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ORIGINAL ARTICLE

Effect of *Gymnema sylvestre* R. Br. leaves extract on certain physiological parameters of diabetic rats

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Abstract *Gymnema sylvestre* R. Br. (*G. sylvestre*) belonging to the family Asclepiadaceae, has been used as a traditional medicine plant in Africa, Australia and Asia especially in India.

Aim of the study: The present study aimed to clarify the effect of *G. sylvestre* leaves extract on several physiological parameters of diabetic rats.

Materials and methods: *G. sylvestre* leaves extract (18 mg/kg body weight) was orally administered for 30 days to normal and streptozotocin (STZ) diabetic rats.

Results: STZ-diabetic rats exhibited a significant increase in plasma glucose, liver function enzymes [alanine aminotransaminase (ALT) and aspartate aminotransaminase (AST)], triglycerides, total cholesterol, LDL-cholesterol malondialdehyde, catalase, reduced glutathione and a significant decrease in insulin, HDL-cholesterol and erythrocyte superoxide dismutase levels. Treatment diabetic rats with *G. sylvestre* leaves extract significantly decreased plasma glucose, ALT, AST, triglycerides, total cholesterol, LDL-cholesterol, malondialdehyde and significantly increased insulin, HDL-cholesterol and erythrocyte superoxide dismutase levels compared to untreated diabetic rats.

Conclusion: It could be concluded that *G. sylvestre* leaves extract treated diabetic rats' complications including hyperglycemia, hypoinsulinemia, hyperlipidemia and oxidative stress.

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1. Introduction

Diabetes mellitus (DM) is a metabolic disorder characterized by hyperglycemia resulting from defective insulin secretion, resistance to insulin action or both (Jarald et al., 2008; Vasi and Austin, 2009; Khan et al., 2012). DM is a widespread disease, associates with chronic micro- and macro-vascular complications (Goycheva et al., 2006; Ahmed et al., 2011; Aralelimath and Bhise, 2012). Total recovery from diabetes has not been reported up to now and this explains the intensive studies done on it.

Untreated diabetes leads to serious complications or even premature death. The treatment of diabetes mellitus is based on insulin and/or oral hypoglycemic drugs (Daisy et al., 2009). These drugs act by various mechanisms to control the blood glucose level, but many side-effects have been reported (Patel et al., 2012). Therefore, there is considerable interest in the field of medicinal plants due to their natural origin and less side effects (Jarald et al., 2008). One of these medicinal plants is *Gymnema sylvestre* (Gurmar), which means sugar killer (Najafi and Deokule, 2011). It is a wild plant that grows in the open forest in India, China, Indonesia, Japan, Malaysia, Sri Lanka, Vietnam, and South Africa (Gurav et al., 2007; Spasov et al., 2008). The leaves of the plant in particular are used as antiviral, diuretic, antiallergic, hypoglycemic, hypolipidemic, antibiotic, in stomach pains and in rheumatism (Saneja et al., 2010).

Most studies on *G. sylvestre* leaves extract focused on its role as hypoglycemic medicinal plant, but the present study demonstrates the effect of *G. sylvestre* leaves extract (18 mg/kg body weight) on several etiological factors of STZ-diabetic rats including hyperglycemia, hypoinsulinemia, hyperlipidemia and oxidative stress at the same time.

2. Materials and methods

2.1. Experimental animals

White male albino rats (*Rattus norvegicus*) weighing 200 ± 10 g were purchased from the Center of Laboratory animals, venoms & crude antisera production, Helwan, Cairo, Egypt. Animals were maintained under laboratory conditions [temperature (20 ± 2 C) and photoperiod (12 h light and 12 h dark cycle)]. Animals were allowed *ad libitum* to food and tap water. Rats were allowed to acclimatize for one week before the onset of the experiment.

2.2. Diabetogenic agent and *Gymnema sylvestre* leaves extract

- 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 (pH 4.5) according to El-Seifi et al. (1993).
- *Gymnema sylvestre* (*G. sylvestre*) 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 *G. sylvestre* leaves extract only. *G. sylvestre* leaves extract was given in a dose of (18 mg/kg body weight) dissolved in 1 ml distilled water according to Paget and Barnes (1964).

Kirtikar and Basu (1998) classified *G. sylvestre* as the following:

Kingdom	Plantae
Subkingdom	Tracheobionta
Super division	Spermatophyta
Division	Magnoliophyta
Class	Magnoliopsida
Subclass	Asteridae
Order	Gentianales
Family	Asclepiadaceae
Genus	<i>Gymnema</i>
Species	<i>sylvestre</i> R. Br.

