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ALPHA AMYLASE ALPHA GLUCOSIDASE AND ANTIDIABETIC ACTIVITY OF GYMNEMIC ACID IN STREPTOZOTOCIN-INDUCED DIABETIC RATS

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Abstract:
Diabetes is being described as one of the worldwide health problems that escalates every day. We found it difficult to discover new antidiabetic drugs from medicinal plants with minimal or no side effects. The current research study involved determining the beneficial effects of the gymnemic acid isolated from plant *Gymnema Sylvester* against diabetes rats induced by streptozotocin.The gymnemic acid was extracted from 75% Gymnemic acid extract of the Gymnema Sylvster plant. Rats were made diabetic through a fructose-containing drinking water mixture and a single dose of streptozotocin. The effects of gymnemic acid on blood glucose levels in blood and diabetes complications were studied after the administration of it to diabetic rats for 21 days—of four-point blood glucose testing (on days 1, 7, 14, and 21). Blood samples were collected from experimental rats at day 21 before euthanasia for collection of the pancreas and liver. Blood samples were used to establish liver function tests, kidney function test, and lipid profiles. BGL reduction on the 21st day was significant as determined using in vivo antidiabetic study while other tests for liver and kidney as well as lipid profile were normal in extract-treated groups. Gymnemic acid was found to have good inhibitory effect on the in vitro alpha-amylase and alpha glucosidase assay. Results obtained in research indicated the presence of appreciable in vitro and in vivo antidiabetic efficacies in Gymnemic Acid.

Keywords: Gymnemic Acid , Alpha Amylase Alpha Glucosidase, Animals , Diabetes

Introduction:

The term “Diabetes Mellitus” refers to a metabolic disorder that arises from a combination of different factors. It leads to the onset of chronic complications, notably hyperglycaemia and disturbances in

carbohydrates, proteins, and f DM is the most frequent clinical disorder affecting about 10 percent people globally [2]. Diabetes is one of the biggest global disease having severe impact on people's health and mortality due to cardiovascular diseases [5]. Controlling not only glucose but lipid levels will prevent serious complications in diabetes that cause heart disease and diseases of blood vessels [6]. In turn, patients with diabetes have lipids, CHD, peripheral vascular disease and stroke [7,8]. Diabetes in rats and rabbits inducted by alloxan or streptozotocin, and in humans poorly controlled diabetes is a specific cause of hyperlipidemia. CHD causes about half of the world's deaths worldwide. Elevation levels of LDL and TG are associated with incidence rates of CHD, while HDL level is associated with reduced risk. Additionally, hyperlipidemias may be caused by genetic deficiencies in the lipoprotein metabolism or by a mixture of genetics and lifestyles [9]. The onset of early atherosclerosis is associated with dyslipidemia and hyperhomocys-teinaemia. Type 2 diabetes in patients leads to arterial disease (e.g. atherogenesis). The liver receives a high burden of abdominal fat in the form of ciruclating free fatty acids that come through the portal circulation. Excessive amounts of the free fatty acids will result in overproduction of triglyceride-rich lipoproteins such as LDL and VLDL while the level of HDL decreases during this process for the type 2 diabetic state [1 Besides, high peripheral or circulating insulin levels could also play a role in dyslipidemia and vasculopathy. It necessitates the data to establish whether hyperinsulinemia is the worst for vascular health in diabetes. Another option could be that hyperglycemia aligns with atherosclerosis.Gymnema Sylvestre R.Br. is an adherent of the milkweed family (Family: Asclediaecae (a tropical forest plant found in India and Africa) and has been shown to possess anti-diabetic properties. This water extract inhibits hyperglycemia and hyperinsulinemia, thereby reducing glucose absorption in the small intestine as well as the oral glucose tolerance test (OGTT). Moreover, Terasawa et al. report that Gymnemic acid water The infusion of gymnemic acid decreases portal immunoreactive gastric inhibitory peptide when stimulated by sugar. The study investigated impact of gymnemic acids containing extract on high K(+)-induced contraction of guinea-pig ileal longitudinal muscles and on glucose transporter mediated by the difference of glucose-evoked transmural potential difference in the in There are many activities of gymnemic acids such as anti-sweetener, anti-diabetic and anti-inflammatory activities.

Material and Method :

Plant Material:

In addition, a plant extract of G. Sylvestre (dry extract, 75%), was procured from Allpure Organics (Delhi, March 2022) and then refined for isolating and identifying acids.

