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Original Research Article

Effect of Postprandial Level of Plasma Glucose Level in Streptozotocin Induced Type 2 Diabetes Albino Rat Treated with Gymnema Sylvestre

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Conflict of interest: Nil

Abstract:

Background: Diabetes mellitus (DM) is a chronic metabolic disorder marked by persistent hyperglycemia due to impaired insulin production, insulin resistance, or both. With the global prevalence of DM expected to rise by 200 million cases by 2040, it presents a critical public health challenge. Metformin, while widely used for type 2 DM management, has side effects that spur interest in alternative treatments like herbal medicine. Gymnema sylvestre (G. sylvestre), a traditional Indian medicinal plant, is recognized for its anti-diabetic properties.

Aim: This study aimed to evaluate the anti-diabetic efficacy of G. sylvestre in a streptozotocin-induced type 2 diabetes rat model and compare its effects to metformin, a conventional treatment for type 2 diabetes.

Methodology: Wistar albino rats were divided into six groups, with type 2 diabetes induced by a high-fat diet and streptozotocin administration. The experimental groups were treated with varying dosages (200 and 400 mg/kg) of G. sylvestre extract and metformin. Blood glucose levels were measured using the oral glucose tolerance test (OGTT) on 0, 14, 19 and 42 days respectively. For this purpose, overnight fasted rats were fed 1.5 g /kg b. w of glucose through oral gavage and its levels were measured at intervals over a span of 2 hours as 0, 60 and 120 mins by glucose analyzer. On 42nd day, overnight fasted rats were sacrificed and blood was collected for the determination of serum insulin, liver enzymes like aspartate transaminase, alanine transaminase, alkaline phosphatase, and bilirubin

Results: Treatment with G. sylvestre extract resulted in a significant reduction in blood glucose levels and improved insulin levels compared to the untreated diabetic group. Higher doses of G. sylvestre showed more pronounced effects. Additionally, liver enzyme profiles indicated improved liver function in the G. sylvestre-treated groups.

Conclusion: G. sylvestre demonstrates significant anti-diabetic potential, suggesting it may serve as an effective alternative or complementary therapy for type 2 diabetes management. Its use could reduce dependence on conventional glucose-lowering medications like metformin, offering a natural and potentially safer treatment option.

Keywords: Diabetes mellitus, Gymnema sylvestre, Metformin, Anti-diabetic, Streptozotocin, Oral glucose tolerance test, Insulin, Herbal medicine, Type 2 diabetes, and Liver function.

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Introduction

Chronic metabolic disease, known as diabetes mellitus (DM), is characterized by persistent hyperglycemia. Since there will likely be an additional 200 million cases of DM by 2040, it is becoming clear that this is a problem for global public health. It could be brought on by decreased insulin production, insulin resistance, or both. Diabetes mellitus is a metabolic illness in which a person's blood sugar levels are elevated due to either insufficient insulin production by the body or an improper insulin response by body cells. For more than 40 years, type 2 diabetes mellitus patients have used metformin as an efficient glucose-lowering medication. Basal and post-prandial hyperglycemia are decreased.

Complications such lactic acidosis, gastrointestinal issues, and vitamin B12 deficiency are caused by it. Due to the negative effects of oral hypoglycemic medications, interest in herbal therapies is currently expanding.

A member of the Asclepiadaceae family, Gymnema sylvestre (G. sylvestre) is an Indian medicinal plant also known as Sirukurunjan in Tamil. Popular anti-diabetic herb G. sylvestre has been utilized for millennia in India as a sugar killer. The most abundant sources of chemical components with therapeutic potential are found in this herb [1]. A long-used remedy for diabetes is a plant called Gymnema sylvestre that is indigenous to the tropical woods of India. Several years ago, Gymnema sylvestre—dubbed a "sugar blocker"—arrived on the American market. 22 patients with type 2 diabetes who were on oral hypoglycemic medications were also given 400 mg of Gymnema sylvestre extract daily. Better blood sugar management was seen in all patients [2-4].