- Common name: Gurmar, *Gymnema*, Gurmari, Gurmarbooti, periploca of the woods, and meshasing.
- Physicochemical properties: *G. sylvestre* leaves extract is soluble in water and other polar solvents as it contains monosaccharides. *G. sylvestre* leaves extract has an acidic nature due to the presence of carboxylic groups. *G. sylvestre* contains more than 20 triterpene saponins that form molecular complexes with proteins, lipids, sterols and tannins (Spasov et al., 2008).

2.3. Induction of 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 (Abdel-Moneim et al., 2002). 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 180 to 200 mg/dl were considered as mild diabetic and included in the experiment according to (Abdel-Moneim et al., 2002).

2.4. Experiment design

Rats under study were classified into five groups (6 rats each):

- **Group I: normal rat group.**

Rats of this group were given intragastrically 1 ml distilled water once a day for 30 days.

- **Group II: rat group injected with citrate buffer.**

Rats of this group were injected with a single intraperitoneal dose of 1 ml citrate buffer (pH 4.5).

- **Group III: rat group supplemented with *G. sylvestre* leaves extract.**

Rats of this group were supplemented with *G. sylvestre* leaves extract (18 mg/kg b.w.) dissolved in 1 ml distilled water once a day for 30 days.

- **Group IV: STZ-diabetic rat group.**

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).

The diabetic rats were housed at the experimental condition for 30 days.

- **Group V: rat group treated with *G. sylvestre* leaves extract post STZ-diabetic induction.**

Rats of this group were treated intragastrically with *G. sylvestre* leaves extract (18 mg/kg b.w.) dissolved in 1 ml dis-

tilled water once a day for 30 days after STZ-diabetic induction.

2.5. Blood sampling

At the end of the experimental period, animals of each group were fasted about 12 h and then anesthetized by diethyl ether inhalation (Sinnet et al., 1984). Blood samples were collected from post caval vein and directly transported to tubes containing ethylenediamine tetra-acetic acid (EDTA) (El-Gomhorya Co. Egypt). All the tubes were centrifuged at 3000 rpm. for 15 min by Hittech® centrifuge and plasma free of hemolysis was separated and frozen at -20°C for the determination of physiological parameters. Packed erythrocytes were washed four times with isotonic solution (0.9% NaCl) and centrifuged for 10 min at 3000 rpm. after each wash to remove the leftover leukocytes. After the last wash erythrocytes were frozen at -20°C for the determination of superoxide dismutase level.

2.6. Determination of biochemical parameters

All parameters except insulin level were determined by Hitachi (902) Automatic Analyzer.

- Plasma glucose level was determined according to the method of Burtis and Ashwood (2006) using reagent kits purchased from Roche Diagnostics (USA).
- Plasma insulin level was measured on a Thermo Scientific Multiskan FC ELISA Reader by rat/mouse insulin ELISA kits purchased from Millipore (USA).
- Plasma ALT and AST levels were determined according to the method of Glick et al. (1986) using kits purchased from Roche Diagnostics (USA).
- Plasma triglycerides, cholesterol, LDL-cholesterol and HDL-cholesterol levels were determined according to the methods of Shephard and Whiting (1990), Trinder (1969), Wieland and Seidel (1983) and Hatch and Lees (1968), respectively using kits purchased from Roche Diagnostics (USA).
- Plasma malondialdehyde, catalase, reduced glutathione and erythrocyte superoxide dismutase levels were determined according to the methods of Satoh (1978), Aebi (1984), Beutler et al. (1963) and Nishikimi et al. (1972), respectively using reagent kits purchased from Bio-diagnostic (Egypt).

2.7. Statistical analysis

Statistical analysis was performed using Statistical Package for Social Science (SPSS) computer program, Version 15.00. The values were analyzed by one-way analysis of variance (ANOVA) followed by Duncan's multiple range test (Duncan, 1957). Data were expressed as mean \pm SD. Values were considered significant at $p < 0.05$.

3. Results

- **Effect of *G. sylvestre* leaves extract on plasma glucose and insulin levels.**

Plasma glucose level was significantly low ($p < 0.05$) in the rat group supplemented with *G. sylvestre* leaves extract (group

III) compared to those of control rat groups (groups I and II, respectively). On the other hand, plasma glucose levels were significantly high in the diabetic rat group (group IV) and the rat group treated with *G. sylvestre* leaves extract post diabetic induction (group V) in relation to those of control rat groups. Plasma glucose level decreased significantly in group V compared to that of group IV (Table 1).

Plasma insulin level was significantly high ($p < 0.05$) in group III compared to those of groups I and II. On the other hand, plasma insulin levels were significantly low in groups IV and V compared to those of groups I and II. Plasma insulin level increased significantly in group V compared to that of group IV (Table 1).