Drugs and chemicals:

The drug sample, Metformin hydrochloride, was a gift from Chemico Laboratories Ltd. Streptozotocin was obtained from Sisco Research Laboratories Pvt. Ltd., Mumbai, India. The acarbose was bought from SRL LTD in Mumbai. The enzymes, Alpha Amylase and Alpha Glucosidase were purchased from SRL LTD Mumbai..

Isolation of Gymnemic acid from Methanol Extract :

About 200gms of methanol soluble crude extract was taken up in 1 percent aqueous KOH solution with continuous stirring for 45 minutes to 1 hour. Thereafter, the solution is passed through filter paper to remove undissolved particles. The gymnemic acids were precipitated by adding diluted HCl gradually while constantly stirring. The precipitated solution was filed under suction and what was left after that was dried. The impure gymnemic acid was obtained by this. Positive results were achieved for steroids, phenolics, and tests for glycoside on the identified gymnemic acid.Identification of the extracted gymnemic acid was performed on its taste removal properties, froth test, acidic test, Liebermann- Burchard colour test, melting point, and optical rotation.

Phenolic test: One pinch of gymnemic acid was put into a clean test tube, where it was dissolved in two milliliters of methanol. Then, three drops of 1%alco.ferric chloride were added.

Steroid test: A little bit of a solution of 2 ml of chloroform and 1 ml of acetic anhydride was mixed with a pinch of gymnemic acid. a couple of microliter drops of conc sulfuric acid was added through one end of the tubing.

Glycoside test: One mg of gymnemic acid was weighed into one dried test tube, and dissolved in two ml of methanol.

In Vitro Antidiabetic Activity

Assessment of alpha amylase inhibition:

The assay components are 120µL of 0.06 M potassium phosphate buffer, 20µL of enzymes, 40 µL of gymnemic acids within the concentration range of 20 – 100µg/ml. Then, after incubation, 40µL of substrate was added and distilled water was added to make the volume of samples equal. Incubation took place in order to mix the reaction blend, which it lasted for 10-15 minutes at a temperature of 37˚C. 40 microliters of DNS I reagent were added and the mixture was heated in a boiling water bath. A 540nm of measure was applied to the sample taken for absorbance by using ELISA reader. These consisted of plain uncompounded samples for use as controls. Aimed as the percent inhibition using the equation below;

$$\text{Inhibition (\%)} = \frac{\text{Abs (540) control} - \text{Abs (540) Test}}{\text{Abs (540) Control}} \times 100$$

Assessment of alpha glucosidase inhibition:

Alpha-glucosidase inhibition was estimated based on Ranilla et al. procedure with certain enhancements. It contained 20µl alpha-glucosidase 0.5 unit/ml; 120µl of 0.1M phosphate buffer (pH 6.9) and 10µl test sample diluted as indicated. After adding a mixed solution into 96-wells place and further incubating it at 37° for 15min; the enzymatic reaction proceeded by addition of 20µL p-nitrophenyl-α-D-glucopyran Then, the reaction was terminated using addition of 80ul of 0.2 M sodium carbonate solution and later absorbance reading was done in a microplate reader (BioTek XS2) at 405nm. Plant phytochemical was used as the lack of reaction system while α-glucosidase used without for calculation of the background absorbance. That is why the following formula was utilized to calculate the inhibitory rate for an herbal sample of α-glucosidase

$$\text{Inhibition (\%)} = \frac{\text{Abs (405) control} - \text{Abs (405) Test}}{\text{Abs (405) control}} \times 100$$

Experimental protocol:

The test samples were gymnemic acid suspended in distilled water. The treatment regimen involved metformin in a dosage of 100 mg/kg and acted as the standard control. The test was done by oral route on all the sample. The administration of diabetes in rats was done by making use of 65 mg/kg of streptozocin freshly dissolved into a normal saline solution. The rats were fed with a normal pellet diet immediately after ip injection of streptozotocin , they had unrestricted access to both food and water. Polydipsia and polyuria of mild degree were recognized as diabetes in the rat. It was also established that after 3 days or 72 hrs of injection; the fasting blood glucose levels will be computed using the well-accepted GOD/POD method with a commercial glucometer based on UV-visible spectrophotometer at 50 Diabetes rats were identified based on their fasting blood glucose which was greater than 180mg/dl.

