

Pharmacological Evaluation of Antidiabetic Activity of *Vitex Negundo* Linn. Leaves Extract and its Zinc Oxide Nanoparticles

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ABSTRACT

The present study was designed to evaluate and compare the antidiabetic potential of *Vitex negundo* Linn. leaves extract and its synthesized zinc oxide (ZnO) nanoparticles. *Vitex negundo*, a medicinal plant traditionally used for various therapeutic purposes, contains bioactive phytoconstituents that may contribute to glycemic control. The powdered leaves were subjected to extraction using suitable solvents, and ZnO nanoparticles were synthesized employing a green synthesis approach using the plant extract as a reducing and stabilizing agent. Characterization of nanoparticles was performed through UV-Visible spectroscopy to confirm their formation, functional groups, and morphology. Antidiabetic activity was assessed using streptozotocin -induced diabetic Wistar rats. Experimental animals were divided into normal, diabetic control, standard, plant extract-treated, and ZnO nanoparticles-treated groups. Parameters such as fasting blood glucose, body weight, lipid profile, and biochemical markers of oxidative stress were recorded. Both the plant extract and ZnO nanoparticles exhibited significant reductions in blood glucose levels and improved lipid profiles when compared with the diabetic group. Notably, the ZnO nanoparticle formulation demonstrated a more pronounced hypoglycemic effect, suggesting enhanced bioavailability and synergistic action between phytochemicals and zinc ions. The study concludes that *Vitex negundo* leaves extract possesses remarkable antidiabetic effects, which are further amplified when formulated as ZnO nanoparticles. These findings support the potential application of green-synthesized ZnO nanoparticles as a novel and effective therapeutic option in the management of diabetes mellitus.

Keywords: *Vitex negundo*, Antidiabetic activity, Zinc oxide nanoparticles, Streptozotocin, Phytomedicine, Green synthesis

INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both.¹ The level of hyperglycaemia associated diabetes increases the risk of microvascular damage (retinopathy, nephropathy and neuropathy). It is associated with reduced life expectancy, significant morbidity due to the related microvascular complications, increased risk of macrovascular complications (ischaemic heart disease, stroke and peripheral vascular disease), and diminished quality of life. ² Glucose blood levels are maintained by insulin which is a hormone released from the pancreas. When these level increases, insulin is produced from the pancreas and maintained the level of glucose. In diabetic patients, the production

of insulin is absent or less which causes hyperglycemia³

1.1 Types of Diabetes Mellitus:

There are mainly two major types of diabetes mellitus.

Type 1: It is also called as Insulin dependent Diabetes Mellitus (IDDM). It is due to failure of body for insulin production. It is childhood disease so it is called as juvenile onset diabetes mellitus ⁴

Type 2: It is also called as Non – Insulin Dependent diabetes Mellitus (NIDDM). In this type of cells are unable for insulin usage. The other name for this type is adult onset diabetes mellitus⁵

1.2 Nanotechnology in Herbal Medicine

Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.



The goal of the applied science and technology discipline of nanotechnology is to create tools and dosage forms that are between 1 and 100 nm in size. The use of nanotechnology in biological disease treatment, diagnosis, monitoring, and control⁶ Recently, systems have been called nanomedicine. Lipids, polysaccharides, and synthetic biodegradable polymers are among the safe components used to create the nanocarriers.⁷ The creation of instruments and dosage forms with sizes ranging from 1 to 100 nm is the aim of the applied science and technology field of nanotechnology. Treatment, diagnosis, monitoring, and management of biological diseases using nanotechnology better distribution of tissue macrophages, prolonged administration, defense against chemical and physical deterioration, etc. Therefore, there may be a future for improving the activity and resolving issues with plant medicines using nanoscale drug delivery systems for herbal medications. In order to combat more chronic diseases including cancer, diabetes, asthma, and others, it is imperative that nanocarriers be incorporated into the regular medical system as an NDDS ⁸.

