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Original research article

Evaluation of Antidiabetic Activity of Gymnemic Acid Phospholipid Complex in Streptozotocin (Stz) Induced Diabetes

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Abstract

Methods: To look for the antidiabetic effect the albino rats were divided into 6 groups, each consisting of 6 animals. A single i.p. injection of streptozotocin at a dose of 50 mg/kg body weight was used to cause diabetes. The rats were given the conventional medicine, glibenclamide, and gymnemic acid at doses of 200mg/kg and 400mg/kg body weight, respectively, until the completion of the trial. On days 7, 14, 21, and 28, blood glucose levels were measured. From day 5 onwards, the standard drug and the extract were fed.

Results: The extract's anti-diabetic properties increased when the dose was increased, and blood glucose levels gradually decreased as the test drug was exposed for longer periods of time. There is a significant drop in liver marker enzymes, indicating that it possesses hepatoprotective properties.

Conclusions: Results obtained in this study substantiate the anti-diabetic activity of gymnemic acid.

Keywords: gymnemic acid, antidiabetic activity, streptozotocin,

INTRODUCTION

Medicinal plants have long been utilised as a source of medicine, with 80–85 percent of populations relying on extracts or active components from these plants as a traditional medicine to address their basic health care needs [1, 2]. Several active components of medicinal plants have been identified for usage as medications or as lead compounds or pharmacological agents. Gymnema Sylvestre (Asclepiadaceae) is a valuable plant that grows slowly in central and peninsular India. According to Kanetkar et al. (2007), The plant's extracts are effective anti-diabetic herbal medication. Gymnemic acids, which give the plant its therapeutic potential, are one of the most important antidiabetic substances.

A vulnerable species, G. Sylvestre (Asclepiadaceae), is a slow-growing, perennial medicinal woody climber found in central and peninsular India. Its leaves, known in India as "Gurmar," are well recognised for their sweet taste suppressing properties and are used to treat diabetic mellitus [3]. The plant's extracts are effective anti-diabetic herbal medication. Gymnemic acids, which give the plant its therapeutic potential, are one of the most important antidiabetic substances. Gymnemic acids are a collection of at least 17 distinct saponins that make up the major ingredients of gymnema [4]. Anti-diabetic, anti-lipidemic, and anti-inflammatory effects have been discovered in gymnemic acids. [5]. The phytochemical prevents glucose from being absorbed into the bloodstream. Gymnemic acid molecules have the same atomic

configuration as glucose molecules. As a result, gymnemic acids load taste bud receptors, preventing sugar molecules in eaten food from activating. Furthermore, the acids bind to receptors in the intestine's absorptive exterior layers, preventing glucose absorption by the intestines. Low blood sugar is the effect of this [5].
Furthermore, the acids have been discovered to increase the production of insulin by the pancreas, which is necessary for glycemic control and the treatment of adult-onset diabetes mellitus [4]. The acids can also work as a laxative, cough suppressant, and diuretic by increasing cholesterol excretion in the faeces. In long-term treatment, dihydroxy gymnemic triacetate, a chemical derived from Gymnema Sylvestre, showed hypoglycemic and hypolipidemic action, suggesting that it could be utilised to treat diabetes [6].

MATERIAL AND METHODS

Sigma-Aldrich provided the Gymnemic acid and Streptozotocin. Agappe Diagnostics Ltd. in India provided the glucose testing kits. Hi-Media (Mumbai, India) and SD Fine Chemicals Limited provided all other chemicals (Mumbai, India).
The following animal species were employed in this research. The rats were put into six groups of equal weight (n=6 in each group). In total, 36 rats were employed in the study: 6 normal rats and 24 diabetic rats. The rats were placed into six groups, each with six rats :

Group I: Served as normal control.
Group II: Rats were induced with STZ (40mg/kg body weight) after 5 days,
Glibenclamide was administrated (5mg/kg body weight) orally for 28 days.
Group III: Rats were induced with STZ (40mg/kg body weight) after 5 days, gymnemic acid was administered (200mg/kg body weight) orally for 28days.
Group IV: Rats were induced with STZ (40mg/kg body weight) after 5 days, gymnemic acid was administered (400mg/kg body weight) orally for 28days.
Group V: Rats were induced with STZ (40mg/kg body weight) after 5 days, gymnemic acid phytosome was administered (200mg/kg body weight) orally for 28days.
Group VI: Rats were induced with STZ (40mg/kg body weight) after 5 days, gymnemic acidphytosomewas administration (400mg/kg body weight) orally for 28days.
Throughout the treatment period, the animals' overall health was monitored. The animals did not die until the end of the experiment.