Experimental Design:

The rats are divided in to 5 groups 6 animals in each.

Group NO	Group Name	Receiver
I	Normal Control	received only vehicle that is distilled water.
II	Diabetic control	rats received only vehicle that is distilled water
III	Herbal Drug 1	received Gymnemic acid (100 mg/kg/day p.o) suspended in distilled water
IV	Herbal Drug 2	received Gymnemic acid (200 mg/kg/day p.o) suspended in distilled water
V	Standard	received Metformin (100 mg/kg p.o) suspended in 2% v/v Tween 80 solution

The grouping is arranged in such a way that group I and group II described as normal and diabetic control ,group III and group IV as herbal extract receiver in doses 100mg/kg and 200mg/kg respectively ,V group received standard drug i.e metformin 100mg/kg .Test sample was given accordingly and blood glucose level was checked on every alternate day like 1,3,5,7,9,till 21st day of antidiabetic study blood glucose measurement was done by using glucose testing kit.

Statistical Analysis

The statistical analysis data were articulated as mean ± standard error mean (SEM). The Significance of differences between the groups was evaluated by applying one-way and multiple way analysis of variance (ANOVA). The test followed by Dunnet's test p values less than 0.05 were considered as significant. All data are articulated as the standard error of the mean. Comparisons between the control and treatment groups were made using analysis of variance followed by a Student- Newman-Keuls t-test using the Graph pad instant statistical program . Analysis of all samples was calculated by , an associated probability (p value) of less than 5% (P<0.05) And it was considered significant.

Result :

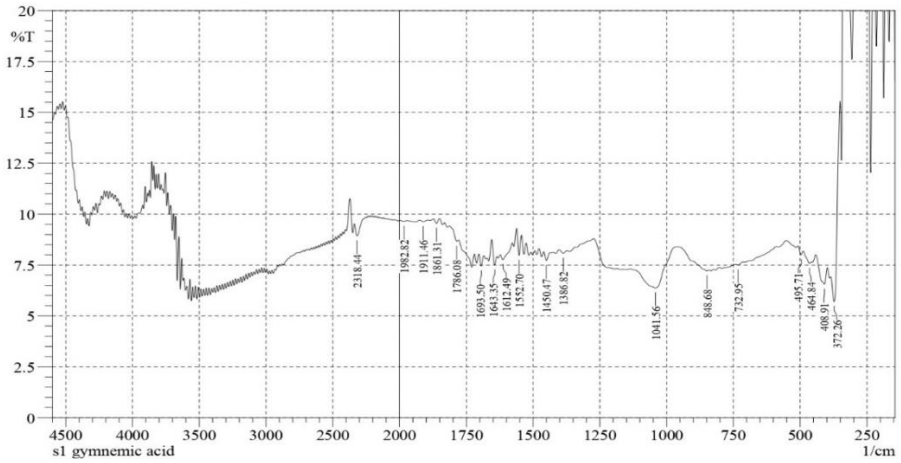
Table I: Represents phytochemical identification results :

Sr.No	Name Of Drug	Liebermann Burchard test	Optical rotation	Melting point
1	Gymnemic Acid	Light green Bluish green	163.500	195^0C198^0C

Table II : Results of phytochemical test :

Sr no	Test	Results
1	Phenolic Test	+++
2	Steroid Test	+++
3	Glycoside Test	+++

Figure :1: IR Spectra of Gymnemic Acid:



Alpha Amylase And Alpha Glucosidase Assay Results :

Concentration-dependent inhibitory effect of alpha amylase was maximum in 100µg/ml in both the Gymnemic acid and Acarbose with IC50 value of 67.12µg and 40.03µg respectively.

Gymnemic acid shows low inhibitory effect when it is compared with Acarbose drug. Table II and Graph II shows the α-glucosidase inhibitory activity of Gymnemic acid and Acarbose. Concentration dependent inhibitory effect of α-glucosidase was shown maximum in 100µg/ml in both Gymnemic acid and Acarbose with IC50 value of 66.27µg and 34.67µg. Gymnemic acid shows less inhibitory effect when compared to that of acarbose drug.Calculation of IC_{50} was done by linear regression equation. This equation used to calculate the IC_{50} where the concentration of the sample is plotted in the y-axis and percent inhibition in the x-axis. From the equation y = a IC_{50} values can be calculated using the following formula IC_{50}= 50-a.