- **Effect of *G. sylvestre* leaves extract on plasma liver function enzymes level.**

Both plasma alanine aminotransaminase (ALT) and aspartate aminotransaminase (AST) levels in group III showed a non significant difference ($p < 0.05$) in relation to those of groups I and II. Plasma ALT and AST levels were significantly high in groups IV and V compared to those of groups I and II. Plasma ALT and AST levels were significantly low in group V compared to those of group IV (Table 2).

- **Effect of *G. sylvestre* leaves extract on plasma lipid profile.**

Plasma triglycerides, cholesterol and LDL-cholesterol levels decreased significantly ($p < 0.05$) in group III compared to those of groups I and II. Plasma triglycerides, cholesterol and LDL-cholesterol levels in groups IV and V were significantly high compared to those of groups I and group II. Plasma triglycerides, cholesterol and LDL-cholesterol levels decreased significantly in group V compared to those of group IV (Table 3).

Plasma HDL-cholesterol levels increased significantly ($p < 0.05$) in group III compared to those of groups I and II. On the other hand, plasma HDL-cholesterol levels were significantly low compared to those of groups I and II. Plasma HDL-cholesterol level was increased significantly in group V compared to that of group IV (Table 3).

- **Effect of *G. sylvestre* leaves extract on antioxidant parameter levels.**

Plasma malondialdehyde (MDA) levels in groups III and V showed a non significant difference ($p < 0.05$) in relation to those of groups I and II. Plasma MDA level was significantly increased in group IV compared to that of groups I and II. Plasma MDA level was significantly decreased in group V compared to that of group IV (Table 4).

Plasma catalase level in group III was significantly low ($p < 0.05$) compared to that of groups I and II. Plasma catalase levels in groups IV and V increased significantly compared to those of groups I and II. Plasma catalase level in group V showed non significant difference compared to that of group IV (Table 4).

Erythrocyte superoxide dismutase (SOD) levels were significantly low ($p < 0.05$) in groups III and IV compared to those of groups I and II. Erythrocyte SOD level in group V showed a non significant difference compared to that of groups I and II.

Table 1 Plasma glucose and insulin levels of normal rats (Group I), rats injected with citrate buffer (Group II), rats supplemented with *G. sylvestre* leaves extract (Group III), STZ-diabetic rats (Group IV) and rats treated with *G. sylvestre* leaves extract post STZ-diabetic induction (Group V).

Parameters	Groups				
	Group I	Group II	Group III	Group IV	Group V
Glucose (mg/dl)	132.86 ± 0.66 ^c	131.66 ± 0.47 ^c	127.54 ± 0.93 ^d	285.77 ± 2.39 ^a	228.06 ± 2.68 ^b
Insulin (ng/ml)	25.71 ± 0.46 ^b	24.71 ± 0.89 ^b	27.97 ± 0.62 ^a	10.36 ± 0.19 ^d	19.00 ± 0.60 ^c

All data expressed as mean ± SD.

^{abcd} Means superscripted with different letters are significantly different at ($p < 0.05$).

Table 2 Plasma liver function enzymes level (alanine aminotransaminase, ALT and aspartate aminotransaminase, AST) of normal rats (Group I), rats injected with citrate buffer (Group II), rats supplemented with *G. sylvestre* leaves extract (Group III), STZ-diabetic rats (Group IV) and rats treated with *G. sylvestre* leaves extract post STZ-diabetic induction (Group V).

Parameters	Groups				
	Group I	Group II	Group III	Group IV	Group V
ALT (U/L)	35.04 ± 0.72 ^c	35.10 ± 0.12 ^c	35.57 ± 0.36 ^c	67.63 ± 0.34 ^a	50.32 ± 0.36 ^b
AST (U/L)	68.33 ± 0.93 ^c	67.57 ± 0.78 ^c	68.03 ± 0.18 ^c	88.81 ± 0.63 ^a	74.37 ± 1.11 ^b

All data expressed as mean ± SD.

^{abc} Means superscripted with different letters are significantly different at ($p < 0.05$).

Table 3 Plasma lipid profile of normal rats (Group I), rats injected with citrate buffer (Group II), rats supplemented with *G. sylvestre* leaves extract (Group III), STZ-diabetic rats (Group IV) and rats treated with *G. sylvestre* leaves extract post STZ-diabetic induction (Group V).