Statistical Method

The statistical significance was determined using the Student's t-test and a probability level of 0.05. The values given are the mean and standard deviation. All statistical analysis was done with a one-way ANOVA and the results were expressed as SD.

Experimental Induction of Diabetes

After an overnight fast, rats were given an intraperitoneal injection of STZ (40 mg/kg BW) freshly produced in 0.1 M sodium citrate buffer (pH 7) to induce diabetes. After 5 days, the rats developed diabetes (fasting blood glucose concentration >300 mg/dl) and were given gymnemic acid dissolved in water as a treatment.

Sample Collection

For the determination of blood glucose, haemoglobin, and glycosylated haemoglobin, blood samples were taken from the orbital plexus by pricking a needle under ketamine anaesthesia. In metabolic cages, rats had free access to water and food. Throughout the experiment, the rats' body weight was measured at regular intervals. All of the animals were starved for 16 hours before being anaesthetized with intraperitoneal injections of ketamine and xylazine (80 mg/kg

and 10 mg/kg body weight, respectively) and sacrificed by cervical decapitation. Blood samples were taken, and the tissue was promptly removed and rinsed in ice-cold physiological saline to eliminate any blood clots or other tissue debris. A slice of 1 cm3 tissue was cut and immediately deposited in phosphate-buffered formal saline pH7.4 for histological examinations.

Separation of Serum

The blood sample was taken in ordinary centrifuge tubes and held in an inclined position for 10 minutes to allow complete coagulation of the blood. The clear supernatant that resulted was pipetted out and stored in tiny vials in the freezer for biochemical research.

Estimation of Blood Glucose

The method used a reagent kit to determine glucose [7]. The enzyme glucose oxidase oxidises glucose to produce D-gluconic acid and H_2O_2. H_2O_2 is oxidised to phenol in the presence of enzymes, which then reacts with 4-amino antipyrine to generate the red hue quinoneimine dye. The colour intensity created is related to the glucose concentration in the sample.
In three separate tubes, 10 l of serum, standard, and distilled water (blank) were collected. One millilitre of enzyme reagent was added, stirred, and maintained at 37 degrees Celsius for 15 minutes. In a UV-Visible spectrophotometer, the colour development was measured at 510 nm against a reagent blank. The results are given in milligrammes per deciliter (mg/dl).

All measurements were done three times and the results were provided as mean values with standard deviations (standard deviations). The significance of the difference between means was determined using the Bonferroni multiple range test, and statistical analyses were performed using a one-way analysis of variance ANOVA test. Differences with a P value of less than 0.05 were deemed statistically significant. The correlation tool in Minitab was used to conduct correlation analyses between different antioxidant assays as well as between total phenolic content and total flavonoid content.

