water.

Induction of STZ- diabetes mellitus

Diabetes mellitus was experimentally induced in rats previously fasted for 12 h by a single intraperitoneal dose (45 mg/kg body weight) of streptozotocin dissolved in citrate buffer (pH 4.5). In order to overcome the hypoglycemic coma that occurs within the first 24 h following STZ injection, animals were given 5% glucose solution instead of drinking water for 2 days

until sustained hyperglycemia was established[22]. Three days after streptozotocin injection, rats were screened for blood glucose levels. Blood samples were withdrawn from the lateral tail vein and glucose concentration was measured from overnight fasted animals (10–12 h). Rats having glucose ranging from 200 to 250 mg/dl were considered as diabetic and included in the experiment.

Experiment design

Rats under study were randomly divided into six groups (6 rats each):

Group I: Healthy control group.

Rats of this group were given intragastrically 1 ml distilled water once a day for 6 weeks.

Group II: Healthy *Gymnema sylvestre* group (healthy Gs)

Rats of this group were supplemented with Gs. sylvestre leaves extract (200 mg/kg b.w.) dissolved in 1 ml distilled water once a day for 6 weeks.

Group III: STZ-diabetic group (Diabetic control)

Rats of this group were injected intraperitoneally with a single dose (45 mg/kg b.w.) of STZ dissolved in citrate buffer (pH 4.5).

Group IV: Diabetic + Gs group

Rats of this group were treated intragastrically with Gs leaves extract (200 mg/kg b.w.) dissolved in 1 ml distilled water once a day for 6 weeks after STZ-diabetic induction.

Group V: Diabetic + GLB group

Rats of this group were treated intragastrically with glibenclamide (0.5 mg/kg/ b.w.) dissolved in 1 ml distilled water once a day for 6 weeks after STZ-diabetic induction.

Blood sample collection

At the end of the experimental period, animals of each group were fasted about 12 h and then anesthetized by diethyl ether inhalation[23], then blood samples were collected via cardiac puncture. Blood samples were collected in two tubes. The first one contained Ethylene Diamine Tetra Acetic acid (EDTA) for collecting blood immediately used for the determination of haemoglobin concentration and glycosylated haemoglobin (HbA1C) level. In the second tube, blood was allowed to stand for 15 minutes at temperature of 37ºc, then was centrifuged at 4000 rpm for 20 min by EBA8 centrifuge (obtained from china) for the

separation of serum. Serum was removed and kept in plastic vials at -20ºc until used for biochemical analyses.

Tissues Sampling

Liver, kidney and pancreas were separated and cleaned, rinsed and washed by saline solution then blotted on filter paper to remove water residue. kidney was weighed immediately . Part of the liver and Kidney were stored frozen at -20ºc until used for tissues biochemical analyses and Part of the pancreas was kept in 10% formalin for histopathological examination.

Biochemical analysis

- The renal AGE level was determined by competitive ELISA[24]. In brief, minced kidney tissue was treated with chloroform and methanol (2:1, v/v) overnight. After washing, the tissue was homogenized in 0.1 N NaOH, followed by centrifugation at 8000 rpm for 15 min at 4 °C. The amounts of AGEs in these alkali-soluble samples were determined by competitive ELISA according to the methods of Makita et al.[24] The AGEs values of samples were measured at a protein concentration of 1 mg/ml and expressed in arbitrary units (AU).
- Glycosylated haemoglobin (HbA1C) estimation was carried out by a modified colorimetric method[25].
- Serum glucose level was determined by the glucose oxidase method[26].
- Insulin level was determined by ELISA method according to Anderson et al.[27]
- The insulin resistance index was calculated according to the Homeostasis Model of Assessment (HOMA-IR) by the following formula[28]:
- Fasting serum insulin (μU/ml) x Fasting serum glucose (mmol/L) / 22.5
- Hepatic glycogen level was assessed by the method given by Van[29].
- Malondialdehyde (MDA) level as one of the main end products of lipid peroxidation was estimated according to Satoh[30].
- superoxide dismutase (SOD) activity was assayed by the method of Nishikimi et al.[31].
- Glutathione Peroxidase (GSPx) activity was assayed by the method given by Rotruck et al.[32]
- Level of reduced glutathione (GSH) was assessed by the method of Ellman[33].
- Triglycerides, cholesterol, LDL-cholesterol and HDL-cholesterol levels were determined according to the methods of Shephard and Whiting[34], and Hatch and Lees[35],