Material and Methods:

Adult Wister albino rats, weighing about 140 to 160 g, have been used for this study. The animals were kept in a polypropylene cage for 12 hours in light and dark cycles at a temperature of 22–25° C and a relative humidity of 50–60%. The animals were fed a regular rat pellet diet and were allowed to drink water ad libitum. The current work was accepted by the animal ethical committee at the Sathyabama Institute of Science and Technology (No. SU/CLATR/IAEC/XI/100/2018) and follows CPCSEA guidelines. The quarantine measures and animal maintenance were carried out according to the recommendation of care and use of experimental animals of the Canadian Council Guide (1993) and the CPCSEA (India) Guidelines for Laboratory Animal Facilities (2003).

Animal Grouping

The acquired species were divided randomly as follows:

Group 1 is for control animals fed with normal rat pellets. Group 2 animals were nourished with a high-fat diet (0.3% yeast powder, 1% d1-methionine, 0.1% sodium chloride, 31% cholesterol, and 67.5% lard oil) for 42 days. Group 3 animals were nourished with a high-fat diet for 2weeks, followed by 40 mg/kg body weight of streptozotocin is administered intraperitoneally (i. p) for five succeeding days to create a type 2 diabetic model. The ethanolic extract of Gymnema sylvestre at a low (200mg/kg) and high (400mg/kg) dosages of body weight for 3 weeks was given to the type 2 diabetic induced animals in groups 4 and 5 respectively, using 18 gauge gavage needles, which is about 2-3 inches in length. Group 6 is positive control animals, were 40 mg/kg body weight of streptozotocin is induced to produce type 2 diabetes and treated with 25 mg/kg b. w. of metformin for 22 days.

Preparation of Drugs and Chemicals

Preparation of Streptozotocin: Streptozotocin solution is freshly prepared by dissolving it in citrate buffered saline (pH 4.5) and is administered into the peritoneal cavity at a low multiple dose of 40 mg/kg body weight for 5 consecutive days with a high-fat diet [5].

Phytochemical Evaluation of Gymnema Sylvestre

Plant materials: The plant material of Gymnema sylvestre was collected and acquired from Chidambaram, Cuddalore district, Tamil Nadu. The plant was properly acknowledged and confirmed with the authentication number as PARC/2018/3985. Around 1.5 kg of the gymnema sylvestre leaves were dried out under shadow and maintained in an arid and cool place. Later, they are sieved through meshes for further studies.

Extraction with ethanol

The coarse leaf powder was packed with 80% ethanol in a soxhlet thimble until the material was totally mashed. Finally, a dark green amorphous powder is obtained after the evaporation of the solvent. Whatmann (No.1) filter paper was used to filter the solvent and dried under reduced pressure at 90 °C by a rotary evaporator to remove sediments and traces of water in the filtrate. Finally, a dark green, thick paste was acquired and stored for further investigation at -20 °C in a freezer. This extraction procedure was carried out according to Farzana et al., 2010 [6].

Biochemical Analysis

OGTT: Before the day of oral glucose tolerance test, the animals was left for overnight fasting. Earlier to the administration of glucose, a baseline blood glucose concentration was taken. All animals were fed with 1.5 g /kg b. w of glucose through oral gavage and its levels were measured at intervals over a span of 2 hours as 0, 60 and 120 mins by glucose analyzer with glucose strip for each mouse in the same order as they were given. From the collected blood samples, the first drop of the blood was discarded and subsequent blood drop was placed on a new test strip and the measurements were recorded.

Insulin Level Analysis

The analytical method was performed by a manual for electro-chemiluminescence immunoassay (ECLIA) (cobas e411 immunoassay analyzer, Roche Diagnostics GmbH, Mannheim, Germany). For separation of serum, the collected blood samples were allowed for 20 minutes to coagulate.

Then, the sample is centrifuged for 15 minutes at 2500 rpm and carefully separated into standard sampling tubes and measured. Measuring insulin in a complex medium without any extraction step is the advantage of this quantification method. The analyzer should be calibrated, controlled, and validated in advance. Automatically, analyzer calculates the concentration of insulin in μU/ml for each sample.

Liver Profile

Assay of Aspartate Transaminase is measure by applying IFCC/KINITIC technique

Principle: The reaction of L-aspartate and alpha-ketoglutarate to L-glutamate and oxaloacetate is catalysed by reversible transaminase. At the same time the malate dehydrogenase (MDH), translate oxaloacetate is reduced to malate with oxidation to NAD and the rate of change in absorbance at 340 nm is directly comparative to AST activity. The enzyme reaction was expressed by IU/L

Assay of Alanine Transaminase (ALT) **was** analyzed by glutamate pyruvate transaminase, IFCC/KINITIC method.

