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ASSESSMENT OF ANTIDIABETIC ACTIVITY OF CLEMATIS HEYNEI AND SOLANUM VIRGINIANUM IN STZ-NICOTINAMIDE-INDUCED DIABETIC RAT MODEL

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ABSTRACT:

Medicinal plants are rich sources of phytochemicals, forming the basis for traditional and modern medicine. Natural antioxidants from plants, such as flavonoids, tannins, and vitamins C & E, help counteract oxidative stress, which is linked to various diseases, including cancer and neurodegenerative disorders. Clematis heynei and Solanum virginianum is also produced beneficial effects in complications of the diabetes mellitus and obesity. Clematis heynei and Solanum virginianum are used for streptozotocin nicotinamide-induced diabetic rats. These plants produces dose-dependently antidiabetic activity. The Clematis heynei and Solanum virginianum has also seems have potent hypolipidemic activity, which lowers the cholesterol, triglyceride, total proteins and increasing high-density lipoproteins levels. The role of antioxidant activities of Clematis heynei and Solanum virginianum and active constituents play a role in preventing diabetic complications and antidiabetic activity of Clematis heynei and Solanum virginianum at 200 mg/kg of the dose was found to be more effective than 100 mg/kg of dose, its produced effects dose-dependently.

KEYWORDS: Clematis Heynei, Solanum virginianum, Lethal Dose, Diabetes, Triglycerides

INTRODUCTION:

Phytochemicals are naturally occurring bioactive compounds in plants that contribute to health benefits [1]. These compounds help in plant defense, stress response, and impart color, flavor, and aroma[2]. Around 4,000 phytochemicals have been classified, with about 50,000 identified chemical structures [3]. Medicinal plants are rich sources of phytochemicals, forming the basis for traditional and modern medicine [4]. India, China, and the U.S. have significant markets for plant-derived drugs, with India's herbal medicine industry valued at $1 billion and exports around $100 million [5,6] Antioxidants inhibit oxidation and neutralize free radicals, preventing cell damage [7]. Natural antioxidants from plants, such as flavonoids, tannins, and vitamins C & E, help counteract oxidative stress, which is linked to various diseases, including cancer and neurodegenerative disorders [8].

Various indigenous medicinal systems including Ayurveda, Siddha, Unani and Allopathy

includes the use of 600 to 700 plant species that were used for different diseases or disorder [9]. On the basis of some other record about 17,000 species of medicinal plants are on the documents from which about 3,000 species of plants are employed in the field of medicines [10]**.** Since the chemical constituents of natural origin like flavonoids, alkaloids, saponins, tannins, alkenyl phenols, glycol-alkaloids, terpenoids, phorbol esters, and sesquiterpenes lactones are simple, they are playing prime significance in development of novel drugs of plant origin plants [11, 12, 13]

Use of plants along with insulin and other synthetic agents is on rise for debates mellitus. Utilization of synthetic drugs alone on the other end has shown severe adverse effects and has failed to treat the basic lesion and diabetic complications. This has increased the use of alternative medicine to treat DM.

The herbal remedies are seemingly effective, imparting less or no adverse effects and are comparatively cheaper than synthetic oral anti-diabetic agents available in the market. A wide range of plant originated chemical constituents in lieu of numerous chemical compounds show their potential against DM under investigation and practical implications. Among these are glycosides, alkaloids, galactomannan peptidoglycans, hypoglycans, gum, polysaccharides, bioflavonoids, amino acids, terpenoids, and inorganic ions. [14-16]

MATERIALS AND METHODS:

Normal saline and other graded chemicals. Drug solutions were prepared fresh and doses are expressed in terms of their free bases. Metformin was used as standard drugs for comparison with various extracts. Streptozotocin (Sigma-Aldrich), Lipid profile estimation kit (Transasia Bio Medical Limited, Mumbai, India) and other chemicals and solvent obtained from Qualigens, India were used.

Animals: Wistar rats

Extraction: The plants Clematis Heynei (Ranunculaceae) and Solanum virginianum (Solanaceae) were collected from local area of Igatpuri, Nasik (M.S.) in the month of August, 2016. The shade dried plant materials were coarsely powdered and proceed to extraction with petroleum ether by maceration. The extraction was continued till the defatting of the material. Dried powdered Clematis heynei and Solanum virginianum has been extracted with different solvents using maceration process for 48 hrs, filtered and dried using vacuum evaporator at 40^0C, and percentage yield of each extract was calculated.

Phytochemical assessments like Detection of alkaloids, carbohydrates, glycosides, Saponins, phenols, flavonoids, proteins, aminoacids and diterpenes were carried out as per the standard methods.

Estimation of total phenolic content

10mg of dried extracts of were dissolved in 10 ml methanol and filter. 2 ml (1mg/ml) of this solution was used for the estimation of phenol.

2 ml of each extract or standard was mixed with 1 ml of Folin-Ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 1 ml (7.5g/l) of sodium carbonate. The mixture was

vortexed for 15s and allowed to stand for 15 min for colour development. The absorbance was measured at 765 nm using a spectrophotometer [17].

Preparation of standard stock solution

10 mg of quercetin was transferred to a 10ml volumetric flask, and prepared stock solution of 1000 ppm by adjusting volume with methanol.