respectively The antiatherogenic index (AAI) was calculated according to the method of Guido and Joseph[36], from total cholesterol and HDL-C as follows:

- AAI= HDL-C x 100 / TC - HDL-C
- The values were expressed as percentage
- Levels of urea and creatinine in serum were evaluated according to the manufacturer's instructions provided in diagnostic kits.[37] [38]

Histopathological Examination

Tissue sample from the pancreas was fixed in 10% buffered-neutral formalin for 6 hours. Fixed pancreas tissue was processed and embedded in paraffin. Sections of 4 mm in thickness were subjected to Hematoxylin and Eosin (H&E) staining before examination.

Statistical analysis

Data were analyzed using one-way analysis of variance (ANOVA) using the Statistical Package for Social Science (SPSS) program, version 17.0 followed by Newman– Keuls post hoc test for multiple comparisons. Differences were considered significant at ($p < 0.05$) level.

3. RESULTS

General observations

During the experimental period including the window period about 20% mortality was observed in STZ induced diabetic group, whereas no mortality was observed in the remaining groups. In STZ-diabetic group rats showed the characteristic signs of diabetes such as polyuria, polydipsia and polyphagia and failure to gain weight.

Effect of Gs leaves extract on body weight

Table (1) illustrates the effect of Gs leaves extract on the change body weight, in both healthy control group and STZ-diabetic rats. The mean body weight of STZ-diabetic group was significantly decreased as compared to normal control rats. The body weight of STZ-diabetic group treated with Gs leaves extract at a dose of 200mg/kg b.w. as well as the Glibenclamide (5 mg/kg b.w.)were significantly increased the body weight as compared to STZ-diabetic animals. However, no significant change was observed in healthy rats treated with Gs leaves extract as compared to normal control rats.

Effect of Gs leaves extract on fasting serum glucose, insulin levels, (HOMA-IR) and liver glycogen

Fasting serum glucose and insulin levels in STZ-diabetic animals exhibited significant hyperglycemia with corresponding hypoinsulinaemia as compared to healthy control rats. However the levels of fasting serum glucose and insulin returned to near normal range in diabetic rats treated with Gs leaves extract at a dose of 200mg/kg b.w.(table 2). This effect of Gs leaves extract was comparable to that of standard drug glibenclamide which showed significant reduction in Fasting serum glucose and elevation in insulin levels. But, no significant changes were observed in healthy rats treated with Gs leaves extract.

In clinical research, HOMA-IR is widely used to assess insulin sensitivity. At the end of the experimental period STZ-diabetic rats showed a significant increase in HOMA-IR ($p<0.05$) as compared to healthy rats. Gs leaves extract ameliorated the insulin sensitivity in group IV (Diabetic + Gs) as was evident from significant decrease of HOMA-IR ($p<0.05$) in STZ-diabetic rats treated with Gs leaves extract. likewise, the Glibenclamide treated group showed a significant reduction of HOMA-IR in STZ-diabetic rats.

On other hand, Glycogen in STZ-diabetic animals was reduced significantly ($p<0.05$) when compared to healthy control groups. However, in treated diabetic group of rats with Gs leaves extract as well as group treated with standard drug Glibenclamide showed markedly significant increase ($p<0.05$) in the levels of glycogen as compared to control diabetic rats.