Table III: Effect of Gymnemic acid and Acarbose on α-amylase activity

Concentration (µg/ml)	**Gymnemic acid**		**Acarbose**	
	% inhibition	(IC_{50})	% inhibition	(IC_{50})
20	20.60 ± 1.68	67.12	22.63 ± 0.78	40.03
40	27.47 ± 1.29		45.67 ± 1.76	
60	54.13 ± 1.63		52.30 ± 1.25	
80	51.63 ± 1.55		65.17 ± 1.89	
100	62.40 ± 1.64		77.87 ± 1.33	

Table IV: Effect of Gymnemic Acid and Acarbose on α- Glucosidase activity

Concentration (µg/ml)	**Gymnemic acid**		**Acarbose**	
	% inhibition	(IC_{50})	% inhibition	(IC_{50})
20	21.67 ± 1.44	66.27	23.63 ± 1.64	34.67
40	27.80 ± 1.59		55.97 ± 1.55	
60	43.10 ± 1.01		67.60 ±1.28	
80	51.43 ± 1.50		76.43 ± 1.40	
100	70.50 ± 1.32		89.20 ± 1.74	

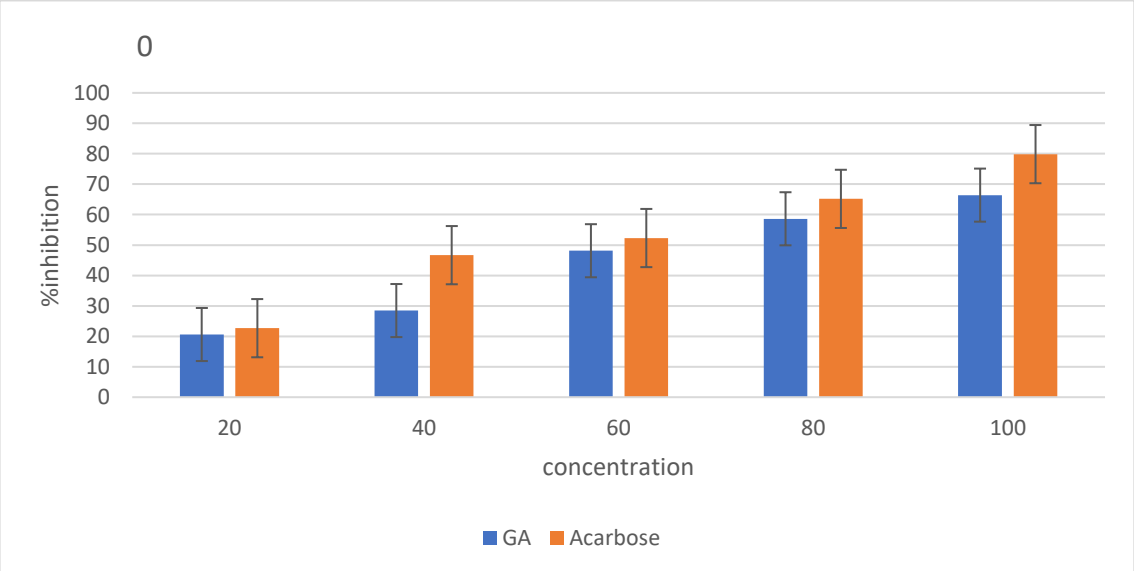


Figure 2: Represents alpha glucosidase activity comparing with Gymnemic Acid and Acarbose is used as standard drug