Parameters	Groups				
	Group I	Group II	Group III	Group IV	Group V
Triglycerides (mg/dl)	76.37 ± 0.66 ^c	77.53 ± 0.42 ^c	61.25 ± 1.17 ^d	101.50 ± 0.45 ^a	81.33 ± 0.58 ^b
Cholesterol (mg/dl)	66.64 ± 0.87 ^c	66.80 ± 0.39 ^c	40.95 ± 0.52 ^d	89.11 ± 0.31 ^a	75.30 ± 1.05 ^b
LDL-cholesterol (mg/dl)	10.57 ± 0.31 ^c	10.43 ± 0.26 ^c	9.16 ± 0.46 ^d	12.46 ± 0.13 ^a	11.43 ± 0.08 ^b
HDL-cholesterol (mg/dl)	30.58 ± 0.47 ^b	30.94 ± 0.21 ^b	34.61 ± 0.55 ^a	23.71 ± 0.46 ^d	28.83 ± 0.51 ^c

All data expressed as mean ± SD.

^{abcd} Means superscripted with different letters are significantly different at ($p < 0.05$).

Table 4 Antioxidant parameter levels of normal rats (Group I), rats injected with citrate buffer (Group II), rats supplemented with *G. sylvestre* leaves extract (Group III), STZ-diabetic rats (Group IV) and rats treated with *G. sylvestre* leaves extract post STZ-diabetic induction (Group V).

Parameters	Groups				
	Group I	Group II	Group III	Group IV	Group V
MDA (nmol/ml)	241.33 ± 3.21 ^{bc}	242.33 ± 5.51 ^{bc}	235.67 ± 2.08 ^c	255.33 ± 5.03 ^a	244.67 ± 5.51 ^b
Catalase (U/ml)	852.67 ± 4.19 ^b	849.00 ± 6.00 ^b	832.33 ± 5.51 ^c	875.67 ± 7.02 ^a	869.00 ± 1.00 ^a
SOD (U/ml)	159.33 ± 9.07 ^a	155.00 ± 6.00 ^a	140.33 ± 2.52 ^b	136.00 ± 2.65 ^b	156.33 ± 3.21 ^a
GSH (mg/dl)	30.63 ± 0.67 ^b	28.73 ± 1.26 ^c	31.67 ± 0.45 ^b	32.70 ± 0.96 ^a	31.23 ± 0.55 ^{ab}

All data expressed as mean ± SD.

^{abc} Means superscripted with different letters are significantly different at ($p < 0.05$).

Erythrocyte SOD level in group V showed a significant increase compared to that of group IV (Table 4).

Plasma reduced glutathione (GSH) level was significantly decreased ($p < 0.05$) in group II compared to that of group

I. On the other hand, plasma GSH levels in groups III and V showed a non significant difference compared to that of group I. Plasma GSH level in group IV increased significantly compared to that of group I. Plasma GSH level in group V

showed a non significant difference compared to that of group IV (Table 4).

4. Discussion

G. sylvestre is an important medicinal plant used in different systems of medicine as a remedy for the treatment of diabetes (Jarald et al., 2008).

The present study showed a significant decrease in plasma glucose level (20.20%) in diabetic rats treated with *G. sylvestre* leaves extract (18 mg/kg body weight) compared to that of untreated diabetic rats. Significant increase in diabetic rats' insulin level post treated with *G. sylvestre* leaves extract confirmed our result. Moreover, glucose level might be decreased in treated diabetic rats as a result of decreasing gluconeogenesis that was indicated by low levels of ALT and AST in treated diabetic rats compared to untreated diabetic rats.

This result was in agreement with Shanmugasundaram et al. (1983) who reported that administration of dried leaf powder of *G. sylvestre* decreased glucose levels as it controlled gluconeogenic enzymes (ALT and AST) and increased glycogen levels in liver, kidney and muscle. Treated 27 type 1 diabetic patients with water extract of *G. sylvestre* leaves (400 mg/day) for 12 months reduced blood glucose level (up to 35%) as a direct effect of increasing exogenous insulin level (up to 50%) (Shanmugasundaram et al., 1990). Chattopadhyay (1998) reported that administration of alcoholic extract of *G. sylvestre* leaves to Swiss albino rats fed by glucose increases the insulin level. Bolkent et al. (2000) explained that the decrease in plasma glucose levels might be due to the increase in insulin levels after administration of gymnemic acid to diabetic rats. Gholap and Kar (2005) discovered that gymnemic acid was capable of reducing corticosteroid hormones in dexamethasone-induced hyperglycemia in mice. Daisy et al. (2009) reported that plasma glucose level reduced more than 50% in diabetic rats treated by dihydroxy gymnemic triacetate (20 mg/kg b.w.) for 40 days compared to untreated diabetic rats and they explain this reduction as a result of increasing plasma insulin (50%), muscle glycogen (77.1%) and liver glycogen (59.09%) content. *In vivo* leaf and *in vitro* callus methanol extract of *G. sylvestre* decreased blood glucose level (72.4%) in diabetic rats (Ahmed et al., 2008) and that might be due to increased liver glycogen content (Ahmed et al., 2010). The activities of phosphorylase enzymes and sorbitol dehydrogenase were increased in diabetic rabbits after treated with *G. sylvestre* extract (Venkatesham et al., 2010).