Antioxidant effect of Gs leaves extract

The induction of diabetes in the rats resulted a significant ($P<0.05$) elevated levels of MDA in the liver and kidney tissues. Furthermore, the diabetic rats exhibited reduced levels of GSH ($P<0.05$) and lower enzymatic activity levels of SOD and GSPx ($P<0.05$) when compared with the healthy control rats(table 3). Treatment of STZ-diabetic rats with Gs leaves extract at a dose of 200mg/kg b.w. for 6 weeks exhibited significant inhibition of the diabetes-induced elevation of MDA in the liver and kidney tissues, at $P<0.05$. However, enhanced levels of GSH ($P<0.05$), as compared with the untreated diabetic rats. Furthermore, the inhibited antioxidant enzymatic activity levels of SOD and GSPx in the liver and kidney tissues were markedly improved ($P<0.05$) in the treated groups with Gs leaves extract (table 3). Similarly, Low levels of GSH, SOD and GSPx in liver and kidney tissues diabetic rats were reverted to near normal values after treatment with standard drug glibenclamide.

Effect of Gs leaves extract on haemoglobin and glycosylated haemoglobin

Table (4) illustrates the levels of total haemoglobin and glycosylated haemoglobin in healthy control rats and treatment animals in each group. STZ-diabetic rats exhibited reduced levels of haemoglobin ($P<0.05$) where as glycosylated haemoglobin levels was increased significantly ($P<0.05$) as compared to healthy control rats. However the level of total haemoglobin and glycosylated haemoglobin returned to near normal range in STZ-diabetic rats treated with Gs leaves extract. Likewise, Glibenclamide treatment group showed markedly significant increase ($p<0.05$) in the levels of total haemoglobin and induced reduction in the glycosylated haemoglobin levels.

Effect of Gs leaves extract on renal weight and advanced glycation end products

Kidney weight and renal AGEs was very elevated in diabetic control rats compared to the control group (Table 4). These levels were reduced to almost in range values by the administration of Gs leaves extract at a dose of 200mg/kg b.w. Symptoms in diabetic animals are increased kidney lipid peroxidation (MDA), reduction in antioxidant defense and increase in renal AGEs. This was in agreement with the present study results that confirmed by Maillard-type measurement, renal AGEs accumulation in streptozotocin induced diabetic rats. For this reasons, we first assessed the effect of Gs leaves extract on renal AGEs accumulation and oxidative stress substance.

Effect of Gs leaves extract on kidney function

Table(5) shows that there is significant ($p<0.05$) increased in the levels of urea and creatinine in serum of STZ-diabetic rats (Group III) as compared to the healthy control rats. However Gs leaves extract treatment in group IV reversed these conditions back to near normal levels. Likewise, Glibenclamide treatment group showed markedly significant decreased ($p<0.05$) in the levels of urea and creatinine as compared to diabetic control rats.

Effect of Gs leaves extract on serum lipid profile

Table (6) shows the level of serum total cholesterol (TC),triglycerides (TG) and Serum Lipid-Lipoprotein Fractions of healthy control rats and experimental animals in each group. Total cholesterol, triglycerides, and Low density lipoprotein (LDL) levels were significantly increased, where as HDL-C level was decreased in STZ-diabetic rats as compared to healthy control rats. Treatment of healthy and STZ-diabetic rats with Gs leaves extract at a dose of 200mg/kg b.w. for 6 weeks resulted in marked decrease in total cholesterol, triglycerides and LDL levels and increase in HDL-C levels as compared to STZ-diabetic rats. STZ-diabetic

rats treated with glibenclamide showed significant ($P < 0.05$) improvement in various parameters on serum lipid profile.