Determination of λ_{max}

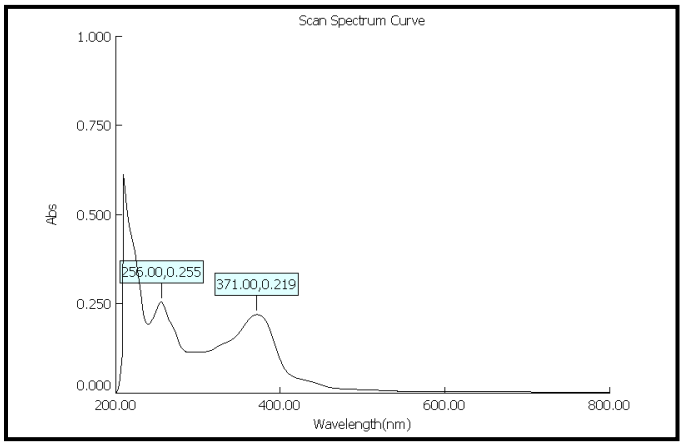

Figure 1: Determination of λ_{max} of extract

Preparation of working standard solution

From stock solutions of Quercetin 1 ml was taken and diluted up to 10 ml. from this solution 0.5, 1.0, 1.5, 2.0, 2.5 ml solutions were transferred to 10ml volumetric flasks and make up the volume up to 10 ml with mobile phase, gives standard drug solution of 5, 10, 15, 20, 25µg/ ml concentration.

Analysis of extracts

10 mg extract was taken in 10 ml volumetric flask and dilute upto the mark with Methanol; resultant solution was filtered through Whatmann filter paper and finally volume made up to mark with same solvent to obtain concentration of 1000 µg/ml. The resulting solution was again filtered using Whatmann filter paper no. 41 and then sonicated for 10 min.

In vivo Antidiabetic activity of

Wistar rats (180–250 g) were group housed (n= 6) under a standard 12 h light/dark cycle and controlled conditions of temperature and humidity (25±2 °C, 55–65%). Rats received standard rodent chow and water ad libitum. Rats were acclimatized to laboratory conditions for 7 days before carrying out the experiments. All the experiments were carried in a noise-free room. Separate group (n=6) of rats was used for each set of experiments. The animal studies were approved by the Institutional Animal Ethics Committee (IAEC Registration No.1329/ac/10/CPCSEA).

Toxicity Study: For the acute oral toxicity and LD_{50} determination the OECD guideline 423 was followed. As per OECD guidelines a stepwise procedure with the use of 3 animals of a single sex per step was followed. Absence or presence of compound related mortality of the animal doses at one step will determine the next step i.e. - No further testing needed, 3 animals were

administered same dose, Additional 3 animals were administered next lower or higher dose levels.

Experimental model

Induction of Diabetes in Rats

Streptozotocin was dissolved in 100 ml citrate buffer (pH 4.5) and calculated amount of the dose (60 mg/kg) of the fresh solution was injected intraperitoneally to overnight fasted rats, 15 min after the intraperitoneal administration of 110 mg/kg of nicotinamide. Blood glucose was checked 48 h later and animals showing blood glucose value more than 250 mg/dl were included in the experiments and termed as diabetic.

Group I- Normal control

Group II- Diabetic rats received only distilled water (negative control)

Group III- Diabetic rats was treated with Metformin (500mg/kg p.o.)

Group IV- Diabetic rats received Methanolic extract of Clematis heynei (100 mg/kg/day p.o.)

Group V- Diabetic rats received Methanolic extract of Clematis heynei (200 mg/kg/day p.o.)

Group VI- Diabetic rats received Methanolic extract of Solanum virginianum (100 mg/kg/day p.o.)

Group VII- Diabetic rats received Methanolic extract of Solanum virginianum (200 mg/kg/day p.o.)

Antidiabetic screening

Blood sampling and glucose estimation

For blood glucose determination, blood was withdrawn by tail snipping technique. For various lipid profile and biochemical parameters estimation, blood was collected from cardiac puncture is a suitable technique. Blood was collected in plain micro centrifuge tube at every second week throughout the study period from all the overnight fasted (16-20 hr.) animals, under anesthesia. Serum was separated from blood sample by centrifugation at 4000 r.p.m. for 10 minutes. Biochemical parameters were studied by biochemistry auto analyzer Hitachi-902.

Estimation of Total Cholesterol (TC)

Specimen collection and preparation yield H_2O_2, which oxidates 4-Aminoantipyrine with phenol to form a colored dye. Serum, heparin or EDTA plasma is suitable for samples. Whole blood, hemolysis is not recommended for use as a sample. Freshly drawn serum is the preferred specimen. Use the suitable tubes or collection containers and follow the instruction of the manufacturer; avoid effect of the materials of the tubes or other collection containers. Centrifuge samples containing precipitate before performing the assay. Stability: 5-7 days at 2–8 ℃, 3 months at -20

Estimation of Triglycerides (TG)

Standard Curve Preparation:

For the colorimetric assay, add 0, 2, 4, 6, 8, 10 µl of the 1 mM Triglyceride Standard into wells individually. Adjust volume to 50 µl/well with Triglyceride Assay Buffer to generate 0, 2, 4, 6, 8, 10 nmol/well of Triglyceride Standard. For the fluorometric assay, dilute the Triglyceride Standard to 0.01- 0.1 mM with the Triglyceride Assay Buffer (Detection sensitivity is 10-100

fold higher for a fluorometric than a colorimetric assay). Follow the procedure as the colorimetric assay.

Sample Preparation: * Prepare test samples to a final volume of 50 µl/well with Triglyceride Assay Buffer in a 96-well plate.

C = Ts / Sv nmol/µl or µmol/ml or mM

Where: **Ts** is triglyceride amount from standard curve (nmol).

Sv is the sample volume (before dilution) added in sample wells (µl).

Estimation of High Density Lipoproteins-Cholesterol (HDL-C)

Serum, EDTA plasma, HDL Cholesterol is reported to be stable in serum for 7 days when stored at 2-8^{0}C. The sample should preferably be of 12 to 14 hours fasting.