This result was supported also by Yoshioka (1986) who demonstrated that Gymnemic acid inhibited Na^+ dependent active glucose transport in the small intestine. Fushiki et al. (1992) suggested that the *G. sylvestre* leaves extract inhibited gastric inhibitory peptide (GIP). Sahu et al. (1996) reported that the gymnemic acid molecules fill the receptor location in the absorptive external layers of the intestine thereby preventing the glucose absorption by the rat intestine. It was suggested that gymnemic acid extract inhibited glucose uptake in the intestine of guinea pig which occurred due to the effect of gymnemic acid extract on the suppression of high K^+ induced contraction in guinea pig ileac longitudinal muscles (Shimizu et al., 1997). The treatment with high concentrations of *G. sylvestre* leaves extract reduced the levels of glucose in albino Wistar rats and that might be due to the effect of gymnemic

acid on the inhibition of glucose uptake in the intestine (sujin et al., 2008). Hypoglycemic potential of *G. sylvestre*, *Tinospora cordifolia*, *Eugenia jambolana* and *Aegle marmelos* might be due to their effect on inhibition of the activity of α -amylase resulting in the delayed digestion of the dietary carbohydrates, so the liberated glucose from the intestine lumen to the circulation decreased (Ahmed et al., 2011).

Plasma insulin levels significantly increased (83.40%) in diabetic rats treated with *G. sylvestre* leaves extract (18 mg/kg body weight) compared to that of untreated diabetic rats. Our result was in accordance with Persaud et al. (1999) who suggested that alcoholic extract of *G. sylvestre* leaves increased insulin release *in vitro* by two mechanisms: (1) the major mode of action was through β -cell plasma membrane permeability, (2) the pores formed by plasma membrane disruption. The number of pancreatic β -cells in the diabetic rats doubled after administration of alcoholic extract of *G. sylvestre* leaves (Shane-McWhorter, 2001). Daisy et al. (2009) reported that the insulin levels in diabetic rats increased (50%) after treatment with dihydroxy gymnemic triacetate and that might be due to the stimulation of insulin secretion from regenerated or residual β -cells by dihydroxy gymnemic triacetate. Liu et al. (2009) suggested that the increase in insulin secretion from mouse β -cells and isolated human islets after the administration of low concentration of *G. sylvestre* leaves extract might be partially dependent on the increase in Ca^+ influx. Degenerative changes in pancreatic β -cells were minimized and normal morphology maintained in alloxan induced diabetic rats after administration of gymnemic acid (Ahmed et al., 2010). Microscopy studies of pancreas of animals receiving *G. sylvestre* extract showed that nuclei of endocrinocytes were significantly enlarged in all sections of the organ with the same volume fraction and area of pancreatic islets. The result agreed completely with investigations proposed that use of *G. sylvestre* increased the endogenous levels of insulin, possibly due to regeneration of pancreas cells (Venkatesham et al., 2010).

In the present study, rats treated with *G. sylvestre* leaves extract post 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. 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 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. *G. sylvestre* inhibited the intestinal absorption of oleic acid in rats, which suggested the possibility of *G. sylvestre* to inhibit lipid absorption (Wang et al., 1998). Shigematsu et al. (2001) reported that administration of *G. sylvestre* leaves extract decreased the total

cholesterol, LDL-cholesterol, VLDL-cholesterol and triglyceride levels in the serum of rats fed with a high fat diet or normal fat diet and that might be due to the effect of *G. sylvestre* in increasing neutral sterols and acid steroids excretion into feces. Luo et al. (2007) discovered the ability of *G. sylvestre* water extract to prevent the genetic obesity by improving the cholesterol metabolism and inhibiting polyphagia. Ishijima et al. (2008) 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*. The intra peritoneal fat and fat drop vacuoles on the epithelium of renal tubules were scattered by administration of the *G. sylvestre* extract (Mahajan et al., 2011). Lipid lowering activity of flavonoids and saponins extracted from *G. sylvestre* might be due to the inhibition of pancreatic lipase activity (Manish et al., 2011).

It was apparent from our results that plasma lipid peroxidation level decreased and erythrocyte superoxide dismutase level increased in rats treated with *G. sylvestre* leaves extract post STZ-diabetic induction compared to untreated diabetic rats.