Histopathological Examination of pancreatic tissues

Pancreatic tissues of normal control and healthy rats treated with Gs leaves extract showed normal distribution of islets of Langerhans within the exocrine part. The acinar cells showed strong staining affinity and are arranged in lobules with prominent nuclei. The islet cells are seen embedded within the acinar cells and surrounded by a fine capsule. Islets are regular with well-defined boundaries. Their cells had oval or rounded nuclei[Fig.1(A&B)]. On the other hand, STZ –Diabetic pancreatic tissue showed shrinkage islets of Langerhans in size (Fig1C), signs of necrosis of β-cell destruction and reduction of number of islet and a similar significant reduction in diameter. Some exocrine acini revealed focal acinar damage represented by cytoplasmic vacuolation and pyknotic nuclei of some acinar cells. In diabetic pancreatic tissues treated with Gs leaves extract signs of recovery were presented where islets appeared more or less normal (Fig1D) with increase in size and diameter of islets Langerhans. Numbers of islets were regained. Acini designated pyramidal cells with basal nuclei and apical acidophilic cytoplasm. Likewise , standard drug Glibenclamide treated STZ induced-DM rats showed marked improvement of the cellular injure(Fig1E) ,as evident from the partial restoration of islet cells, reduced β-cell damage, more symmetrical vacuoles and an increase in number of islet cells.

Table 1: Effect of Gs leaves extract on body weight change in healthy and STZ – Diabetic Rats

Groups parameters	Healthy control	Healthy Gs	Diabetic control	Diabetic +Gs	Diabetic + GLB
Initial Body weight (g)	155.55±3.21 [a]	156.45±2.49 [a]	156.10±4.15 [a]	156.05± 2.42 [a]	156.18± 3.24 [a]
Final body Weight(g)	197.67±5.01 [a]	198.00±3.95 [a]	129.67±3.50 [d]	187.67 ± 5.57 [b]	181.17±4.07 [c]

Values are expressed as means ± S.D, n=6

There is no significant difference between means have the same letter in the same row ($P<0.05$)

Table 2: Effect of Gs leaves extract on fasting serum glucose, insulin , insulin resistance index (HOMA-IR) and liver glycogen in healthy and STZ – Diabetic Rats

Groups parameters	Healthy control	Healthy Gs	Diabetic control	Diabetic +Gs	Diabetic + GLB
fasting serum glucose (mg/dL)	85.25±2.1 ^{c}	83.49±4.23 ^{c}	267.66 ± 5.18 ^{a}	118.50±2.79 ^{b}	121.86±4.07 ^{b}
Insulin (U/ml)	13.10±0.35 ^{a}	13.32±0.44 ^{a}	5.82±0.42 ^{d}	11.01±0.02 ^{b}	10.88±0.04 ^{c}
HOMA-IR	49.59±1.79 ^{c}	50.03±3.41 ^{c}	69.31±5.74 ^{a}	57.98±1.78 ^{b}	59.03±1.91 ^{b}
Liver glycogen (mg/g tissue)	44.15 ± 0.67 ^{b}	45.55 ± 0.72 ^{a}	19.62 ± 0.61 ^{e}	40.83 ± 0.76 ^{c}	38.62±1.19 ^{d}

Values are expressed as means ± S.D, n=6

There is no significant difference between means have the same letter in the same row (P<0.05)

Table 3: Effect of Gs leaves extract on liver and kidney oxidative stress in healthy and STZ – Diabetic Rats