CALCULATIONS

HDL Cholesterol in mg/dl = $\frac{\text{Abs. T}}{\text{Abs. S}}$ x 25 x 2

Where 2 is the dilution factor due to the deproteinization step

Estimation of Total Protein (TP)

Pipette into three test tubes: Sample, standard, physiological solution and biuret reagent (ml). Mix properly and allow standing for 30 min at room temperature. Measure the absorbances of the sample and the standard at 546 nm against the blank. Calculate the total protein concentration:

$$\text{Total protein concentration (g/l)} = \frac{\textbf{sample}}{\textbf{A standard}} \text{ X c standard (70 g/l)}$$

Estimation of SGPT

This assay is intended for use with serum. Reports indicate that ALT in serum remains stable at 4 - 8°C for a minimum of seven days.4 Hemolyzed specimens should not be used as erythrocytes contain seven times the ALT activity of serum.

Estimation of SGOT

Reconstitute one vial of Enzyme Reagent (A1) with 10ml of SGOT

Diluent for 6 x 10 ml and 20 ml for 5 x 20 ml. Mix gently to allow dissolution.

Working reagent: This working reagent is stable for at least 4 weeks when stored at 2-80C.

Sample Material:

Serum. Free from hemolysis. SGOT (AST) is report to be stable in serum for 3 days at 2-8^{0}C.

Procedure Assay

Pipette into a clean dry test tube labelled as Test (T):

Addition Sequence	Test (T) 37°C
Working Reagent	1.0 ml
Sample	0.1 ml

Mix well and read the initial absorbance A0 after 1 min. and repeat the absorbance reading after 1, 2 and 3 minutes. Calculate the mean absorbance change per min.

Statistical analysis

Variables of interest were entered and all data analyzed using Graph Pad Instant 3.06 software version 14 for (version 2007, Microsoft Corp, Seattle, Washington). All statistical analysis is expressed as mean ± standard error of the mean (SEM). Data were analyzed by two-way ANOVA, where applicable $P \leq 0.05$, $P \leq 0.01$, $P \leq 0.001$ was considered statistically significant, compared with vehicle and Diabetic control.

RESULTS AND DISCUSSION

In vivo Antidiabetic activity of Clematis heynei and Solanum virginianum

Acute toxicity studies:

The acute oral toxicity studies and selection of doses was carried out as per guidelines of 423 (OECD) received from Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Healthy wistar rats of either sex weighing between 180-250 gm were used for acute toxicity study to determine LD50 of Clematis heynei and Solanum virginianum. Randomization of selected healthy animals were carried and marked for identification. Acclimatization of animals were done for 7 days prior to dosing. In acute toxicity study no toxic symptoms were observed for Clematis heynei and Solanum virginianum up to dose 2000 mg / kg body weight. All animals behaved normally. No neurological or behavioural effect could be noted. No mortality was found up to 14 days study.

As represented in Table 1and Figure 2, body weights of animals in all groups were performed at the initial and end of the study. Body weight of animals was significantly ($p<0.05$) maintained in all treatment groups (Metformin 500 mg/kgp.o., Clematis heynei and Solanum virginianum 100 and 200 mg/kg/p.o., 190.40±10.45; 195.00 ± 9.70 and 196.00 ± 9.70) during study as compared to control group (195.30±8.37).

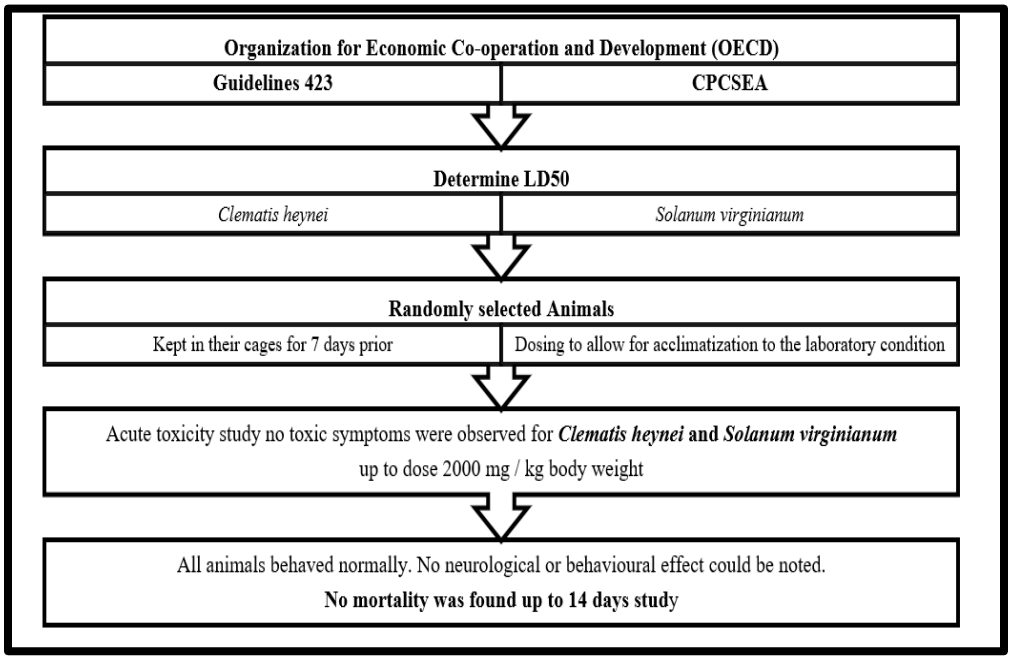

Table 1: Change in body weight

Group	Treatments	Body weight (g)	
		Onset of study	End of study
I	Normal Control (Normal saline)	210.15±8.83	230.18±8.93
II	Diabetic Control (Normal saline)	220.20± 10.00	195.30±8.37
III	STZ-NZ +Metformin (500mg/kg p.o.)	225.22± 8.26	190.40±10.45*
IV	STZ-NZ +Clematis heynei (100 mg/kg p.o.)	235.15± 6.00	199.20±7.50
V	STZ-NZ +Clematis heynei (200 mg/kg p.o.)	227.10± 5.00	195.00 ± 9.70*
VI	STZ-NZ +Solanum virginianum (100 mg/kg p.o.)	231.15± 6.00	198.00 ± 9.60
VII	STZ-NZ +Solanum virginianum (200 mg/kg p.o.)	233.10± 5.00	196.00 ± 9.70*

Values are expressed as mean ± S.E.M. (n = 6).Values are statistically significant at $p<0.05$ vs. control group respectively (One-way ANOVA followed by Dunnett's test).

