



International Journal of Medical Research & Health Sciences

www.ijmrhs.com

Volume 4 Issue 3

Coden: IJMRHS

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ISSN: 2319-5886

Received: 4th May 2015Revised: 20th June 2015Accepted: 30th June 2015

Research article

ANTIDIABETIC AND HYPOLIPIDEMIC ACTIVITY OF GYMNEMA SYLVESTRE IN DEXAMETHASONE INDUCED INSULIN RESISTANCE IN ALBINO RATS

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ABSTRACT

Background: *Gymnema sylvestre* plant was widely used for medicinal purpose. The plant leaves were traditionally used to treat diabetes. **Aim:** To determine the antidiabetic and hypolipidemic activity of *Gymnema sylvestre* in dexamethasone induced insulin resistance in Albino rats. **Objectives:** The present study was undertaken to evaluate antidiabetic and hypolipidemic activity of *Gymnema sylvestre* leaf aqueous extract against dexamethasone induced insulin resistance in Albino rats. **Materials and Methods:** Animals were divided into five groups. Normal control and diabetic control group received gum acacia (2%) orally for 12days, and normal saline (i.p.), dexamethasone (8mg/kg/i.p.) from day 7- day12 respectively. Two test groups (*Gymnema sylvestre* leaf aqueous extract 2 and 4gm/kg/p.o./12days) and standard control received metformin (2gm/kg/p.o./12 days). The two test groups, standard control group received dexamethasone (8mg/kg/i.p) from day 7- day 12 respectively. The antidiabetic and hypolipidemic activity was estimated by measuring serum glucose, insulin, lipid levels and histopathological evaluation of liver tissue. Results were analyzed by using one way ANOVA followed by Scheffe's multiple comparison test. **Results:** Treatment with aqueous extract of *Gymnema sylvestre* (2 and 4gm/kg/p.o) significantly ($p < 0.01$) altered the elevated glucose, lipid, insulin levels and also improved the histopathology of liver in dexamethasone induced insulin resistance rats. **Conclusion:** Treatment with aqueous extract of *Gymnema sylvestre* improved the altered glucose, insulin and lipid profile in insulin resistance rats.

Keywords: *Gymnema sylvestre*, Glucocorticoids, Insulin Resistance, Diabetes, Dyslipidemia.

INTRODUCTION

Gymnema sylvestre belongs to family asclepidaceae. It is a native plant in south west of India, Australia and Africa. It is also known as Meshashringi in sanskrit, in Hindi: Gurmar, Kannada: Sannagerasehabmu and Telugu: Podapatri. From ancient times it is used to treat diabetes, hypercholesterolemia, asthma, eye complications and inflammation.^[1]

The major pathogenic factor in the development of type -2 diabetes is the cellular resistance of muscle, liver and or fat cells to the action of insulin.^[2]

Glucocorticoids were widely used for several clinical conditions. With prolonged exposure of glucocorticoids, insulin secretion will increase to compensate for the excess of glucose in blood due to development of insulin resistance in peripheral tissues.^[3] Insulin resistance is associated with development of various complications such as diabetes, cardiovascular events and hepatic steatosis etc.^[3-5]

Since ages *Gymnema sylvestre* plant preparation was used in different formulations for the treatment of

diabetes. Chewing the leaves of *Gymnema sylvestre* was reported to suppress the sweet taste.^[6] *Gymnema sylvestre* plant leaves improve the enzyme activity responsible for glucose uptake and utilization and also increases the permeability of cells to insulin.^[6] It was also used in the treatment of obesity and hypercholesterolemia.^[7]

Dexamethasone is a potent Glucocorticoid with minimal mineralocorticoid action. Several studies were conducted with the use of dexamethasone for induction of insulin resistance. It induces insulin resistance in rodents in a relatively short period of time.^[8, 9] Hence, dexamethasone was selected in present study for induction of insulin resistance. There is scarce data on insulin sensitizing action of *Gymnema sylvestre* plant leaves. Hence, the present study was undertaken to investigate protective role of aqueous extract of *Gymnema sylvestre* on serum glucose, insulin and lipid profile in dexamethasone induced insulin resistant rats.

MATERIALS AND METHODS

Study design: Experimental animal based study

Ethics approval: Institutional animal ethics committee permission was taken prior to study.