Groups parameters	Healthy control	Healthy Gs	Diabetic control	Diabetic +Gs	Diabetic + GLB
MDA-Liver (nmol/g tissue)	21.32±2.39 ^{d}	20.74±1.26 ^{d}	48.47±1.84 ^{a}	25.44±1.06 ^{c}	27.33±0.80 ^{b}
MDA-kidney (nmol/g tissue)	16.59±0.86 ^{d}	14.36±0.64 ^{e}	34.89±1.67 ^{a}	18.08±1.28 ^{c}	19.73±0.95 ^{b}
GSH-Liver (mg/g tissue)	43.31±0.31 ^{b}	45.49±0.49 ^{a}	24.64± 0.48 ^{e}	41.60±0.36 ^{c}	38.60±0.46 ^{d}
GSH-kidney (mg/g tissue	25.11 ± 0.39 ^{b}	25.86 ± 0.53 ^{a}	6.17 ± 0.45 ^{e}	21.65 ± 0.37 ^{c}	20.59 ± 0.34 ^{d}
SOD-Liver (U/g tissue)	7.77 ± 0.41 ^{ab}	8.61± 1.03 ^{a}	4.10 ± 0.83 ^{c}	7.51 ± 0.67 ^{b}	7.04 ± 0.80 ^{b}
SOD-Kidney (U/g tissue)	14.52 ± 0.85 ^{b}	15.42± 0.51 ^{a}	7.12 ± 0.55 ^{e}	13.70 ± 0.360 ^{c}	12.90 ± 0.46 ^{d}
GSPx-Liver (U/g tissue)	7.76 ± 0.61 ^{a}	8.43± 0.68 ^{a}	4.38 ± 0.48 ^{c}	6.37 ± 0.68 ^{b}	6.23 ± 0.52 ^{b}
GSPx-Kidney (U/g tissue)	6.10 ± 0.56 ^{b}	6.61 ± 0.44 ^{a}	3.41 ± 0.21 ^{d}	4.65 ± 0.37 ^{c}	4.43 ± 0.27 ^{c}

Values are expressed as means ± S.D, n=6

There is no significant difference between means have the same letter in the same row (P<0.05)

Table 4: Effect of Gs leaves extract on the formation of advanced glycation end products (AGEs), Haemoglobin, Glycosylated Haemoglobin (HbA1c) and Renal Weight in healthy and STZ – Diabetic Rats

Groups parameters	Healthy control	Healthy Gs	Diabetic control	Diabetic +Gs	Diabetic + GLB
Haemoglobin (gm/dL)	11.36±0.35 [b]	11.80±0.36 [a]	7.13±0.28 [e]	10.54±0.29 [c]	10.08±0.36 [d]
Glycosylated haemoglobin HbA1c (%)	3.55±0.25 [d]	3.16±0.17 [d]	13.71±0.28 [a]	4.09±0.30 [c]	4.54±0.59 [b]
AGEs-kidney (AU)	14.49 ± 0.77 [c]	11.85 ± 0.43 [d]	25.83 ± 0.85 [a]	16.25 ±1.05 [b]	17.20 ±0.78 [b]
Relative kidney weight (g %)	1.46 ± 0.087 [c]	1.40 ± 0.051 [c]	2.03 ± 0.015 [a]	1.57± 0.056 [b]	1.61± 0.039 [b]

Values are expressed as means ± S.D, n=6

There is no significant difference between means have the same letter in the same row (P<0.05)

Table 5: Effect of Gs leaves extract on kidney function in healthy and STZ – Diabetic Rats

Groups parameters	Healthy control	Healthy Gs	Diabetic control	Diabetic +Gs	Diabetic + GLB
Serum Urea (mg/dl)	21.38 [c]	20.89 [c]	46.96 [a]	25.33 [b]	26.28 [b]
Serum Creatinine (mg/dl)	0.83 [c]	0.76 [c]	2.12 [a]	1.19 [b]	1.36 [b]

Values are expressed as means ± S.D, n=6

There is no significant difference between means have the same letter in the same row (P<0.05)

Table 6: Effect of Gs leaves extract on lipid profile in healthy and STZ – Diabetic Rats