Principle: SGPT catalyse the chemical action of α ketoglutarate and L- alanine to pyruvate and L-glutamate. Thereafter, lactate dehydrogenase converts pyruvate to lactate and NADH to NAD by oxidation. Reduction in oxidation rate is proportionate to SGPT activity at 340 nm. The enzyme action was symbolized as IU/L

Analysis of Alkaline Phosphatase (ALP) was analyzed by the IFCC/KINITIC procedure

Principle: p-nitro phenyl phosphate (p-NPP) into p-nitrophenol and phosphate was catalysed by alkaline Phosphatase. In alkaline medium p-nitro phenol is yellow compound which absorbs light at 405 nm. The elevation in the rate of absorbance occur at 405 nm is relative to action of alkaline phosphatase in specimen. The enzymatic action was denoted as IU/L

Principle: Diazotized sulfanilic acid is formed when sodium nitrite reacts with sulfanilic acid. Azobilirubin is produced by the combination of bilirubin together with diazo and dimethyl sulfoxide either directly or indirectly. The colour concentration formed corresponds to the concentration of total bilirubin in the test sample. The enzyme activity was expressed as mmol/L

Result:

4. **OGTT**

The oral glucose tolerance test is a routine screening method for the confirmation of type 2 diabetes. All the animal groups were allowed fasting overnight, and the blood samples were taken at three different times: 0 min, 60 min, and 120 min. subsequent ingestion of the glucose water. The plasma glucose levels were analyzed on different days of the experiments, such as the 14th, 19th, and 42nd days. The plasma levels of OGTT are shown in Tables 1–3 and Graphs 1–3. All the groups are shown in the same way.

On the 14th day of the experiment (prior to diabetes induction): The animals in all groups, viz. groups 2, 3, 4, 5, and 6, fed with high-fat diet, expressed high blood glucose levels ($p<0.05$) at 0 min. After 60 min. (one hour), glucose levels were intensively increased in these experimental animals, and they did not produce any statistical significance with control animals (group 1). However, there was a significant gradual decrease in blood glucose levels, bringing them back to below 200 mg/dl after 120 min (two hours). For statistical significance, experimental groups were interrelated with control animals.

On the 19th day of the experiment (following diabetes induction): On the 19th day, the experimental groups, like/viz., group 3, group 4, group5 and group 6, were At 0 min after administration of streptozotocin, we found a markedly increased level of blood glucose. Nevertheless, the high-fat diet groups without streptozotocin administration (group 2) also exhibited high glucose levels at 0 min with more significance compared to the control group. Similarly, 60 min (one hour) later, the glucose level shot up in all groups. The experimental animals in groups 2, 3, 4, 5, and 6, expressed increased glucose levels while compare to the control group (group 1). After 120 min. (two hours), the control animal alone was brought back to the normal glucose level. The other groups failed to decrease their glucose levels and remained elevated when compared to the control group (group 1).

42nd day of the experiment (Termination of the experiment): On the last day of the experimental period, gymnema extract and metformin were given to the treatment groups, viz., groups 4, 5, and 6, an hour before OGTT. The control animals showed gradual increase of plasma glucose level that reached peak at 60 min. (after one hour) and finally it reversed to normal after 120 min (two hour) as appeared in 0 min. But the high fat diet animals (group2) and streptozotocin induced diabetic animals (group 3) showed elevated glucose level at all three different time point that is 0 min, 60 min and 120 min. and expressed highly statistical significance when comparing with control group (group1).

These groups (group 2 and group 3) seem to have persistence of high glucose level even after 120 min. Animals treated with lower and higher dosage of Gymnema treated (group 4 and group 5) and

metformin treated (group 4) specified indicated progressive increasing of glucose level, which reached high at 60 min. (after one hour) and reversed back ($p<0.001$) towards normalcy after 120 min (two hours).Interestingly the higher dosage of Gymnema treated animals (group 5) showed regulated their high glucose level more significantly ($P<0.001$) even after 60 min (one hour) than the lower dose of Gymnema (group 4) and metformin treated (group 4) ($P<0.01$) animals.