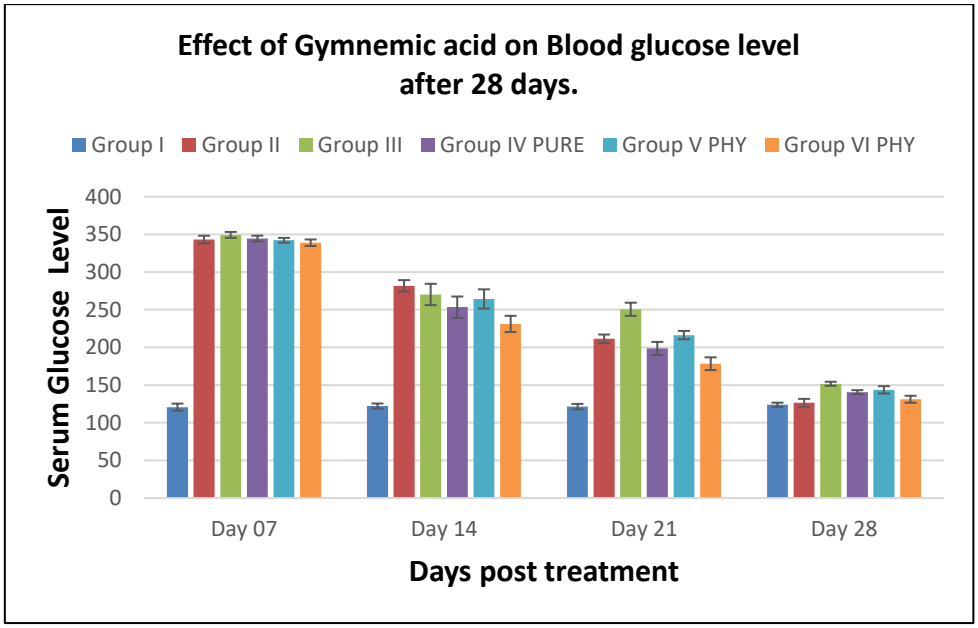
RESULTS

The levels of blood glucose in the control and test groups at day-7 (after 2 days of medication administration) and at the end of 04 weeks are shown in [Table-1]. At the end of the trial, all of the test groups had significantly lower levels of the same ($p < 0.05$). The diabetic rats who received a lesser dose of gymnemic acid (200mg/kg body weight) had a 38 % reduction in blood glucose and a larger dose (400mg/kg body weight) had a 61 % reduction, whereas the conventional medication glibenclamide only had a 63% reduction. From the start of the trial until the end, neither the normal control nor the diabetic control groups showed any significant changes.

Table 1: Effect of Gymnemic acid on the Blood glucose level.

Group	Serum Glucose - Days post-treatment			
	Day 07	Day 14	Day 21	Day 28
Group I	120.66 ± 4.72	122.16 ± 3.40	121.34 ± 3.63	123.83 ± 2.83
Group II	343.16 ± 5.05	281.75 ± 75	211.50 ± 5.57	126.46 ± 5.23
Group III PURE	349.33 ± 3.92	270.25 ± 14.17	250.58 ± 8.72	151.73 ± 2.67
Group IV PURE	344.35 ± 3.92	253.35 ± 14.17	198.58 ± 8.72	140.55 ± 2.67
Group V PHY	342.16 ± 3.19	264.33 ± 12.72	216.33 ± 5.54	143.66 ± 4.88
Group VI PHY	338.91 ± 4.35	231.16 ± 10.75	178.33 ± 8.48	131.16 ± 4.67

$p < 0.05$ by comparison with normal rats.