Groups parameters	Healthy control	Healthy Gs	Diabetic control	Diabetic +Gs	Diabetic + GLB
HDL (mg/dl)	34.82±1.02 [b]	37.63±0.61 [a]	24.66±0.67 [e]	33.00± 0.78 [c]	32.00±0.28 [d]
LDL (mg/dl)	24.50±0.66 [d]	20.95±1.13 [e]	63.43±0.98 [a]	30.52±1.03 [c]	32.47±0.91 [b]
CH (mg/dl)	79.68±2.04 [c]	78.05±2.03 [c]	117.05±1.71 [a]	82.32±2.10 [b]	84.49±2.34 [b]
TGS (mg/dl)	95.98±1.00 [c]	94.51±1.19 [c]	143.35±1.29 [a]	99.46± 2.23 [b]	100.41±2.04 [b]
AAI	77.91±6.10 [b]	93.34±5.72 [a]	26.84±1.25 [e]	67.06±4.63 [c]	60.96±2.73 [d]

Values are expressed as means ± S.D, n=6

There is no significant difference between means have the same letter in the same row (P<0.05)

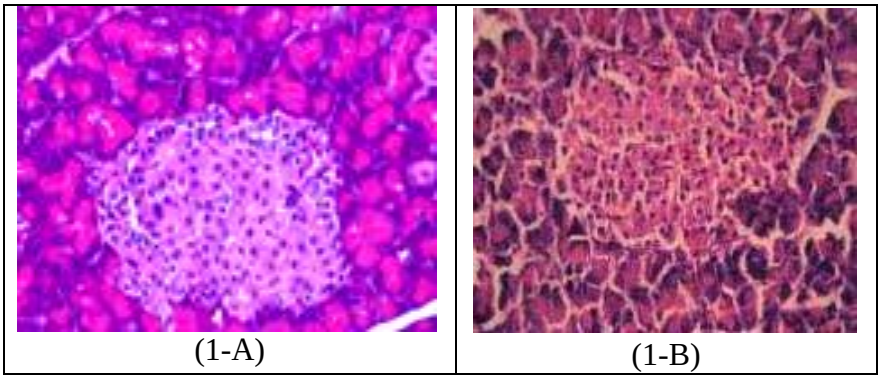

Fig. 1 (A-E): Pancreatic sections of albino rats in experimental groups, stained with (H&E X200

DISCUSSION

Diabetes mellitus (DM) is characterized by hyperglycemia associated with impairment in insulin secretion and/or insulin action as well as alteration in intermediary metabolism of carbohydrates, proteins and lipids. The present study was conducted to determine the antidiabetic, antioxidant, antiglycation, hypolipidemic as well as effects of Gs leaves extract, in STZ-diabetic rats. Our results showed that oral treatment of diabetic rats with a Gs leaves extract restored the elevated blood glucose levels to normal range indicating marked antidiabetic activity of the STZ-diabetic rats. Gymnemic acid is the active principle of Gs leaves extract responsible for the antihyperglycaemic effect of the plant. The possible mechanisms by which gymnemic acid exerts its antidiabetic effect have been reported to be through promotion of regeneration of islet cells, secretion of insulin, inhibition of glucose absorption from intestine, increased utilization of glucose through activation of enzymes responsible for utilization of glucose by insulin-dependent pathways, increase in phosphorylase activity and decrease in gluconeogenic enzymes and sorbitol dehydrogenase[39]. The immune histochemical and special staining technique which showed

an increase in the β-cells upon Gs leaves extract supplementation point out the possible regeneration of β-cells from precursor cells or trans differentiation of α-cells to β-cells[40,41].

Likewise, Our result was in accordance with Aralelimath et al.[42] who suggested that of Gs leaves extract increased insulin release in vitro by two mechanisms: (1) the major mode of action was through b-cell plasma membrane permeability, (2) the pores formed by plasma membrane disruption.

On other hand, The improvement seen in glibenclamide treated rats could be attributed to stimulation of insulin release that rapidly follows the drug's binding to a surface membrane receptor and a subsequent rise in cytoplasmic free calcium concentration. It has been reported that prolonged exposure to glibenclamide maintains high intracellular calcium levels and several calcium dependent signalling pathways, which is responsible for activation of translation and subsequent protein synthesis in beta cells under sustained glibenclamide influence[43]. In the present study, G. sylvestre was found to be more effective than glibenclamide in alleviation of STZ induced diabetes in rats.