Although all the treatment groups' viz., group 4, 5 and 6 were showing maintenance of plasma glucose level, the high dose of gymnema (group 5) was effectively maintaining the glucose level at all three different point of time i. e, at 0, 60 and 120 min respectively.

Table 1: Oral Glucose Tolerance Test (OGTT) in mg/dl 14th Day (Pre-Diabetic)

	0	60	120
Group 1	114.25 ± 3.065	189.25± 10.641	115.25± 4.956
Group 2	151.25 ± 8.260$^{@**}$	224.25± 12.835	180.75± 9.844$^{@***}$
Group 3	148.00 ± 4.813$^{@*}$	218.25± 17.974	177.00± 6.245$^{@***}$
Group 4	146.75 ± 6.498$^{@*}$	214.50± 16.815	166.50± 6.994$^{@***}$
Group 5	148.25 ± 7.836$^{@*}$	228.75± 19.189	171.50± 8.451$^{@***}$
Group 6	153.25 ± 6.102$^{@**}$	226.25± 12.809	179.25± 6.981$^{@***}$

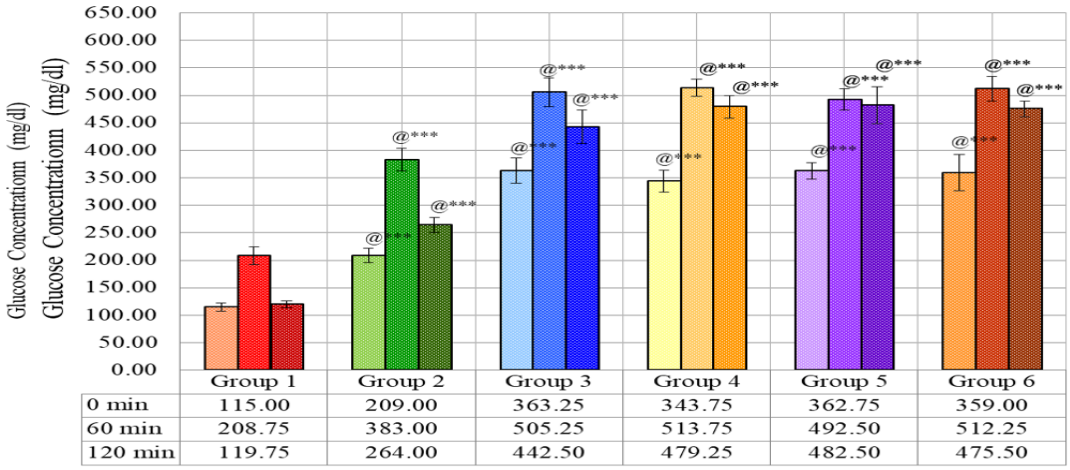
Table 2: 19thDay (Diabetic)

	0	60	120
Group 1	115.00 ± 7.360	208.75 ± 16.132	119.75 ± 6.524
Group 2	209.00 ± 13.172$^{@***}$	383.00 ±20.547$^{@***}$	264.00 ± 13.832$^{@***}$
Group 3	363.25 ± 22.816$^{@***}$	505.25 ± 26.030$^{@***}$	442.50 ± 30.653$^{@***}$
Group 4	343.75 ± 19.512$^{@***}$	513.75 ± 15.697$^{@***}$	479.25 ± 20.742$^{@***}$
Group 5	362.75 ± 14.969$^{@***}$	492.50 ± 18.875$^{@***}$	482.50 ± 33.696$^{@***}$
Group 6	359.00 ± 32.972$^{@***}$	512.25 ± 22.119$^{@***}$	475.50 ± 14.385$^{@***}$

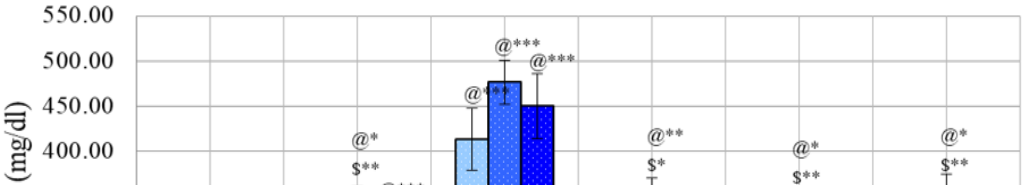
Table 3: 42nd Day (Post-Diabetic Treatment)