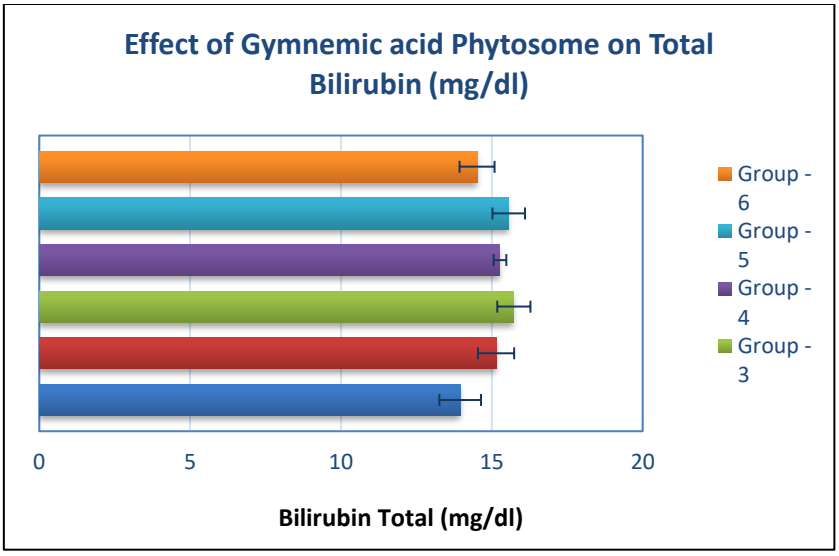


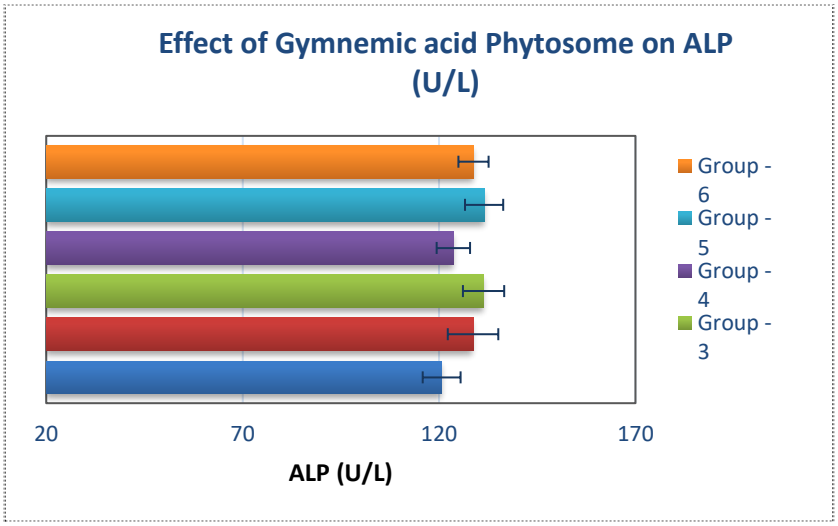
Graph 1: Effect of gymnemic acid on change in the blood glucose level of rat

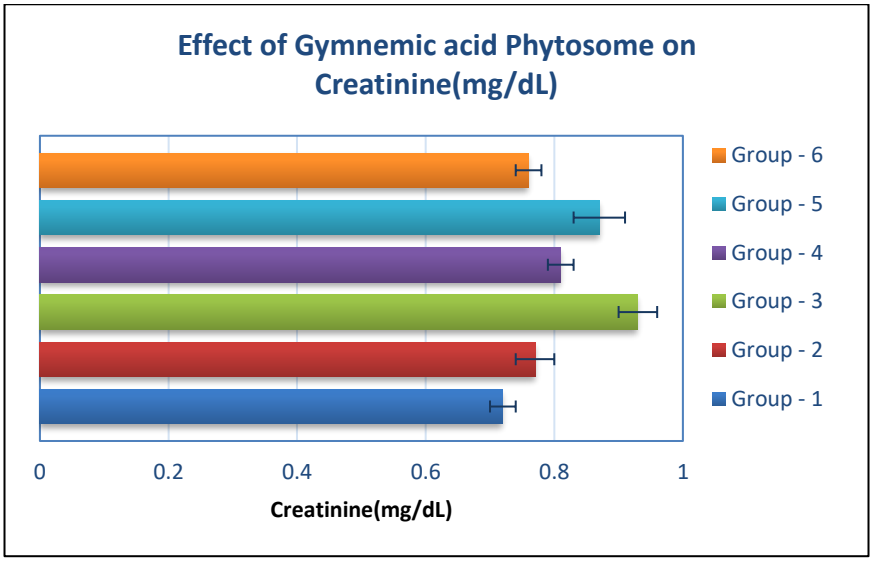
Table 2: Effect of long-term treatment of Gymnemic acid on Serum level of bilirubin, creatinine, SGOP, SGPT and ALP in a different group of experimental animals.

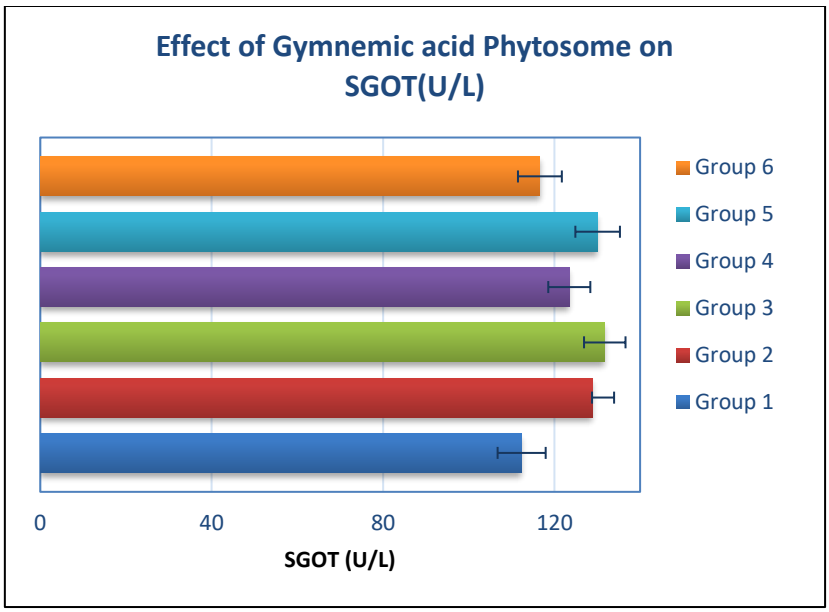
Groups	Bilirubin Total (mg/dl)	ALP (U/L)	Creatinine (mg/dl)	SGOT(U/L)	SGPT (U/L)
Group - 1	13.95 ± 0.69	120.66 ± 4.82	0.72 ± .02	112.35 ± 5.61	70.29 ± 3.23
Group - 2	15.14 ± 0.60	128.65 ± 6.43	0.77 ± .03	128.81 ± 5.12	74.26 ± 2.89
Group - 3	15.73 ± 0.55	131.33 ± 5.25	0.93 ± 0.03	131.75 ± 4.84	75.84 ± 3.12
roup - 4	15.27 ± 0.21	123.64 ± 4.25	0.81 ± 0.02	123.47 ± 4.91	70.32 ± 3.16
roup - 5	15.56 ± 0.54	131.47 ± 4.86	0.87 ± 0.04	130.09 ± 5.20	75.84 ± 2.84
roup - 5	14.51 ± 0.58	128.77 ± 3.84	0.76 ± 0.02	116.61 ± 5.12	70.96 ± 3.85