Thus, Insulin plays a crucial role in lowering blood glucose level by enhancing glycogenesis in liver and muscles[19]. Therefore, the present results showed markedly increased liver glycogen content in diabetes rats treated with Gs leaves extract.

It has been known that glycation (non-enzymatic glycosylation) is the result of a sugar molecule such as fructose or glucose binding to a protein or lipid molecule without the controlling action of an enzyme. In diabetic patients, glycation occurs excessively. Among various metabolic development implicated in the pathogenesis of diabetes vascular complication, advanced glycated end products (AGEs) has been known to be compatible with the level of hyperglycemia.

Several studies have suggested that AGEs stimulates production of ROS, including superoxide anions, hydrogen peroxide and hydroxyl radicals, in a variety of cell types[8 ,9,10].

Oxidative stress serves a vital function in the pathogenesis of both types of diabetes mellitus and is known to be induced by hyperglycemia following the autoxidation of monosaccharide's and proteins[3] Several animal and clinical trials have shown that diabetes is associated with weakening of antioxidant defense system[6]. The imbalance created between the oxidants and antioxidants disturbs the equilibrium leading to oxidative stress.

GSH constitutes is the first line of defense against free radicals at the cellular and tissue levels to protect against the toxic effects of lipid peroxidation. Hence, severe GSH depletion leaves cells more vulnerable to oxidative damage by radicals and increases protein thiolation or oxidation of SH groups. Likewise, superoxide dismutase (SOD) and glutathione peroxidase (GSPx) helps in scavenging of free radicals thus strengthening the defense system.

Our findings show that STZ-diabetic rats increased intracellular generation of AGEs and ROS which is reported as increased lipid peroxides. Results showed a significant reduction in antioxidant enzymatic activity($p \leq 0 \cdot 05$) in hepatic and renal tissues in control diabetic rats as compared to healthy control group. On other hand, the present study showed an elevation of AGEs was attributed to inhibition of antioxidant enzyme activities which reducing ROS generation in cells.

Some herbs are being utilized as antioxidants for preventing oxidative damage in living systems and to delay the onset of degenerative diseases[44,45,46,47].

The gymnema leaves extract has shown antioxidant activity in vitro by inhibiting DPPH (2,2- diphenyl-1-picrylhydrazyl), scavenging super oxide, hydrogen peroxide and their reduction by the presence of flavonoids, phenols, tannins (Phenolic compounds) and triterpenoids[48,49]. Treatment with Gs. leaves extract in STZ diabetic rats(G4) restoring the antioxidant enzyme activities to normal with reduced AGEs and inhibited lipid peroxidation which attributed to ROS generation in cells.

Similarly, In diabetic conditions the erythrocytes are more prone to oxidative stress and hence exhibit high glycosylated hemoglobin levels. In the present study, treatment with *Gymnema sylvestre* extract decreased the, glycosylated hemoglobin level (HbA1c) when compared to diabetic control rats.

In the present study, rats treated with Gs. sylvestre leaves extract in STZ-diabetic induction showed a significant decrease in triglyceride, cholesterol and LDL-cholesterol and showed a significant increase in HDL-cholesterol as compared to that of untreated diabetic rats in G3. 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 result was in agreement with Aralelimath and Bhise[42] 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 Pothuraj et al.[50] who reported that G. sylvestre decreases total cholesterol, LDL-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. G. sylvestre inhibited the intestinal absorption of oleic acid in rats, which suggested the possibility of Gs. sylvestre to inhibit lipid absorption[51]. Shigematsu et al.[52] reported that administration of Gs. sylvestre leaves extract decreased the total cholesterol, LDL-cholesterol and triglyceride levels in the serum of STZ-diabetic rats that might be due to the effect of G. sylvestre in increasing neutral sterols and acid steroids excretion into feces. Kumar et al.[53] discovered the ability of Gs. sylvestre water extract to prevent the genetic obesity by improving the cholesterol metabolism and inhibiting polyphagia. Ishijima et al.[39] suggested that gymnemic acid might have some pharmacological activities including antidiabetic activity and lipid lowering effects via the inhibition of glycerol-3-phosphate dehydrogenase activity in vitro. Lipid lowering activity of flavonoids and saponins extracted from G. sylvestre might be due to the inhibition of pancreatic lipase activity[54].