	0	60	120
Group 1	112.25 ± 7.642	218.00 ± 8.436	117.50 ± 2.398
Group 2	201.25 ± 15.596$^{@*}$	335.50 ± 24.982$^{@*\$**}$	312.00 ± 29.969$^{@***}$
Group 3	413.25 ± 34.091$^{@***}$	476.50 ± 24.473$^{@***}$	450.00 ± 35.590$^{@***}$
Group 4	164.50 ± 6.602$^{\$***}$	352.00 ± 18.457$^{@**\$*}$	180.75 ± 10.980$^{\$***}$
Group 5	166.00 ± 6.285$^{\$***}$	335.00 ± 23.274$^{@*\$**}$	155.00 ± 6.455$^{\$***}$
Group 6	178.25 ± 18.870$^{\$***}$	340.00 ± 34.701$^{@*\$**}$	176.75 ± 8.499$^{\$***}$

Table 6-8 Shows level of Oral Glucose Tolerance Test (OGTT) of all the animals groups at0min, 60 min, 120 min. during 14thday, 19th day and 42nd days of the experimental periods.

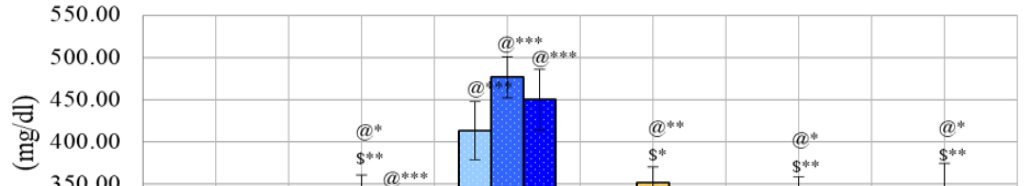


Graph 1: 14th Day Oral Glucose Tolerance Test



	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
0 min	112.25	201.25	413.25	164.50	166.00	178.25
60 min	218.00	335.50	476.50	352.00	335.00	340.00
120 min	117.50	312.00	450.00	180.75	155.00	176.75

Graph 2: 19th Day Oral Glucose Tolerance Test



	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
0 min	112.25	201.25	413.25	164.50	166.00	178.25
60 min	218.00	335.50	476.50	352.00	335.00	340.00
120 min	117.50	312.00	450.00	180.75	155.00	176.75

Graph 3: 42nd Day Oral Glucose Tolerance Test

Serum insulin: The serum insulin level was analyzed on different days of the experiments, like the 0th, 14th, 19th, and 42nd days.

The insulin level was evidently increased in all groups, viz., groups 2, 3, 4, 5, and 6 on the 14th day, followed by the high-fat diet. On the 19th day of the experiment, animals induced diabetes with streptozotocin in groups 3, 4, 5, and 6 exhibited raised insulin levels.