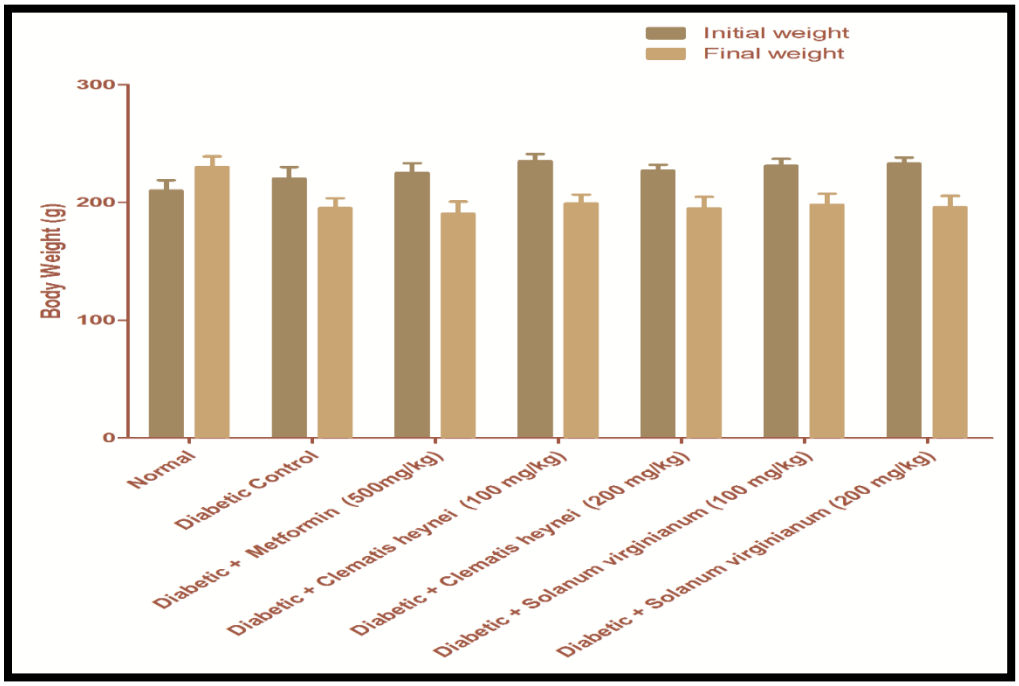

Figure 2: Mean Body Weight Change

As shown in Table 2 and Figure 3, Blood glucose level of animals in all groups was recorded at 0, 7th, 14th and 21th day. Progressive decrease in blood glucose level was found in all

treatment groups during study. At the end of experiment Metformin 500 mg/kgp.o., Clematis heynei and Solanum virginianum 100 and 200 mg/kg/p.o. (108.87±20.89(Met.),180.88±11.14; 134.93±18.31(MECH) and 155.59±11.22, 136.57±12.19(MESV)) treated group blood glucose level was decrease significantly (P≤0.05, P≤0.01, P≤0.001) at 21st days, respectively.

Table 2: Antidiabetic activity of Clematis heynei and Solanum virginianum on blood glucose level in STZ-Nicotinamide induced diabetic rats

Group	Treatments	Blood glucose (mg/dl)			
		Day 0	Day 7	Day 14	Day 21
I	Normal Control (Normal saline)	89.23±3.98	91.18±6.57	93±4.56	92.74±4.52
II	Diabetic Control (Normal saline)	281.07±4.54	288.48±6.35	294.72±4.57	306.74±12.52
III	STZ-NZ +Metformin (500mg/kg p.o.)	279.3±11.03	193.82±9.88***	141.7±5.94***	108.87±20.89***
IV	STZ-NZ +Clematis heynei (100 mg/kg p.o.)	291.08±4.73	270.03±3.82**	243.08±4.32***	180.88±11.14***
V	STZ-NZ +Clematis heynei (200 mg/kg p.o.)	280.72±6.38	237.67±5.81***	191.12±4.41***	134.93±18.31***
VI	STZ-NZ +Solanum virginianum (100 mg/kg p.o.)	285.78±3.88	255.22±6.17***	210.53±5.35***	155.59±11.22***
VII	STZ-NZ +Solanum virginianum (200 mg/kg p.o.)	288.82±4.24	252.15±9.06***	191.62±10.55***	136.57±12.19***

Values are expressed as mean±S.D. (n=6). * P≤0.05 ** P≤0.01 *** P≤0.001 compared with the diabetic control group (two-way ANOVA followed by Dunnett's test)