Raring of animals: The present study was conducted on male albino Wistar rats (weight 230-300gms). Rats were obtained from central animal house of the institution. Animals were maintained under standard conditions, as provided by Committee for the Purpose of Control and supervision on experimental animals (CPCSEA), at temperature (23±2)^o C, humidity 50±5%, 12:12hr light-dark cycles. Animals were maintained in polypropylene cages (UN Shah Manufacturers), rat pellets (Hindustan lever ltd, Mumbai) and water were given ad-libitum.

Drugs and Chemicals: Dexamethasone injection was obtained from Zydus pharmaceuticals, Mumbai. Metformin was obtained from USV Limited, Mumbai, India. Ketamine injection was obtained from Neom laboratories limited, Mumbai, India.

Reagents and kits: The biochemical parameters glucose, lipid profile were measured by using commercially available kits (Erba Mannheim, Transasia Biomedicals LTD.). Serum Insulin levels were estimated by using Ultra sensitive rat insulin ELISA kit from Gen X Bio Health Sciences private limited, New Delhi.

Collection of plant material: Leaves of *Gymnema sylvestre* were obtained from the local market in Vijayapura. The leaves were identified and authenticated by Dr. Sunil Kumar KN, Senior Research officer, Pharmacognosy, SDM Centre for research in Ayurveda & Allied sciences, Udupi.

Preparation of the aqueous extract of *Gymnema sylvestre*: Weighed accurately 450gms of the *Gymnema sylvestre* leaves and kept in a round bottom flask. 500ml of distilled water was added and allowed to stand for 24hours. The contents were filtered and the extract was concentrated by distillation. The solvent was removed by evaporation on a water bath. It was completely dried under vacuum. Around 120gms of dried extract was obtained.^[10]

Phytochemical analysis: *Gymnema sylvestre* aqueous extract was subjected to preliminary phytochemical analysis for detection of major chemical constituents such as alkaloids, phenols, resins, tannins, terpenoid and saponins etc.^[11-13]

Grouping:

Animals were divided into 5 groups (n=6). Study was conducted for a period of 12 days. Pilot study was conducted initially to determine the antidiabetic doses of *Gymnema sylvestre*.

Group I served as normal control

Group II diabetic control, received gum acacia (2% p.o.)

Group III received 2gm/kg/p.o., and group IV 4gm/kg/p.o. of *Gymnema sylvestre* aqueous extract for 12days respectively.

Group V rats were treated with a standard drug metformin (2gm/kg/p.o/12days).

All the groups except normal control group received dexamethasone (8mg/kg/i.p.) from day7- day 12.^{[14-}

^{15]} At the end of the study period, fasting blood was collected by retro-orbital sinus puncture for estimation of biochemical parameters. Later rats were anesthetized with intra peritoneal ketamine injection,^[16] sacrificed by cervical dislocation and liver tissues were collected for histopathological investigations.

Biochemical estimation: Blood samples were centrifuged at 2000RPM and serum was separated. Serum was used for following biochemical investigations.

Serum glucose levels were measured by glucose oxidase and peroxidase (GOD-POD) method,^[13, 17] triglycerides by Glycerol Phosphate Oxidase-peroxidase (GPO-POD),^[13, 17] Total Cholesterol

(CH), High density lipoproteins (HDL) and Low density lipoproteins (LDL) - Cholesterol by Oxidase-Peroxidase (CHOD-PAP) methods. [13, 17-20] Very low density lipoproteins (VLDL) cholesterol was estimated by using the formula, VLDL = triglycerides / 5. [17-20] All the biochemical investigations were done by using fully automated analyzer (ERBA-EM 200). [17-20]

Serum Insulin levels were estimated by Enzyme Linked Immunosorbant Assay (ELISA) method by using ELISA reader. [20]

Histopathology: A small portion of liver was fixed in formalin (10%) solution. Liver sections were made 5µm thick, and stained with hemotoxylin and eosin (H&E) stain. After staining, the sections were observed under microscope at 10X and photographs were taken.

Statistical analysis: The data was presented in Mean ± SEM. Results were analyzed by one-way ANOVA followed by Scheffe multiple comparison tests using SPSS software. Statistical significance was assumed if $p < 0.01$.

RESULTS

Phytochemical analysis: The preliminary phytochemical analysis of *Gymnema sylvestre* aqueous extract showed presence of alkaloids, phenols, saponins, tannins, and terpenoids.