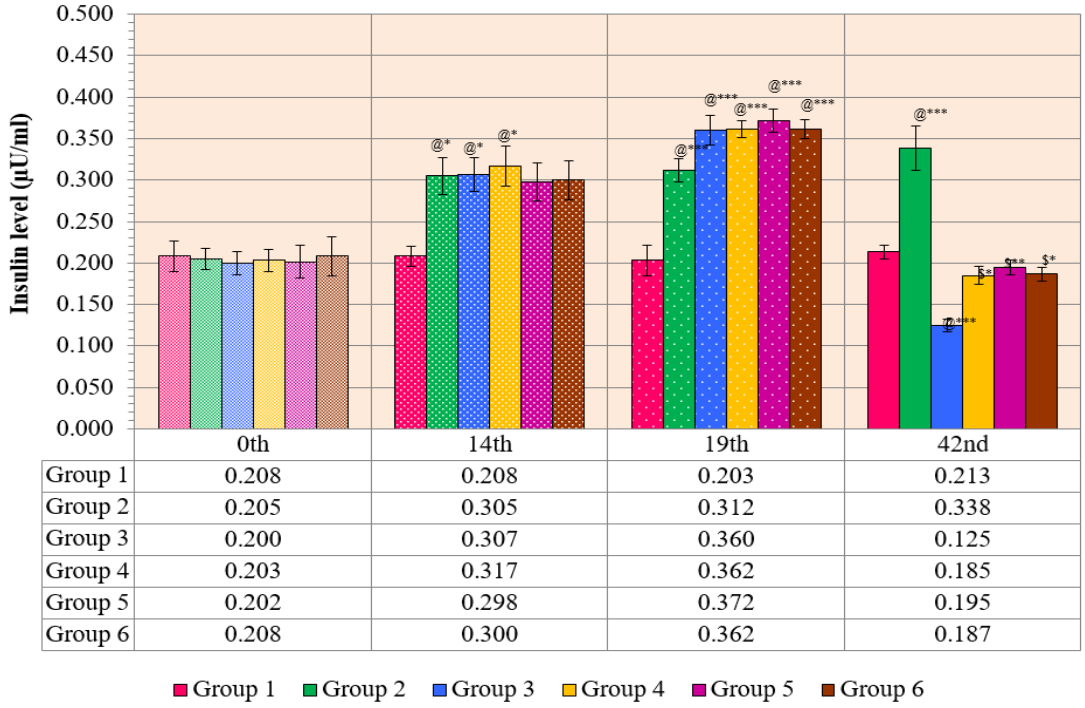
Similarly, the animals in Group 2 on a high-fat diet had increased insulin secretion in their serum. On both the 14th and 19th days of sample analysis, all experimental groups were expressing a considerable increase in ($p<0.001$) insulin levels compared to group 1. At the termination of the experimentation (42nd day), the high-fat diet animals (group 2) exhibit a persistent elevated level of insulin ($p<0.001$).

Similarly, streptozotocin-induced diabetes (group 3) was found to have a lower (p 0.05) insulin level. These results (group2 and group3) were related to the control animals (group1). The treatment of Gymnema sylvestre with lower (group 4) and higher dosage (group 5), and the treatment of metformin (group 6) animals was shown to increase the level of insulin secretion when compared with streptozotocin-induced diabetic

animals (group 3). Interestingly, the higher dosage of Gymnema treated animals (group 5) exhibited a highly significant increase (P<0.01) in insulin levels than the lower dose of Gymnema (group 4) and metformin treated animals (P<0.05).

Table 4: Shows serum insulin level of various groups in 0th, 14th, 19thand 42nd days of the experiment,

Groups	0th day	14th day	19th day	42nd day
Group 1	0.208 ± 0.019	0.208 ± 0.012	0.203 ± 0.019	0.213 ± 0.008
Group 2	0.205 ± 0.013	0.305 ± 0.022@*	0.312 ± 0.014@***	0.338 ± 0.027@***
Group 3	0.200 ± 0.014	0.307 ± 0.021@*	0.360 ± 0.018@***	0.125 ± 0.008@***
Group 4	0.203 ± 0.014	0.317 ± 0.024@*	0.362 ± 0.010@***	0.185 ± 0.011$*
Group 5	0.202 ± 0.019	0.298 ± 0.023	0.372 ± 0.014@***	0.195 ± 0.009$**
Group 6	0.208 ± 0.024	0.300 ± 0.024	0.362 ± 0.012@***	0.187 ± 0.008$*



Graph 4: representing the serum insulin level

Liver profile test: In a liver profile test, the streptozotocin induced diabetic animals (group 3) elevated the levels of liver enzymes, as well as bilirubin than control. However, the same diabetic induced animals showed significant changes in the level of ALP (p<0.05) than the other groups. Treatment with lower and higher dosages of Gymnema sylvestre (group 4 and 5) and metformin (group 6) showed decreased levels of AST, ALT, ALP and bilirubin when comparable to diabetic induced animals (group 3). However they are not statically significance. Nevertheless, the high dose of gymnema (group 5) exhibits notable reduction in AST, ALT, and ALP levels than the lower dosage (group 4) and metformin (standard diabetic drug) administration (group 6). The level of bilirubin was increased in higher dosage of gymnema (group5) than the lower dose. However it did not show any statistical significance. Though there was moderate increase in all the enzyme levels in high fat diet animals (group 2), they was no considerable changes on the status of Liver profile test when compared with control animals (group 1).