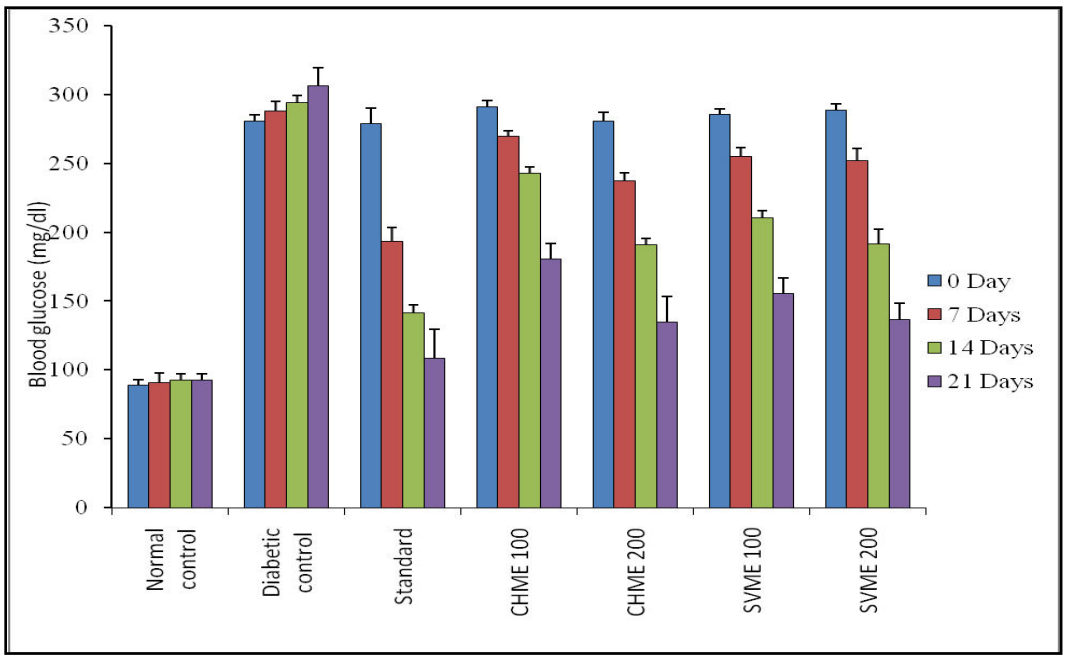

Figure 3: Antidiabetic activity of Clematis heynei and Solanum virginianum on blood glucose level in STZ-Nicotinamide induced diabetic rats. Values are expressed as mean±S.D. (n=6).

In Clematis heynei and Solanum virginianum 100 and 200 mg/kg/p.o. (135.0 ± 5.00; 138.0 ± 5.00; 122.5 ± 4.00; 125.5 ± 5.00) treated group total cholesterol decreased significantly ($p < 0.05$) treated group total cholesterol also decreased significantly ($p < 0.05$). In 500 mg/kg metformin (115.0 ± 5.00) treated group total cholesterol decreased significantly ($p < 0.05$), respectively as compared with control group (200.0 ± 5.00), as shown in Table 3 and Figure.4

Table 3: Effect of Clematis heynei and Solanum virginianum on total cholesterol level in STZ-Nicotinamide induced diabetic rats

Group	Treatments	Total Cholesterol (mg/dl)
I	Normal Control (Normal saline)	81.50 ± 5.00
II	Diabetic Control (Normal saline)	200.0 ± 5.00
III	STZ-NZ +Metformin (500mg/kg p.o.)	115.0 ± 5.00***
IV	STZ-NZ +Clematis heynei (100 mg/kg p.o.)	135.0 ± 5.00
V	STZ-NZ +Clematis heynei (200 mg/kg p.o.)	122.5 ± 4.00**
VI	STZ-NZ +Solanum virginianum (100 mg/kg p.o.)	138.0 ± 5.00
VII	STZ-NZ +Solanum virginianum	125.5 ± 5.00**

	(200 mg/kg p.o.)	

Values are expressed as mean ± S.E.M. (n = 6).Values are statistically significant at $p<0.05$ (One-way ANOVA followed by Dunnett's test).

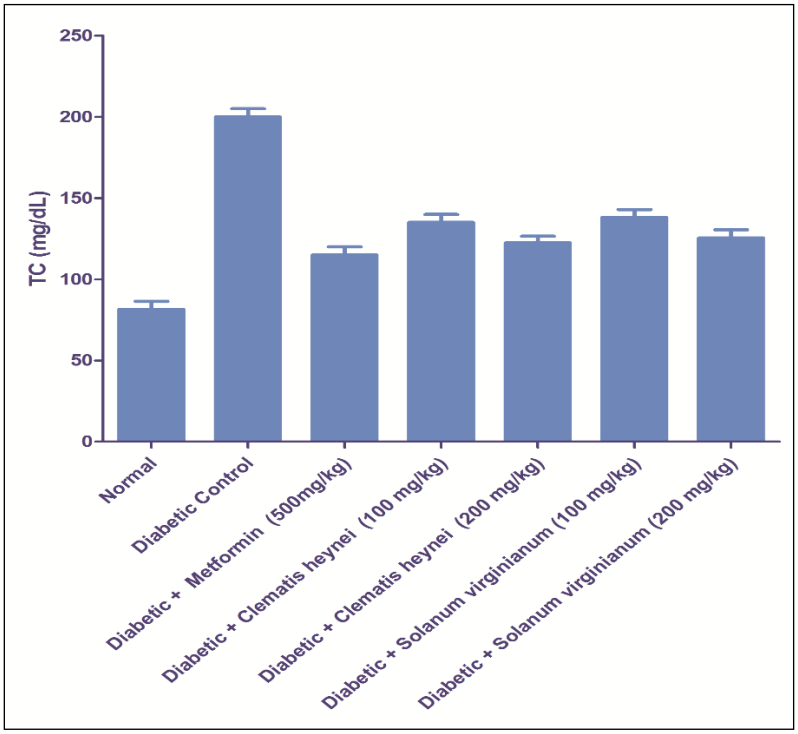

Figure 4: Effect of Clematis heynei and Solanum virginianum on total cholesterol level in STZ-Nicotinamide induced diabetic rats

In Clematis heynei and Solanum virginianum 100 and 200 mg/kg/p.o. (91.00 ± 7.00; 96.00 ± 7.00; 85.50 ± 5.50; 82.00 ± 6.00) treated group triglyceride decreased significantly ($p < 0.05$). In 500 mg/kg m 4etformin (80.00 ± 5.00) treated group triglyceride decreased significantly ($p < 0.05$), respectively as compared with control group (140.5 ± 6.00), as shown in Table no 4 and Figure.5