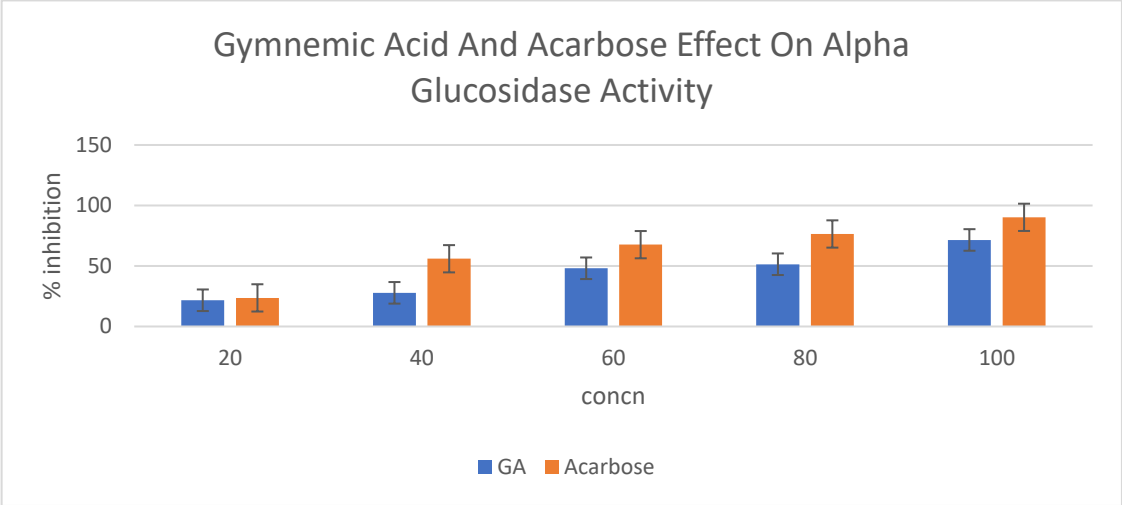


Figure 3: Represents Alpha amylase inhibitory activity of Gymnemic Acid comparing with standard Acarbose drug.

Animal Study Result:

Upon administration of Gymnemic acid, significant changes were recorded in blood glucose levels, triglycerides, total cholesterol levels, urea and creatinine levels both in acute and in chronic study groups. It was observed that the higher dosage of Gymnemic acid exhibited increased reduction in the values of parameters compared to low dosage administration. The values of the glucose levels in blood observed by treating diabetes induced rats with Gymnemic acid was comparable to the values obtained by treating with metformin. Recorded values showed a dose dependent reduction of blood glucose levels, total cholesterol, triglycerides and urea levels in the streptozotocin induced diabetic rats treated with Gymnemic acid.

Table V: Effect of extract On Weight After Every 7 Days:

Days	Normal	DC	Metformin 100 mg/kg	100 mg/kg GA	200mg/kg GA
0th Day	164 ±0.12	166±0.12	170.4±0.34	163±0.34	161±0.47
7th Day	169.2±1.23	169.2±0.41	152.4±1.43	165.8±0.41	177.2±.0.67
14thday	223±0.43	226.2±0.12	210.8±2.13	221.4±0.32	220±1.22
21th Day	228.4±0.89	236.6±1.43	225.8±1.65	221.4±1.42	224±2.31

Table VI : Effect of extract on Post Prandial Blood Sugar

Days	NC	DC	Metformin	100mg/kg GA	200mg/kg GA
0th Day	108.4±0.23	342±1.43	355±0.65	342.2±1.43	374.6±2.31
7th Day	108±0.12	343.8±2.43	296.8±0.54	307.8±2.31	340±3.21
14th Day	105.8±0.43	348±0.23	178.6±1.45	189.2±2.41	238.8±1.65
21ST Day	105.8±0.56	336±0.56	152±2.23	169.2±1.44	134.4±2.13

Table No VI : Effect of extract on Liver Function Test

Sr No	NC	DC	Metformin	100mg/kg GA	200mg/kg GA
SGOT	33.8±0.67	212.96±1.56	47.6±0.43	77±1.23	82.2±2.14
SGPT	38.8±1.23	28.6±2.11	30.6±2.13	29.2±2.56	22.8±0.23
Bilirubin	0.58±3.12	1..324±0.65	0.46±0.67	0.34±1.76	0.6±1.43
Total Protein	6.9±0.63	28.6±0.89	8±2.11	10±1.45	8.76±2.54

Table No VII : Effect of extract on Kidney Function Test :

Sr No	NC	DC	Metformin	100mg/Kg GA	200mg/Kg GA
Creatinine	0.54±1.45	5.88±1.45	1.72±0.32	2.22±0.98	1.84±0.56
BUN	9.88±2.31	47.81±3.21	12.4±0.87	14.6±0.35	14.8±0.17
BUN/Creatinine Ratio	10.6±0.13	93.72±2.45	14.2±0.45	16.94±0.52	18.98±0.65
Uric Acid	1.76±0.56	1.18±1.45	1.71±1.45	1.64±0.16	1.68±0.73i

Table No VIII : Effect of extract on Lipid profile:

Name	Normal	DC	Metformin	GA100mg/Kg	GA200mg/Kg
Total Cholesterol	119.6±1.89	271.8±0.98	149.2±1.54	181±1.98	155±1.43
Triglyceride	129.4±2.31	721.6±0.43	149.2±2.31	149±2.31	132.4±2.41
HDL	42±0.23	15.8±0.43	35.6±1.65	29.6±0.91	36.8±2.31
LDL	74.8±1.56	151.7±0.23	98.6±2.11	120.2±0.23	116.6±0.35
CH/HDL	3.5±2.43	5.82±2.87	3.14±2.34	4.95±1.12	3.76±0.54
VLDL	29±1.67	12.4±0.26	29.8±1.21	71.6±0.28	47±0.11

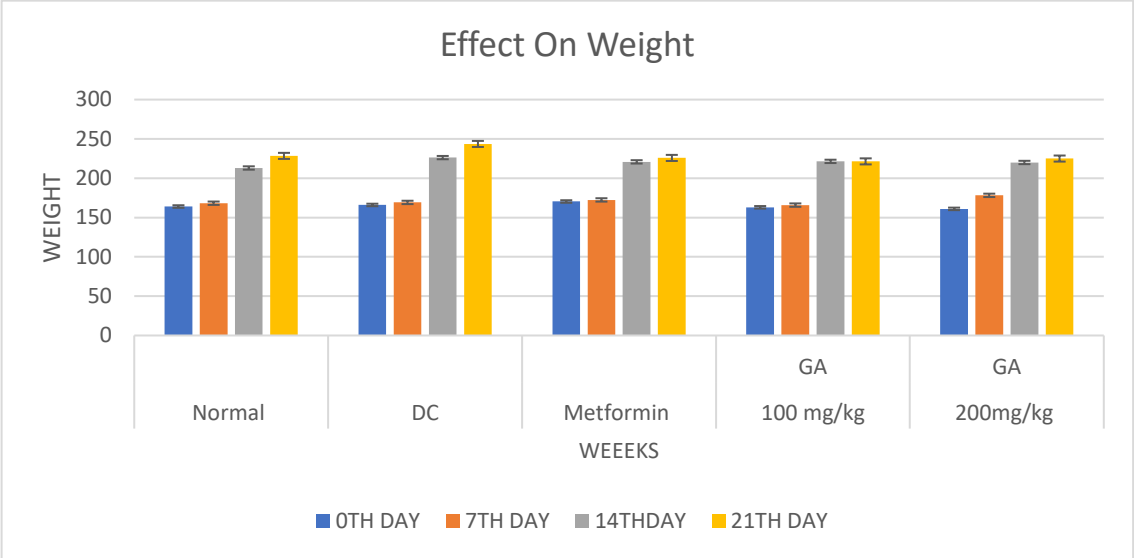


Figure 4: Represents effect of antidiabetic activity on weight of rats comparing with diabetic control rats (DC).

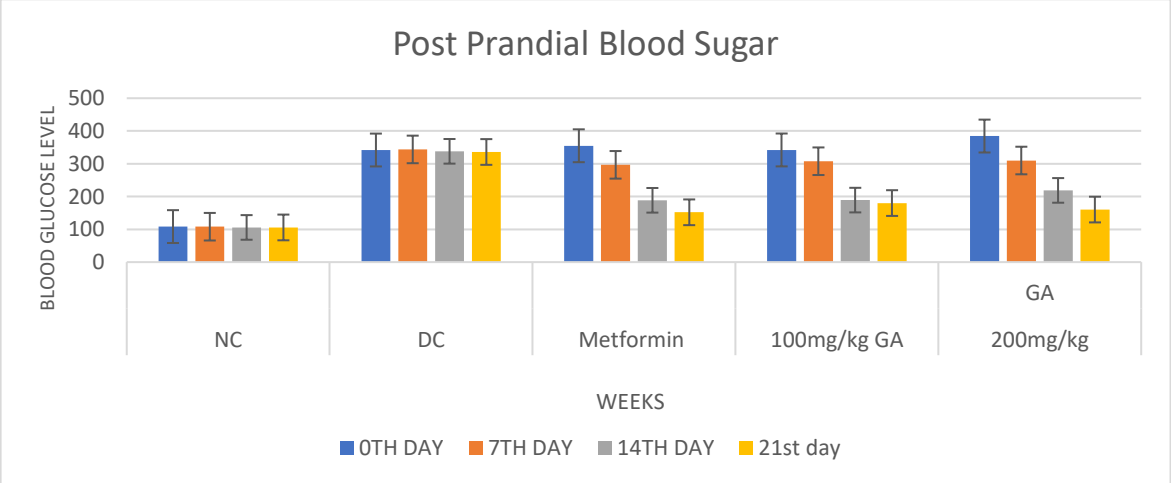


Figure 5: Represents effect of antidiabetic activity of extract on post prandial blood glucose level on different study group comparing with diabetic control (DC)