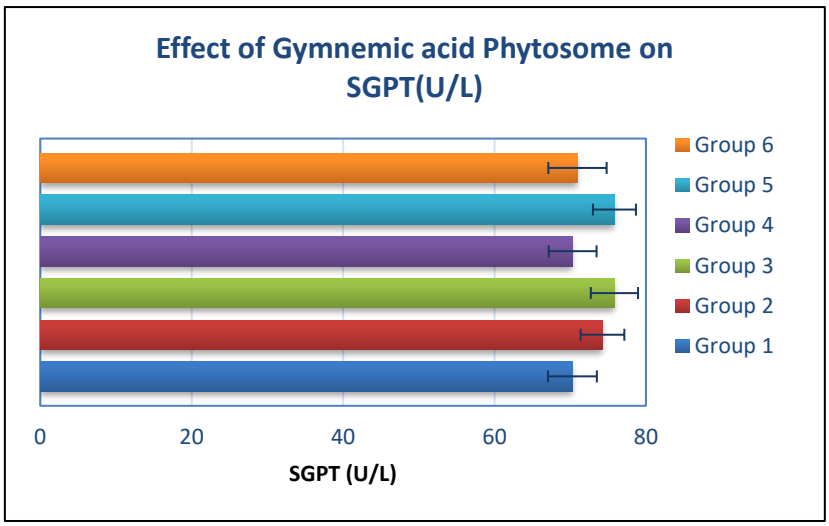
The effect of gymnemic acid on several markers such as ALP, SGOT, SGPT, serum creatinine, and serum bilirubin was studied further, and it was discovered that increasing the dose had superior therapeutic results. A greater dose of gymnemic acid significantly lowers increased levels of ALP, SGOT, SGPT, serum creatinine, and serum bilirubin, as seen in Table 2. The serum levels of bilirubin, creatinine, ALP, SGOT, and SGPT have all decreased, according to the mean values of numerous marker enzymes.


Effect of Gymnemic acid Phytosome on Total Bilirubin (mg/dl)
Group - 6
Group - 5
Group - 4
Group - 3
0
5
10
15
20
Bilirubin Total (mg/dl)


Effect of Gymnemic acid Phytosome on ALP (U/L)
Group - 6
Group - 5
Group - 4
Group - 3
20
70
120
170
ALP (U/L)


Effect of Gymnemic acid Phytosome on Creatinine(mg/dL)
Group - 6
Group - 5
Group - 4
Group - 3
Group - 2
Group - 1
0
0.2
0.4
0.6
0.8
1
Creatinine(mg/dL)





DISCUSSION

The goal of this study was to look into gymnemic acid's anti-diabetic properties as well as its effect on liver enzymes, as diabetes is a serious health issue that affects large populations around the world.

At a dose of 400 mg/kg body weight, gymnemic acid showed considerable antihyperglycemic effect in streptozotocin-induced diabetic mice.

As a result, the medicine has had a positive impact on the liver as well as the treatment of diabetes.

CONCLUSION

The study concluded that the results obtained in this study substantiate the anti-diabetic activity of gymnemic acid.

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