Biochemical parameters: Treatment with dexamethasone (8mg/kg/i.p.) significantly increased the serum glucose, insulin, total cholesterol, TG's, LDL, VLDL and decreased HDL levels in comparison to control group. It indicates the dexamethasone induced diabetes, dyslipidemia due to insulin resistance. [Table 1]

Treatment of rats in group III, IV with aqueous extract of *Gymnema sylvestre* significantly reduced the elevated serum glucose, insulin, total cholesterol, TG's, LDL, VLDL and raised the HDL levels in comparison to dexamethasone (8mg/kg/i.p) treated group. [Table 1] There was no significant difference in serum glucose, insulin and lipid profile between Metformin treated rats and *Gymnema sylvestre* (2,4gm/kg/p.o.) aqueous extract treated rats. [Table 1]

Table: 1 showing antidiabetic and hypolipidemic activity of *Gymnema sylvestre* leaf aqueous extract

Groups	Glucose (mg/dl)	Insulin (ng/ml)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)	TGS (mg/dl)	CH (mg/dl)
Group-I	96.83±1.22	3.0±0.18	26.50±1.75	14.35±1.25	10.27±0.25	51.39±1.26	84.11±1.85
Group- II	272.25±1.82*	19.46±0.33*	6.77±0.30*	98.78±1.17*	32.67±0.36*	163.36±1.79*	196.71±1.61*
Group-III	146.20 ±3.16†	7.6 ±0.68†	20± 1.39†	48.4± 3.24†	16.18±0.77†	80.93± 3.85†	118.55 ±2.44†
Group-IV	143.82 ±2.41†	7.25± 0.53†	20.1± 1.30†	46.31± 2.14†	15.79±0.64†	79.0± 3.22†	119.63 ±2.76†
Group-V	137.54±2.23†	5.11± 0.31†	24.01± 1.63†	42.73±2.27†	14.55±0.50†	72.76±2.5†5	110.89±2.58†

Values are expressed in mean ± SEM, * $p < 0.01$ vs. normal control, † $p < 0.01$ vs. diabetic control

Histopathological observations:

The control group and metformin treated groups showed normal hepatocytes, hepatic parenchyma. All the sinusoids appear normal. Periportal (Zone 1), mid zone (Zone 2) and centrilobular area (Zone 3) appears normal. [Fig 1 and Fig 2 respectively]

The histopathological examination of dexamethasone 8mg/kg/i.p. treated group showed increase in the size of hepatocytes, cytoplasm is vesicular to clear. Fat deposition was observed in Zone 2, Zone 3. [Fig 3]

The liver sections of rats treated with aqueous extract of *Gymnema sylvestre* (2gm/kg/p.o) showed normal architecture of liver, dilation and congested vein. Fat deposition was observed only in zone 3. [Fig 4]

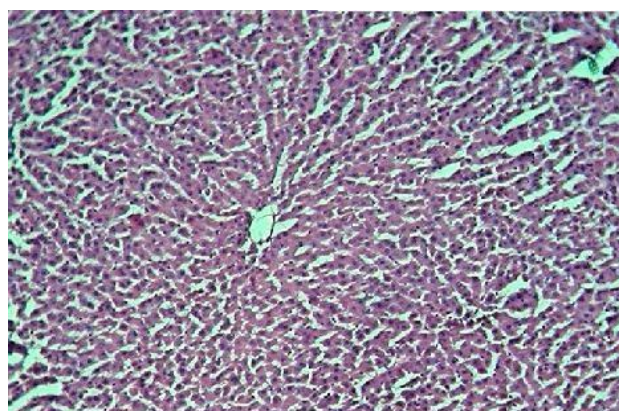


Fig 1: Gum acacia treated rat's shows normal hepatic architecture [H&E 10X]

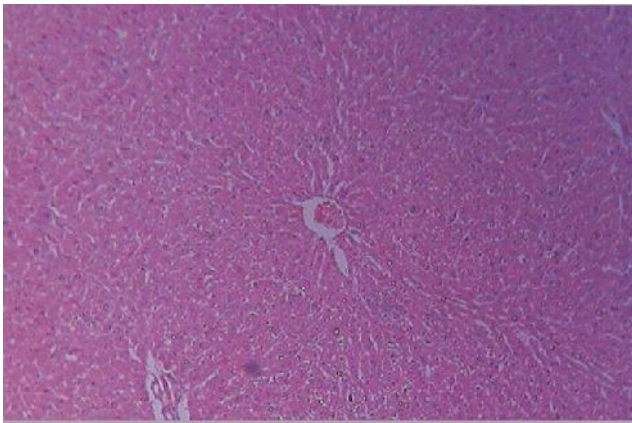


Fig 2: Treatment with Metformin (2gm/kg/p.o.) shows normal hepatic architecture[H& E10X]