It was apparent from our results that serum lipid Severe hyperglycemia observed in STZ diabetic rats is favourable for increased non-enzymic glycation of LDL-C. There are reports that LDL-C increases its atherogenic potential after chemical modification including glycation[55,56,57]. These explains the role of LDL in premature development of atherosclerosis under diabetic conditions. Atherogenic Index indicates deposition of foam cells, plaque, fatty infiltration or lipids in heart, coronaries, aorta, liver and kidney[9]. The higher atherosclerotic index greater the risk of these organs to oxidative damage. Thus, greater atherogenecity in diabetic control group can be attributed to increased oxidative insult under hyperglycemic conditions. The antihyperlipidemic and anti atherogenic property of Gs leaves extract is evident by the corrected dislipidemia and improved AAI in G4. The protection by the administration of Gs leaves extract against the atherogenecity in insulin deficient conditions can be attributed to its hypolipidemic and antioxidant properties.

Numerous epidemiological studies suggest that herbs/diets rich in phytochemicals and antioxidants execute a protective role in health and disease[58,59]. Flavonoids, sterols,

triterpenoids, alkaloids, saponins and phenolics are reported as bioactive antidiabetic principles[11,60,61,62]. Triterpenoids can regenerate damaged β-cells in the alloxan and STZ induced diabetic rats[40,42]. Polyphenols inhibit lipid peroxidation by acting as chain breaking peroxyl radical scavengers and can protect LDL from oxidation and also improvement serum lipid profile[11]. Thus Gs leaves extract exhibited a protective role as antidiabetic, antiglycation, antioxidant and anti hyperlipidimic in experimental diabetes rats.

STZ diabetic rats showed a significant increase in serum urea and creatinine levels as compared to control animals. the increase in serum urea and creatinine levels may be due to hyperglycemia that causes osmotic dieresis and depletion of extracellular fluid volume ; several studies also have shown an increase correlation between serum urea and creatinine in diabetic paitients[63]. Treatment with Gs leaves extract found to decrease serum urea and creatinine levels . This may be correlated with decrease in glucose levels by Gs leaves extract and thereby decrease in osmotic dieresis and depletion of extracellular fluid volume.

On other hand, STZ diabetic rats (G3) has shown to induce free radical production and cause tissue injury. The pancrease is especially susceptible to the action of streptozotocin –induced free radical damage. Gs leaves extract possesses strong antioxidant property[42] can act as free radical scavenger and protect ß cells from damage. Similarly, the damage of pancreas in STZ diabetic rats and regeneration of ß cells by glibenclamide was observed in these study. The regeneration of ß cells of the STZ – destructed islets is probably due to the fact that pancreas contain stable cells which have the capacity of regeneration. Therefore, the surviving cells can proliferate to replace the lost cells. The total ß cells mass reflects the balance between the renewal and loss of these cells.[40]

CONCLUSIONS

In conclusion, A possible reason for this might be high content of phytochemicals in Gs leaves extract which attenuates the effect of STZ in diabetic rats and suggest their ability to boost antioxidant levels in diabetic conditions. Gs leaves extract have an antidiabetic, antiglycation, antioxidant and antihyperlipidemic potential in STZ diabetic rats. The bioactive compounds present in the Gs leaves extract have can quench free radicals, and protect the cellular and tissue damage by oxidative stress. Gs leaves extract have can be used as a promising functional food for various chronic diseases.

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