Ohmori et al. (2005) discovered the antioxidant ability of *G. sylvestre*, when they studied the antioxidant activity of six teas against free radicals and LDL oxidation in 10 healthy volunteers ranging from 20 to 22 years of age. *In vitro* *G. sylvestre* alcoholic leaf extract showed antioxidant ability by inhibiting 1,1-diphenyl-2-picrylhydrazyl (DPPH) and scavenging superoxide and hydrogen peroxide (Rachh et al., 2009). Administration of *G. sylvestre* extract to diabetic rats increased superoxide dismutase activity and decreased lipid peroxide by either directly scavenging the reactive oxygen species, due to the presence of various antioxidant compounds, or by increasing the synthesis of antioxidant molecules (albumin and uric acid) (Vasi and Austin, 2009). Fazal et al. (2011) found that the maximum scavenging activity of ethanolic extract of *G. sylvestre in vitro* was 54.4% at concentration 250 µg/ml. Some poly herbal ayurvedic formulations like Hyponidd and Dihar containing *G. sylvestre* extract had shown antioxidant activity by increasing superoxide dismutase, glutathione and catalase levels in rats (Mahajan et al., 2011). Kang et al. (2012) reported that feeding *G. sylvestre* extract to diabetic rats decreased lipid peroxidation levels by 31.7% in serum, 9.9% in liver and 9.1% in kidney. It also decreased the activity of glutathione peroxidase in cytosolic liver and glutamate pyruvate transaminase in serum to normal levels.

5. Conclusion

It can be concluded that, *G. sylvestre* leaves extract exhibit antidiabetic activity, so it is recommended to use it as a complementary medicine in diabetes mellitus. Hence, clinical studies on *G. sylvestre* leaves extract as a complementary medicine are needed.

References

- Abdel-Moneim, A., Al-Zayat, E., Mahamoud, S., 2002. Effect of some antioxidants on streptozotocin diabetic rats. J. Egypt Ger. Soc. Zool. 38 (A), 213–245.
- Aebi, H., 1984. Catalase *in vitro*. Methods Enzymol. 105, 121–126.
- Ahmed, A.B.A., Rao, A.S., Rao, M.V., 2008. Role of *in vivo* and *in vitro* callus of *Gymnema sylvestre* (Retz) R. Br. in maintaining the normal levels of blood glucose and lipid profile in diabetic Wistar rats. Biomedicine 28 (2), 134–138.
- Ahmed, A.B.A., Rao, A.S., Rao, M.V., 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.
- Ahmed, F., Siddaraju, N.S., Urooj, A., 2011. *In vitro* hypoglycemic effects of *Gymnema Sylvestre*, *Tinospora cordifolia*, *Eugenia jambolana* and *Aegle marmelos*. J. Nat. Pharm. 2 (2), 52–55.
- Aralelimath, V.R., Bhise, S.B., 2012. Anti-diabetic effects of *Gymnema sylvestre* extract on streptozotocin induced diabetic rats and possible β -cell protective and regenerative evaluations. Dig. J. Nanomater. Biostruct. 7 (1), 135–142.
- Beutler, E., Duron, O., Kelly, B.M., 1963. Improved method for the determination of blood glutathione. J. Lab. Clin. Med. 61, 882–888.
- Bolkent, S., Yamardag, R., Tabakogluoguz, A., Ozsoy-sacaon, O., 2000. Effects of chord (*Beta vulgaris* L. Var. cicla) extract on pancreatic β -cells in streptozotocin-diabetic rats: a morphologic and biochemical study. J. Endocrinol. 73, 251–259.
- Burtis, C.A., Ashwood, E.R., 2006. Tietz Textbook of Clinical Chemistry and Molecular Diagnostics, fourth ed. W. B. Saunders Company, Philadelphia, pp. 444–451.
- Chattopadhyay, R.R., 1998. Possible mechanism of antihyperglycemic effect of *Gymnema sylvestre* leaf extract, part I. Gen. Pharmacol. 31 (3), 495–496.
- Daisy, P., Eliza, J., Farook, K.A.M., 2009. A novel dihydroxy gymnemic triacetate isolated from *Gymnema sylvestre* possessing normoglycemic and hypolipidemic activity on STZ-induced diabetic rats. J. Ethnopharmacol. 126, 339–344.
- Duncan, B.D., 1957. Multiple range tests for correlated and heteroscedastic means. Biometrics 13, 359–364.
- El-Seifi, S., Abdel-Moneim, A., Badir, N., 1993. The effect of *Ambrosia maritime* and *Cleome droserifolia* on serum insulin and glucose concentrations in diabetic rats. J. Egypt Ger. Soc. Zool. 12 (A), 305–328.
- Fazal, H., Ahmad, N., Khan, M.A., 2011. Physico-chemical, phytochemical evaluation and DPPH-scavenging antioxidant potential in medicinal plants used for herbal formulation in Pakistan. Pak. J. Bot. 43, 63–67.
- Fushiki, T., Kojima, A., Imoto, T., Inoue, K., Sugimoto, E., 1992. An extract of *Gymnema sylvestre* leaves and purified gymnemic acid inhibits glucose-stimulated gastric inhibitory peptide secretion in rats. J. Nutr. 122, 2367–2373.
- Gholap, S., Kar, A., 2005. Gymnemic acid from *Gymnema sylvestre* potentially regulates dexamethasone-induced hyperglycemia in mice. Pharm. Biol. 43 (2), 192–195.
- Glick, M.R., Ryder, K.W., Jackson, S.A., 1986. Graphical comparisons of interferences in clinical Chemistry instrumentation. Clin. Chem. 32, 470–474.
- Goycheva, P., Gadjeva, V., Popov, B., 2006. Oxidative stress and its complications in diabetes mellitus. Trakia J. Sci. 4 (1), 1–8.
- Gurav, S., Gulkari, V., Duragkar, N., Patil, A., 2007. Systemic review pharmacognosy, phytochemistry, pharmacology and clinical application of *Gymnema sylvestre* R. Br. Pharmacogenomics Rev. 1, 338–343.
- Hatch, F.T., Lees, R.S., 1968. Practical methods for plasma lipoprotein analysis. Adv. Lipid Res. 6, 1–68.
- Ishijima, S., Takashima, T., Ikemura, T., Izutani, Y., 2008. Gymnemic acid interacts with mammalian glycerol-3-phosphate dehydrogenase. Mol. Cell. Biochem. 310, 203–208.
- Jarald, E., Joshi, S.B., Jain, D.C., 2008. Diabetes and herbal medicines. Iranian J. Pharmacol. Ther. 7 (1), 97–106.
- Kang, M., Lee, M., Choi, M., Min, K.S., Shibamoto, T., 2012. Hypoglycemic activity of *Gymnema sylvestre* extracts on oxidative