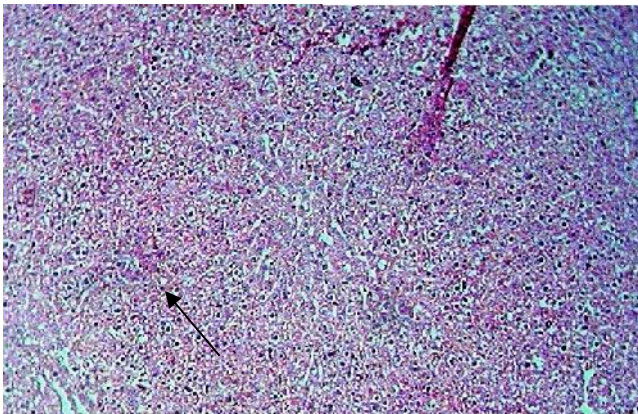


Fig 3: Dexamethasone (8mg/kg/i.p.), shows fat deposition in liver [H& E 10x]



Fig 4: Treatment with *Gymnema sylvest্রে* (2gm/kg/p.o.) shows partial improvement. [H& E 10X]

High doses of *Gymnema sylvest্রে* (4gm/kg/p.o.) completely restored the pathological changes induced by dexamethasone. Rat livers showed normal hepatocytes, sinusoids. There were no apparent signs of fat deposition, liver appear normal. [Fig 5]

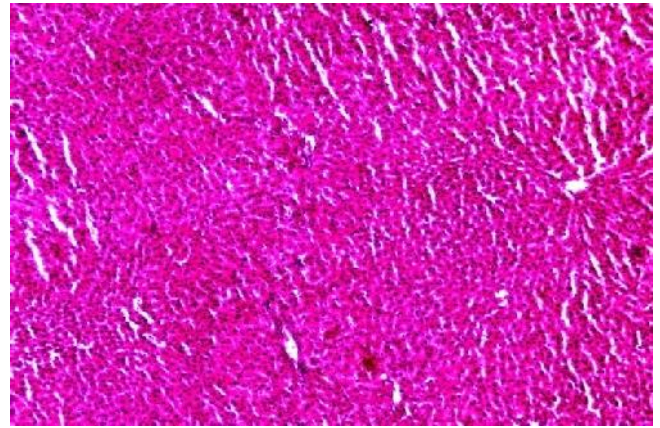


Fig 5: Treatment with *Gymnema sylvest্রে* (4gm/kg/p.o.) shows complete improvement. [H& E 10x]

DISCUSSION

Several theories were postulated for the development of adult onset of diabetes, one of the possible mechanisms is the Insulin resistance. Dexamethasone induces insulin resistance which leads to the development of hyperinsulinemia, hyperglycemia, dyslipidemia and hepatic steatosis. Insulin resistance develops before the clinical appearance of complications. In several clinical conditions, higher doses of dexamethasone is used. Prophylactic treatment of insulin resistance can prevent the development of secondary complications.^[21]

In current study, dexamethasone administration resulted in insulin resistance and leading to diabetes, dyslipidemia and hepatic steatosis. Treatment with aqueous extract of *Gymnema sylvest্রে* (2,4gm/kg/p.o.) significantly reduced the elevated serum glucose, insulin, lipid levels and also improved the liver pathology changes induced by dexamethasone.

Glucocorticoids (GCs) in general increases blood glucose levels by various mechanisms; increased hepatic glucose production (gluconeogenesis), decreased peripheral glucose uptake into muscle and adipose tissue, breakdown of muscle and fat to provide additional substrates for glucose production.^[22] Prolonged GC exposure is associated with development of severe insulin resistance and metabolic dysfunction; however the precise molecular mechanism is not clearly defined.^[22] The present study also confirms same findings, as dexamethasone significantly increased the serum glucose, insulin levels in insulin resistant animals.