Table 4: Effect of Clematis heynei and Solanum virginianum on triglyceride level in STZ – Nicotinamide induced diabetic rats

Group	Treatments	Triglyceride (mg/dl)
I	Normal Control (Normal saline)	75.00 ± 8.00
II	Diabetic Control (Normal saline)	140.5 ± 6.00
III	STZ-NZ +Metformin (500mg/kg p.o.)	80.00 ± 5.00**
IV	STZ-NZ +Clematis heynei (100 mg/kg p.o.)	91.00 ± 7.00*

V	STZ-NZ +Clematis heynei (200 mg/kg p.o.)	85.50 ± 5.50*
VI	STZ-NZ +Solanum virginianum (100 mg/kg p.o.)	96.00 ± 7.00*
VII	STZ-NZ +Solanum virginianum (200 mg/kg p.o.)	82.00 ± 6.00**

Values are expressed as mean ± S.E.M. (n = 6). Values are statistically significant at $p<0.05$ (One-way ANOVA followed by Dunnett's test).

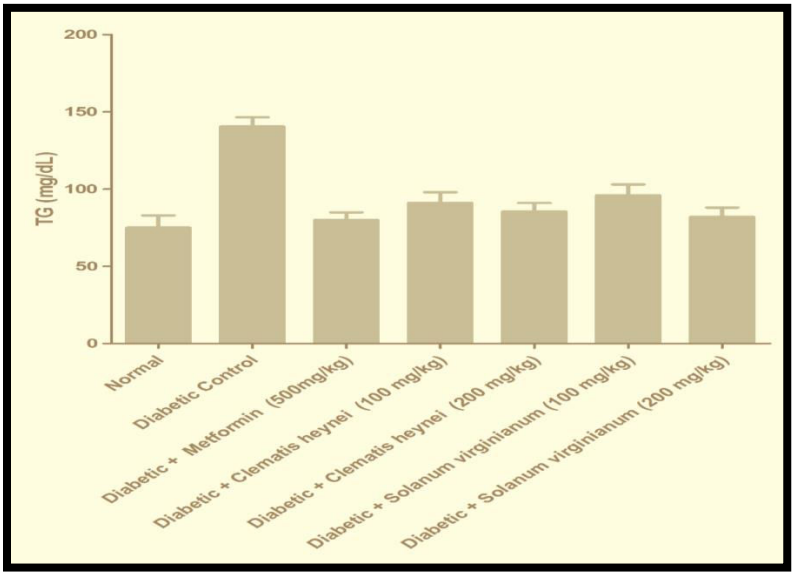

Figure 5: Effect of Clematis heynei and Solanum virginianum on triglyceride level in STZ-Nicotinamide induced diabetic rats

As shown in Table 5 and Figure 6, in Clematis heynei and Solanum virginianum 100 mg/kg (36.10±1.80; 38.10±1.50) treated group high density lipoprotein (HDL) increased significantly ($p < 0.05$), and Clematis heynei and Solanum virginianum 200 mg/kg (45.00±1.70; 47.00±1.30) treated group HDL also increased significantly ($p < 0.001$). In 500 mg/kg p.o. metformin (51.60±2.00) treated group HDL increased significantly ($p<0.001$), respectively as compared with control group (27.48±2.87).

Table 5: Effect of Clematis heynei and Solanum virginianum on HDL in STZ-Nicotinamide induced diabetic rats

Group	Treatments	HDL (mg/dl)
I	Normal Control (Normal saline)	51.87±1.13
II	Diabetic Control (Normal saline)	27.48±2.87
III	STZ-NZ +Metformin (500mg/kg p.o.)	51.60±2.00***

IV	STZ-NZ +Clematis heynei (100 mg/kg p.o.)	36.10±1.80*
V	STZ-NZ +Clematis heynei (200 mg/kg p.o.)	45.00±1.70**
VI	STZ-NZ +Solanum virginianum (100 mg/kg p.o.)	38.10±1.50*
VII	STZ-NZ +Solanum virginianum (200 mg/kg p.o.)	47.00±1.30**

Values are expressed as mean ± S.E.M. (n = 6).Values are statistically significant at $p<0.05$ (One-way ANOVA followed by Dunnett's test).

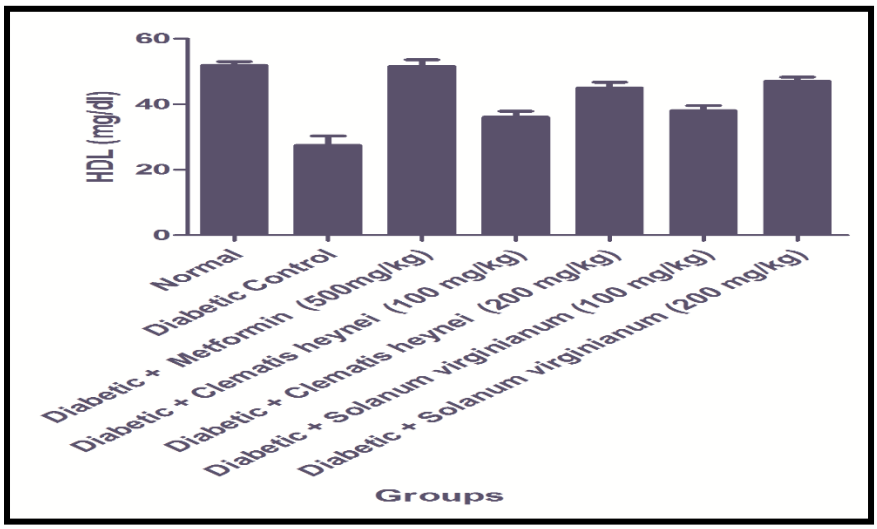

Figure 6: Effect of Clematis heynei and Solanum virginianum on HDL in STZ-Nicotinamide induced diabetic rats

As shown in Table 6 and Figure 7, in Clematis heynei and Solanum virginianum 100 mg/kg (97.00 ± 5.00; 96.50 ± 5.00) treated group total protein (TP) significantly decreased, and Clematis heynei and Solanum virginianum 200 mg/kg (86.00 ± 7.00; 87.00 ± 6.00) treated group TP also decreased significantly ($p<0.01$). In 500 mg/kg p.o. metformin (80.00 ± 9.00) treated group TP was significantly decreased ($p<0.001$), respectively as compared with control group (138.00± 6.00).