Data on the status of Liver profile test, are presented in Table 5.

Table 5: Liver Profile Test

	AST (IU/L)	ALT(IU/L)	ALP (IU/L)	Bilirubin (mg/dl)
Group 1	127.42 ± 6.79	454.35 ± 37.82	72.65 ± 3.43	0.387 ± 0.053
Group 2	138.62 ± 5.04	474.53 ± 31.16	85.93 ± 7.64	0.405 ± 0.066
Group 3	161.02 ± 16.38	700.80 ± 65.40	96.02 ± 9.52@*	0.497 ± 0.062
Group 4	144.30 ± 14.30	602.92 ± 72.98	87.47 ± 10.33	0.390 ± 0.042
Group 5	131.47 ± 7.73	587.55 ± 38.41	72.48 ± 2.33	0.412 ± 0.063
Group 6	136.75 ± 14.14	600.83 ± 54.63	78.33 ± 3.71	0.432 ± 0.051

Discussion

Diabetes mellitus is a composite metabolic chaos that brings about multiple problems. It demands proper management of glycemic control and multifactorial risk-reduction strategies [7]. Uncontrolled or poorly managed diabetes would worsen its associated complications and could lead to the risk of long term complications, resulting in frequent hospitalization and premature death. Though there are various therapeutic approaches currently being used for the intervention of this metabolic disorder, herbal medicine has received significant attention because of its lack of or reduced side effects. In fact, the herbal remedy is a fair approach to the discovery of a new drug to improve diabetes outcomes. In the present study, the medicinal herb Gymnema sylvestre was used as a therapeutic drug against diabetic conditions.

Oral Glucose Tolerance Test

This analysis is a standard diagnostic method which gives information about insulin resistance [8]. Thus, the type of diabetes could be categorized based on the OGTT results. In addition, it represents a more physiological pattern of alteration in insulin, glucose, and incretin hormones, which collectively give the estimation of B-cell function [9-10].

The OGTT value in control animals displayed a gradual increase of OGTT glucose that reached its peak at 60 min. (after one hour) and returned to normal after 120 min. (two hours), as appeared at 0 min., indicating effective maintenance of their glucose level at all three different points in time (i. e. at 0, 60, and 120 min.) and over three consecutive days (14th, 19th, and 42nd day).

On the 14th day of the experiment (before induction), the animals in all groups that were fed a high fat diet expressed marginally increased levels of OGTT glucose at the initial (0 min) and intensively increased after 60 min (one hour), then steadily dwindled, bringing them back to below 200 mg/dl after 120 min (two hours). On the 19th day of the experiment (after induction of diabetes), the diabetic induction animals (Groups 3, 4, 5, and 6) were found to have a markedly elevated level of OGTT glucose at 0 min after the administration of streptozotocin. Similarly, 60 min (one hour) later, the OGTT glucose level shot up in all groups and kept on increasing till 120 min (two hours).

On the 42nd day of the experiment (the terminal period of the experiment), the high fat diet animals (Group 2) and untreated diabetic animals (Group 3) showed persistence of elevated glucose levels at all three different time points, i. e., 0 min, 60 min, and 120 min. The OGTT value demonstrates an uncontrolled blood glucose level and impaired glucose tolerance [11]. These results are further supported by the other investigation [12-13]. The impaired glucose tolerance could be due to dysfunction of B-cell secretion as a result of a loss of B-cell mass [14]. The aforementioned statements can be linked to other findings of the current study, such as histological, histomorphometrical, and immunohistocheimstrical analysis of pancreatic islets.

The lower (200 mg) and higher (400 mg) dosages of Gymnema treated (Group 4 and Group 5) metformin treated (Group 4) animals showed progressive increases in OGTT values, reaching a peak at 60 min. (after one hour) and finally reversing back towards normalcy after 120 min (two hours). These results indicate improvement in glucose tolerance. But the higher dosage of Gymnema (Group 5) showed more recognisable regulation of their high glucose levels at all three different points in time, i. e., at 0, 60 and 120 min. Some studies stated, the administration of a high dose of Gymnema sylvestre, potentially lowered the fasting and oral glucose tolerance test intensities. [15,16].