According to review of literature *Gymnema sylvestre* plant leaves were helpful in treatment of diabetes. Several studies have been conducted to identify the possible mechanisms of *Gymnema sylvestre* in improvement of hyperglycemia. It was reported that *Gymnema sylvestre* induces hypoglycemia.^[1] Leaves of *Gymnema sylvestre* improve the glucose utilization by increasing the activity of enzymes responsible for glucose utilization by insulin dependent pathways. It increases phosphorylase activity, decrease in gluconeogenic enzymes and sorbitol dehydrogenase enzyme.^[1, 23] Our study supports the earlier findings that the aqueous extract of *Gymnema sylvestre* leaves significantly decreased elevated serum glucose levels. It is believed that one of saponin form of *Gymnema sylvestre* plant leaves, Gymnemic acid was responsible for development of hypoglycemia. Gymnemic acid molecules by filling the receptors on absorptive layers of intestine decreases absorption of glucose, resulting in low blood sugar levels.^[24] In the present study aqueous extract of *Gymnema sylvestre* (2 and 4gm/kg/p.o.) significantly decreased the elevated insulin levels. Dexamethasone administration led to increase in insulin level in comparison to control group due to insulin resistance. The possible mechanism responsible for improvement in insulin resistance by *Gymnema sylvestre* may be due to improvement in the permeability of cells to the insulin action.^[23] This might result in significant decrease in the circulating insulin levels. Further studies are required to know the exact mechanism by which it improves insulin resistance. In the current study, treatment with aqueous extract of *Gymnema sylvestre* significantly improved the altered lipid profile. As per review of literature, glucocorticoids administration leads to lipid disturbances. It elevates triglycerides, total cholesterol and LDL cholesterol levels, and reduces HDL levels which may be secondary cause of dyslipidemia. The Mechanisms responsible for glucocorticoid induced dyslipidemia could be impaired catabolism of LDL, increase in the activity of lipoprotein lipase and subsequent increase in LDL and VLDL levels due to increased plasma insulin.^[25] *Gymnema sylvestre* plant leaves were helpful in treatment of obesity and hypercholesterolemia. Studies were conducted for the possible role of *Gymnema sylvestre* plant leaves in dyslipidemia. In

the present study, it significantly decreased circulating cholesterol, triglycerides, LDL and VLDL levels. It promotes the fecal excretion of cholesterol and cholic acid derived bile acids. It also decreases serum triglycerides and cholesterol and improves hypertriglyceridemia and hypercholesterolemia. These actions might be due to phytochemical constituent saponins.^[26] Isolation of specific phytochemical constituent, was not possible hence, the whole leaf aqueous extract were used in present study. One of the possible mechanism for improvement in serum cholesterol by *Gymnema sylvestre* may be due to increase in fecal fermentation leading to production of organic acids, propionic acids, acetic acid.^[26] Positive correlation was found between propionic acid and serum cholesterol. Injection of propionic acid decreased blood cholesterol in rats.^[27]

Gymnema sylvestre (2,4gm/kg/p.o.) significantly decreased elevated CH, TG's, LDL, VLDL and increased HDL level. It decreases the activity of hormone sensitive lipase (converted neutral fats into free fatty acids) and decrease of cholesterologenesis and fatty acid synthesis.^[27] In the present study *Gymnema sylvestre* increased the HDL level, which is correlating with other studies.^[6] These increased HDL levels will be helpful in preventing various cardiovascular complications due to dyslipidemia. Presence of sitosterol in leaf of aqueous extract might also be responsible for lipid lowering action. It decreases the absorption of lipids.^[28]

Glucocorticoids can contribute to fatty liver production, through a combination of increased fatty acid synthesis and decreased fatty acid oxidation in liver.^[29] Raised free fatty acid levels have been associated with the development of hypertension, skeletal muscle insulin resistance, and fatty liver, this last being considered as the hepatic consequence of the metabolic syndrome due to specific hepatic insulin resistance.^[30] Present study supports the earlier finding that, the dexamethasone (8mg/kg/i.p) administration resulted in development of hepatic steatosis due to insulin resistance. Fat deposition was observed in zone 2, zone 3. [Fig 3] Treatment with aqueous extract of *Gymnema sylvestre* in high doses (4gm/kg/p.o.) significantly improved pathological changes in liver (Fig 5). This might be due to the decrease in circulating fatty acid levels, leading to

decrease in the fat deposition in liver induced by dexamethasone.

CONCLUSION

Dexamethasone is used in high doses in several clinical conditions. This might lead to development of Insulin resistance leading to various complications. Pretreatment with *Gymnema sylvestre* significantly prevented the development of insulin resistance and associated complications diabetes, dyslipidemia and hepatic steatosis.

Identification of cellular mechanism of insulin resistance and, Isolation of specific constituent responsible for hypoglycemic effect of extract will be more helpful, which remains the limitations of present study.

Acknowledgement: We are sincerely thankful to the Dr. Sucheta shetty and members of Central research laboratory, NITTE University, Mangalore for their great support in estimation of insulin levels by ELISA method.

Conflict of Interest: Nil

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