Table 6: Antidiabetic effect of Clematis heynei and Solanum virginianum on serum lipid profile i.e. total protein (TP) level in STZ-Nicotinamide induced diabetic rats

Group	Treatments	TP (g/dl)
I	Normal Control (Normal saline)	75.00± 8.00
II	Diabetic Control	138.00± 6.00

	(Normal saline)	
III	STZ-NZ +Metformin (500mg/kg p.o.)	80.00 ± 9.00***
IV	STZ-NZ +Clematis heynei (100 mg/kg p.o.)	97.00 ± 5.00
V	STZ-NZ +Clematis heynei (200 mg/kg p.o.)	86.00 ± 7.00**
VI	STZ-NZ +Solanum virginianum (100 mg/kg p.o.)	96.50 ± 5.00
VII	STZ-NZ +Solanum virginianum (200 mg/kg p.o.)	87.00 ± 6.00**

Values are expressed as mean ± S.E.M. (n = 6). Values are statistically significant at $p<0.05$ (One-way ANOVA followed by Dunnett's test).

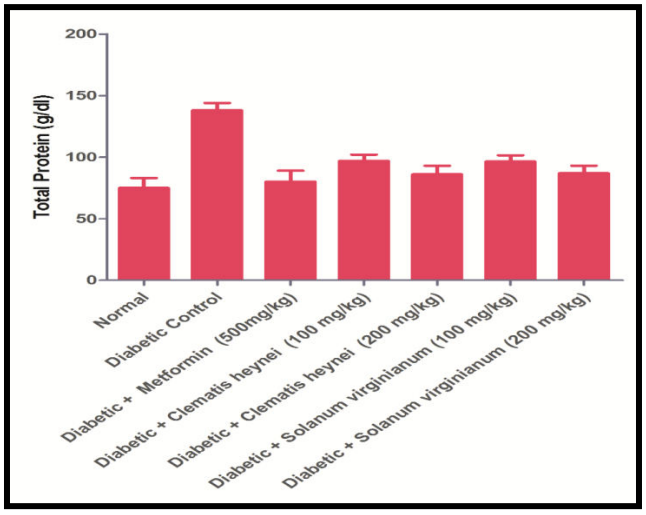

Figure 7: Effect of Clematis heynei and Solanum virginianum on TP in STZ-Nicotinamide induced diabetic rats

After end days of experiment, serum transaminase such as SGOT level was significantly ($p < 0.001$) elevated in diabetic control group. As shown in Table and Figure, in Clematis heynei and Solanum virginianum 100 mg/kg (78.50 ± 5.50; 79.00 ± 5.00) treated group SGOT significantly decreased, and Clematis heynei and Solanum virginianum 200 mg/kg (72.00 ± 5.00; 71.50 ± 5.50) treated group SGOT also decreased significantly ($p<0.01$). In 500 mg/kg p.o. metformin (68.00 ± 4.00) treated group SGOT was significantly decreased ($p<0.001$), respectively as compared with control group (120.5 ± 7.50) as shown in Table 7 and Figure 8

Table 7: Effect of Clematis heynei and Solanum virginianum on SGOT in STZ-Nicotinamide induced diabetic rats

Group	Treatments	SGOT (IU/L)
I	Normal Control (Normal saline)	57.00 ± 5.00

II	Diabetic Control (Normal saline)	120.5 ± 7.50
III	STZ-NZ +Metformin (500mg/kg p.o.)	68.00 ± 4.00**
IV	STZ-NZ +Clematis heynei (100 mg/kg p.o.)	78.50 ± 5.50*
V	STZ-NZ +Clematis heynei (200 mg/kg p.o.)	72.00 ± 5.00*
VI	STZ-NZ +Solanum virginianum (100 mg/kg p.o.)	79.00 ± 5.00*
VII	STZ-NZ +Solanum virginianum (200 mg/kg p.o.)	71.50 ± 5.50*

Values are expressed as mean ± S.E.M. (n = 6).Values are statistically significant at $p<0.05$ (One-way ANOVA followed by **Du**nnett's test).

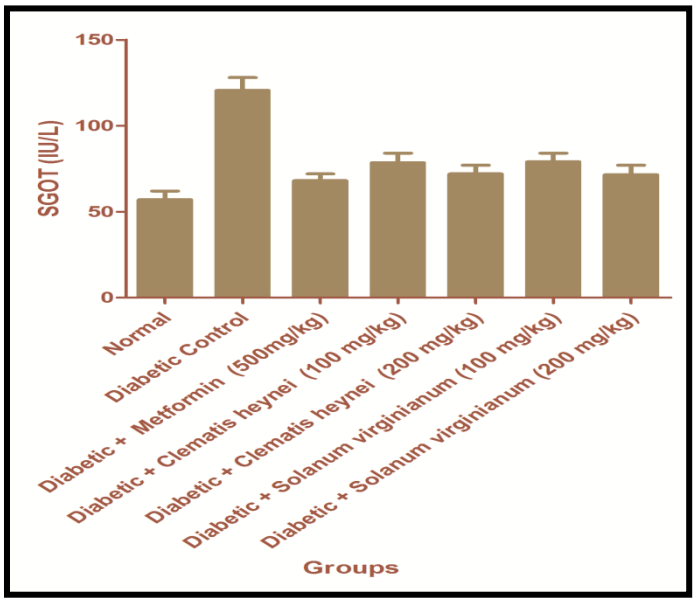

Figure 8: Effect of Clematis heynei and Solanum virginianum on SGOT in STZ-Nicotinamide induced diabetic rats