- stress and antioxidant status diabetic rats. *J. Agric. Food Chem.* 60 (10), 2517–2524.
- Khan, V., Najmi, A.K., Akhtar, M., Aqil, M., Mujeeb, M., Pillai, K.K., 2012. A pharmacological appraisal of medicinal plants with antidiabetic potential. *J. Pharm. Bioall. Sci.* 4 (1), 27–42.
- Kirtikar, K.R., Basu, B.D., 1998. *Indian Medicinal Plants*, second ed. International Book Distributors, Dehradun, India, p. 1625.
- Liu, B., Asare-Anane, H., Al-Romaiyan, A., Huang, G., Amiel, S.A., Jones, P.M., Persaud, S.J., 2009. Characterisation of the insulinotropic activity of an aqueous extract of *Gymnema sylvestre* in mouse β -cells and human islets of Langerhans. *Cell. Physiol. Biochem.* 23, 125–132.
- Luo, H., Kashiwagi, A., Shibahara, T., Yamada, K., 2007. Decreased bodyweight without rebound and regulated lipoprotein metabolism by gymnemate in genetic multifactor syndrome animal. *Mol. Cell. Biochem.* 299, 93–98.
- Mahajan, D., Krishna, A.R., Gothandam, K.M., 2011. Ethnobotanical and pharmacological study of *Gymnema sylvestre*. *Pharmacologyonline* 3, 785–798.
- Mall, G.K., Mishra, P.K., Prakash, V., 2009. Antidiabetic and hypolipidemic activity of *Gymnema Sylvestre* in alloxan induced rats. *Global J. Biotechnol. Biochem.* 4 (1), 37–42.
- Manish, K., Aditi, K., Renu, A., Gajraj, S., Poonam, M., 2011. Anti-obesity property of hexane extract from the leaves of *Gymnema sylvestre* in high fed cafeteria diet induced obesity rats. *Int. Res. J. Pharm.* 2 (8), 112–116.
- Najafi, S., Deokule, S.S., 2011. Studies on *Gymnema sylvestre* – a medicinally important plant of the family Asclepiadaceae. *Trakia J. Sci.* 9 (2), 26–32.
- Nishikimi, M., Roa, N.A., Yogi, K., 1972. Measurement of superoxide dismutase. *Biochem. Biophys. Res. Commun.* 46, 449–454.
- Ohmori, R., Iwamoto, T., Tago, M., Takeo, T., Unno, T., Itakura, H., Kondo, K., 2005. Antioxidant activity of various teas against free radicals and LDL oxidation. *Lipids* 40 (8), 849–853.
- Paget, G.E., Barnes, J.M., 1964. Toxicity tests. In: Laurence, D.R., Bachrach, A.L. (Eds.), *Evaluation of drug activities: pharmacometrics*. Academic Press, London and New York, pp. 135–166, vol. 1.
- Patel, P., Harde, P., Pillai, J., Darji, N., Patel, B., 2012. Antidiabetic herbal drugs a review. *Pharmacophore* 3 (1), 18–29.
- Persaud, S.J., Al-Majed, H., Raman, A., Jones, P.M., 1999. *Gymnema sylvestre* stimulates insulin release *in vitro* by increased membrane permeability. *J. Endocrinol.* 163, 207–212.
- Rachh, P.R., Patel, S.R., Hirpara, H.V., Rupareliya, M.T., Rachh, M.R., Bhargava, A.S., Patel, N.M., Modi, D.C., 2009. *In vitro* evaluation of antioxidant activity of *Gymnema sylvestre* R. BR. Leaf extract. *Rom. J. Biol. Plant Biol* 54 (2), 141–148.
- Sahu, N., Mahato, S.B., Sarkar, S.K., Poddar, G., 1996. Triterpenoid saponins from *Gymnema sylvestre*. *Phytochemistry* 41, 1181–1185.
- Saneja, A., Sharma, C., Aneja, K.R., Pahwa, R., 2010. *Gymnema Sylvestre* (Gurmar): a Review. *Sch. Res. Libr.* 2 (1), 275–284.