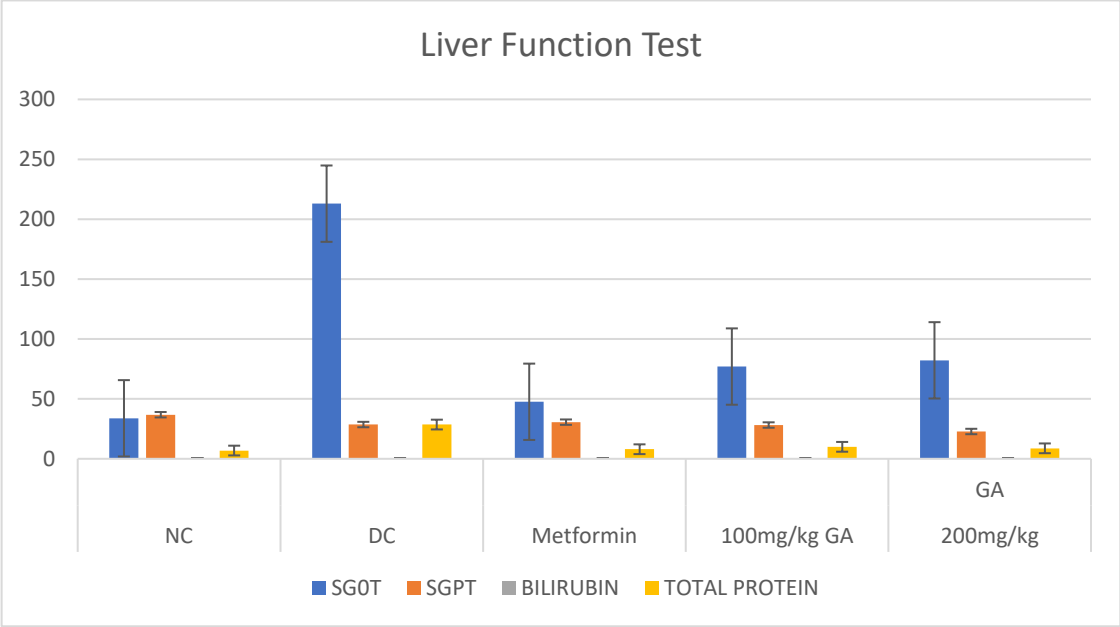


Figure 6: Represent effect of antidiabetic activity on liver function test comparing with (DC) i.e diabetic control group.

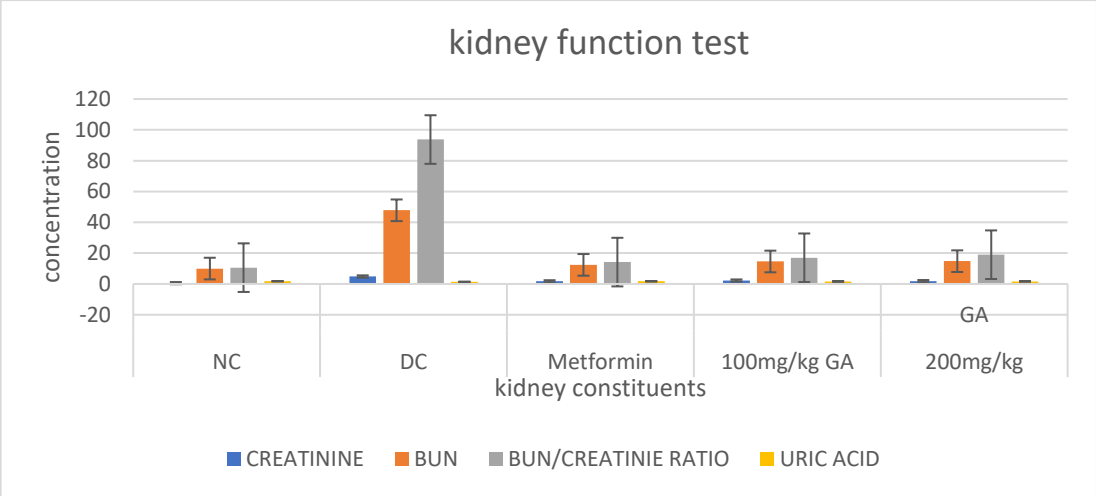


Figure 7: Represents effect of antidiabetic activity of extract on kidney function test on different study group comparing with diabetic control (DC)