Similarly, at the end days of experiment the serum transaminase such as SGPT level was significantly ($p < 0.001$) elevated in diabetic control group. As shown in Table and Figure, in Clematis heynei and Solanum virginianum 100 mg/kg (70.00 ± 2.50; 69.50 ± 2.50) treated group SGPT significantly decreased, and Clematis heynei and Solanum virginianum 200 mg/kg (60.00 ± 5.00; 61.00 ± 6.00) treated group SGPT also decreased significantly ($p<0.01$). In 500 mg/kg p.o. metformin (55.00 ± 5.00) treated group SGPT was significantly decreased ($p<0.001$), respectively as compared with control group (118.0 ± 6.00). as shown in Table 8 and Figure 9

Table 8: Effect of Clematis heynei and Solanum virginianum on SGPT in STZ-Nicotinamide induced diabetic rats

Group	Treatments	SGPT (IU/L)
I	Normal Control (Normal saline)	45.00 ± 5.00
II	Diabetic Control (Normal saline)	118.0 ± 6.00
III	STZ-NZ +Metformin (500mg/kg p.o.)	55.00 ± 5.00**
IV	STZ-NZ +Clematis heynei (100 mg/kg p.o.)	70.00 ± 2.50*
V	STZ-NZ +Clematis heynei (200 mg/kg p.o.)	60.00 ± 5.00**
VI	STZ-NZ +Solanum virginianum (100 mg/kg p.o.)	69.50 ± 2.50*
VII	STZ-NZ +Solanum virginianum (200 mg/kg p.o.)	61.00 ± 6.00**

Values are expressed as mean ± S.E.M. (n = 6).Values are statistically significant at $p<0.05$ (One-way ANOVA followed by Dunnett's test).

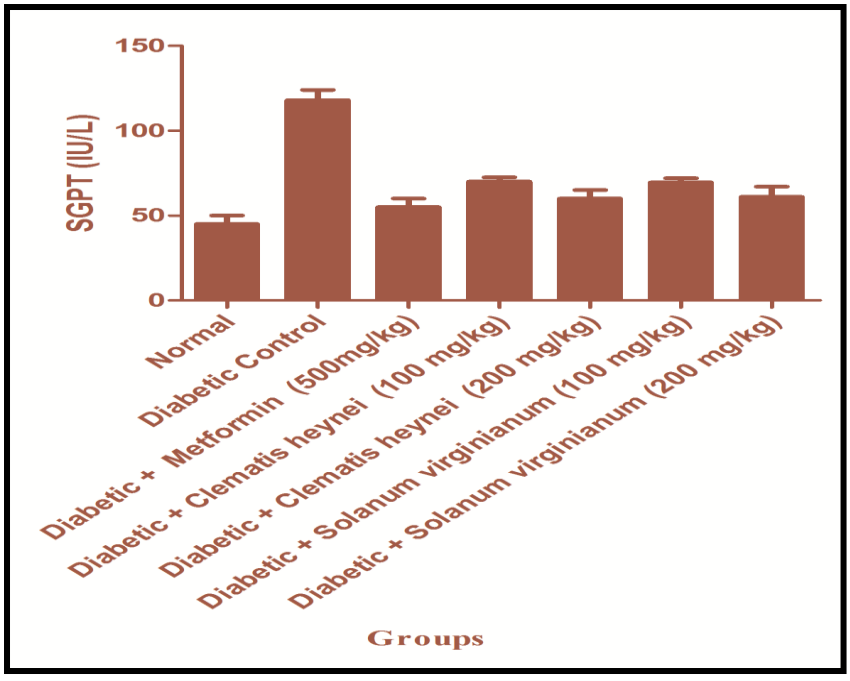

Figure 9: Effect of Clematis heynei and Solanum virginianum on SGPT in STZ-Nicotinamide induced diabetic rats

CONCLUSION:

Diabetes Mellitus (DM) is the metabolic disease with abnormal glucose homeostasis, due to defects in secretion or action of insulin. It has high prevalence globally and showed potentially

morbid conditions and has become prime medical concern. Animal models play a vital role in conceptualizing these types of diseases. Diabetes in experimental animals develops either by using surgical, chemical, genetic method or by spontaneously and illustrates numerous clinical features or associated phenotypes of the diseases. For induction of diabetes in the experimental animals, Streptozotocin (STZ) is a widely employed chemical. In the experimental animals Nicotinamide play a protective role against destruction of pancreatic- cells in STZ induced diabetes model. Other findings of the study recommended that the Clematis heynei and Solanum virginianum is also produced beneficial effects in complications of the diabetes mellitus and obesity. The present study findings indicated that the usefulness of the Clematis heynei and Solanum virginianum streptozotocin nicotinamide -induced diabetic rats. Our study suggested that Clematis heynei and Solanum virginianum dose-dependently produced antidiabetic activity. In this study might be helpful to understand the role of Clematis heynei and Solanum virginianum in the clinical treatment of Diabetes Mellitus.

The Clematis heynei and Solanum virginianum has also seems have potent hypolipidemic activity, which lowers the cholesterol, triglyceride, total proteins and increasing high-density lipoproteins levels. The mechanism has pointed towards, inhibiting cholesterol and triglyceride synthesis. Our study indicates the role of antioxidant activities of Clematis heynei and Solanum virginianum and active constituents play a role in preventing diabetic complications and antidiabetic activity of Clematis heynei and Solanum virginianum at 200 mg/kg of the dose was found to be more effective than 100 mg/kg of dose, its produced effects dose-dependently.

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