- Satoh, K., 1978. Serum lipid peroxide in cerebrovascular disorders determined by a new colorimetric method. *Clin. Chim. Acta* 90 (1), 37–43.
- Shane-McWhorter, L., 2001. Biological complementary therapies: a focus on botanical products in diabetes. *Diabetes Spectr.* 14, 199–208.
- Shanmugasundaram, K.R., Panneerselvam, C., Samudram, P., Shanmugasundaram, E.R.B., 1983. Enzyme changes and glucose utilization in diabetic rabbits: the effect of *Gymnema sylvestre* R.Br. *J. Ethnopharmacol.* 7 (2), 205–234.
- Shanmugasundaram, E.R., Rajeswari, G., Baskaran, K., Rajesh, K.B.R., Radha, S.K., Kizar, A.B., 1990. Use of *Gymnema sylvestre* leaf extract in the control of blood glucose in insulin-dependent diabetes mellitus. *J. Ethnopharmacol.* 30 (3), 281–294.
- Shephard, M.D.S., Whiting, M.J., 1990. Falsely low estimation of triglycerides in lipemic plasma by the enzymatic triglyceride method with modified trinder's chromogen. *Clin. Chem.* 36 (2), 325–329.
- Shigematsu, N., Asano, R., Shimosaka, M., Okazaki, M., 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.
- Shimizu, K., Iino, A., Nakajima, J., Tanaka, K., Nakajyo, S., Urakawa, N., Atsuchi, M., Wada, T., Yamashita, C., 1997. Suppression of glucose absorption by some fractions extracted from *Gymnema sylvestre* leaves. *J. Vet. Med. Sci.* 59 (4), 245–251.
- Sinet, M., Muffat, J.M., Henzel, D., Renaut, G., Pocidal, J.J., 1984. Performance of hypothermic isolated rat heart at various levels of blood acid–base status. *J. Appl. Physiol.* 56 (6), 1526–1532.
- Spasov, A.A., Samokhina, M.P., Bulanov, A.E., 2008. Antidiabetic properties of *Gymnema sylvestre* (a review). *Pharm. chem. J.* 42 (11), 626–629.
- Sujin, R.M., Subin, R.M., Mahesh, R., Mary, R.V.J., 2008. Antidiabetic effect of *Gymnema sylvestre* (asclepiadaceae) powder in the stomach of rats. *Ethnobotanical leaflets* 12, 1158–1167.
- Trinder, P., 1969. Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. *Ann. Clin. Biochem.* 6, 24–27.
- Vasi, S., Austin, A., 2009. Effect of herbal hypoglycemic on oxidative stress and antioxidant status in diabetic rats. *The Open Diabet. J.* 2, 48–52.
- Venkatesham, A., Vasu, K., Rao, S., 2010. New molecular screening from a folk lore medicine *Gymnema*, using its active moieties for lead optimization. *Int. J. Pharm. Technol.* 2 (4), 574–583.
- Wang, L.F., Luo, H., Miyoshi, M., Imoto, T., Hiji, Y., Sasaki, T., 1998. Inhibitory effect of gymnemic acid on intestinal absorption of oleic acid in rats. *Can. J. Physiol. Pharmacol.* 76 (10–11), 1017–1023.
- Wieland, H., Seidel, D., 1983. Quantitative lipoprotein electrophoresis. In: Lewis, A., Boca Raton (Eds.), *Handbook of Electrophoresis*. CRC Press, pp. 83–102.
- Yoshioka, S., 1986. Inhibitory effects of gymnemic acid and an extract from the leaves of *Zizyphus jujuba* on glucose absorption in the rat small intestine. *J. Yonago Med. Assoc.* 37, 142–154.