The enhancement of the OGTT level in diabetic animals could be the active compound, Gymnemic acid, which exists in Gymnema sylvestre. The ethanolic extract used for the present study contains a high amount of gymnemic acid content. This is affirmed by the result of HPTLC shown elsewhere. To agree with our statement, an investigation has shown a reduction in glucose levels in the blood and improved OGTT after gymnemic acid administration in diabetic animals (11). An earlier study also reported the activity of gymnemic acid IV in streptozotocin administrated experimental animals which prevent the cause of diabetes [17].

Insulin Level

β-cells of the islets of Langerhans secrete a peptide hormone, insulin, which is composed of 51 amino acids. Insulin is the most important hormone for the metabolism of glucose, lipids, and protein. The action of assimilating glucose from the blood stream and passing it to the liver, adipose tissue, and skeletal muscle cells is promoted by insulin [18]. On the 14th day, the insulin levels increased following high-fat diet supplementation in groups 2, 3, 4, 5, and 6 animals. Likewise, on the 19th day, experimental animal groups after streptozotocin administration also expressed elevated insulin levels. These hyperlipidaemia and diabetic conditions might be associated with increasing blood glucose levels and could cause increased insulin secretion to promote absorption of increasing glucose from the blood. But on the 42nd day, the high fat diet animals (Group 2) exhibit persistently elevated insulin levels that clearly

indicate insulin resistance due to hyperlipidaemia. The reduced level of insulin in diabetes animals could be a result of deficient insulin secretion from β-cells. In agreement with the above statements, previous research stated that the impaired secretion of insulin leads to the pathogenesis of diabetes [19]. The treatment of Gymnema sylvestre (200 mg and 400 mg) and metformin normalizes insulin secretion. Interestingly, the higher dosage of Gymnema (400 mg) showed pronounced recovery. An earlier investigation reported the anti-diabetic properties of Gymnema sylvestre. They exhibited several phytoconstituents such as gymnemic acids, gurmarin, and gymnema saponin. These components act together to normalize blood glucose levels by escalating insulin release and regeneration of pancreatic islet cells. [20].

Liver profile test

In our experimental study, the marker enzymes such as aspartate transaminase, alanine transaminase, alkaline phosphatase, and bilirubin levels in serum are analyzed to estimate liver function for our experimental study. The present investigation found a higher number of marker enzymes (AST, ALT, and ALP) alongside of bilirubin in diabetic-induced animals (Group 3).

Aspartate transaminase and alanine transaminase are recognised marker enzymes utilized as biomarkers to diagnose liver toxicity [21]. The higher level of transaminase in the serum of diabetic animals suggested the destruction of hepatocytes [22]. ALP is another liver marker enzyme frequently used to assess the reliability of the plasma membrane and endoplasmic reticulum [23]. Similarly, bilirubin is a good indicator for accessing the physical condition of the liver [24].

Therefore, these indicators show the malfunction of the liver under hyperglycemic conditions. The treatment of Gymnema sylvestre with higher and lower dosages (Group 4 and Group 5) and metformin (Group 6) restored AST, ALT, and ALP and bilirubin levels.

However, in higher dosages (Group 5), the recovery of these liver parameters was pronounced. Substantiated by our results, previous studies also demonstrated the recuperation activity of Gymnema sylvestre in these liver parameters of diabetic animals [25].

Conclusion: From the study it was concluded that medicinal plant gymnema sylvestre 400mg/kg has significant drug in reduction of blood sugar level in type 2 diabetes. Futher studies to be carried out to find out the effectiveness of drug in preventing the complication of type 2 diabetes mellitus.

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Ethical statement:

Institutional ethical committee accepted this study. The study was approved by the institutional animal ethics committee, Melmaruvathur Adhiparasakthi Institute of Medical Sciences and Research in Melmaruvathur.

Authors' contributions:

Arumugam Rajalakshmi - conceptualization, data curation, investigation, methodology, project administration, visualization, writing—original draft, writing—review and editing; Govindarajan Sumathy -conceptualization, methodology, writing—original draft, writing—review and editing; Elumalai Prithiviraj - conceptualization, visualization, supervision, writing—original draft; Mohamed Ismail basha- methodology, writing—original draft, writing, review and editing. All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work. All authors have read and agreed to the published version of the manuscript**.**

Data Availability:

All datasets generated or analyzed during this study are included in the manuscript.

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