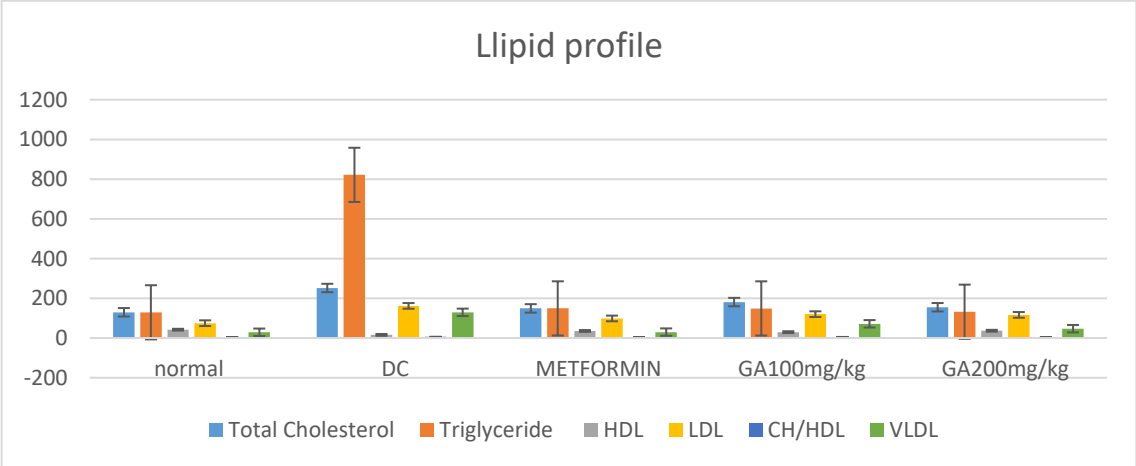


Figure 8: Represents effect of antidiabetic activity of extract on lipid profile level on different study group comparing with diabetic control (DC)

Discussion :

Diabetes is found to be a lethal and life-threatening disease like cardiovascular and cancer disease. Organ failure is the major problem following damage, dysfunction, and failure. Chronic hyperglycemia works as a silent killer for the kidneys, eyes, heart, and nervous system. Due to disorders in lipid metabolism, there are high chances of coronary heart disease and peripheral vascular disease. Nowadays herbal plants are majorly used for antidiabetic activity on animals and patients. still, plenty of plants require documented justification for confirmed antidiabetic activity. Among all these herbal studies *Gymnema Sylvester* was found to be a miraculous remedy for antidiabetic activity. Triterpenid saponin fraction i.e a bunch of Gymnemic Acid is the major component responsible for antidiabetic activity.In our study by experimenting on animals we have concluded that gymnemic acid in defined dose is reducing blood glucose level statisticaly. The studies are comparable with standard drug a Metformin .By performing this study it can be concluded that metformin is having multiple sideeffects but if given with gymnemic acid the dose can be decreased and side effects also can be reduced. So we can conclude the beneficial effects of gymnemic acid for antidiabetic activity.

Conclusion :

Gymnemic acid pronounced component of *Gymnema Sylvester* was found to be a potent component for reducing blood glucose level. It also plays a vital role to reduce lipid profile , which protects from risk of cardiovascular diseases mainly coronary arterial disease . Hence it acts as an alternative or a helper medicine with metformin to reduce blood glucose level and chronic complications of type 2 diabetes.

Conflict of interest statement

We declare that we are not having any conflict of interest.

DECLARATIONS

Ethics approval and consent to participate: Not Applicable.
Consent for publication: Not Applicable.
Availability of data and material: The data collected and reported in the review has been cited accordingly as per the ethics.
Competing interests: Authors declare no competing interests.
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Authors' contribution:

1. ASB: Revision of final draft.
2. SIS: Literature search and data analysis.
3. BAY: Article drafting and critical revision.
4. LNA: Idea for the article.

"all authors have read and approved the manuscript"

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