MATERIAL AND METHOD

2.2 Collection and authentication of Vitex Negunda Linn.

Vitex Negunda Linn. plant were collected from Thiruvalla, Kerala. The plant material was identified and authenticated by Mrs. A. M. Gaharwar, Assistant Professor of Vasantrao Naik College of Agricultural Biotechnology, Yavatmal.

2.3 Experimental Animals

Healthy male Sprague dawley rats approximately 8 weeks of age weighing about 200 to 210 gm used for the pharmacological screening. The animals were purchased from NIN Hyderabad. The animals were housed in polypropylene cages with wire and mesh top and husk bedding and maintained under the standard environmental conditions (25 ± 20 °C, relative humidity 5 % light dark cycle for 12 hours each) and the fed with the standard pellet diet and water were used for the entire study. Animal study protocol was duly approved by IAEC and P. Wadhwani college of Pharmacy, Yavatmal with research project

No.650/PO/Re/S/2002/CPCSEA/2025/14. The rats were treated according to the rule and regulation of IAEC and CPCSEA.

2.3 Procurement of diagnostics kits and chemicals

Diagnostics kits for estimation of glucose, urea, creatinine, triglyceride and cholesterol were obtained from Ambika diagnostics. Streptozotocin was obtained from M.P. Biomedical, Glibenclamide was generously obtained from Cipla Pvt Ltd, Mumbai. All other chemicals used were of laboratory grade.

METHODS:

Extraction

The leaves of Vitex Negunda Linn. were collected, dried in shade and coarsely powdered. The powdered leaves were defatted with petroleum ether and then subjected to maceration with methanol.⁹

2.5 Phytochemical Screening¹⁰

Test for Carbohydrates

Dissolve 2 gm extract in 5 ml distilled water and filter it. The filtrates were used to test for the presence of carbohydrates.

Molish test

A few drops of the extract was treated with molish reagent (Alpha – naphthol in alcohol) and a drop of concentrated sulphuric acid was added. Purple color was obtained. This confirms the presence of Carbohydrates.¹¹

Test for Alkaloids Hager's test

Filtrate was treated with saturated aqueous solution of picric acid. Presence of alkaloids were confirmed by the formation of yellow coloured precipitate.¹²

Test for saponin Foam test

Small quantity of the extract was shaken with 2 ml of water. Persistence of foam produced for 10 min indicated the presence of saponins.

Test for tannin

Take 0.5 gm of the dried powdered plant. Boil 0.5 gm sample in 20 ml of water in a test tube. Filter the above mixture. Add few drops of 0.1% ferric chloride. Development of brownish green color or blue-black coloration indicates presence of tannin.¹³

Test for terpenoids Salkowski Test:

Mix 2 ml of chloroform to extract solution carefully added con. H₂SO₄ to form a layer. A reddish brown colouration of the interface indicated the presence of terpenoids.¹⁴

Test for Glycosides Keller Killani Test

To 50 mg of each extracts, 2 ml of glacial acetic acid, 1 ml FeCl₃ solution were added, heated and the cooled. This was transferred to a test tube containing 2 ml conc. H₂SO₄ and observed.

Test for steroids Salkowski Test

To 100 mg of each extracts, 2 ml of CHCl₃, 2 ml of conc. H₂SO₄ was added, mixed thoroughly and both the layers were observed for colour.¹⁵

Test for phenol Ferric chloride test

Treat the extract with 3-4 drops of ferric chloride solution. Formulation of bluish black colour indicated the presence of phenols.

Test for Amino acid Ninhydrin Test

Add ninhydrin reagent to the test solution and boiled for few minutes. Formation of blue colour indicated the presence of amino acids.¹⁶

Test for flavonoids Ferric chloride test

Add a few drops of ferric chloride solution to the extract solution. Development of intense green colour indicates the presence of flavonoids.

Lead acetate test

Treat the extract with few drops of lead acetate solution. Formation of yellow precipitate indicate presence of flavonoid.¹⁷

Anthraquinone

1 ml of filtrate + 10 ml Benzene filter and 5 ml of ammonia to the filtrate and shake well gives pinkish colour.¹⁶

2.5 Synthesis of Zinc Oxide Nanoparticles

This synthesis method involved dissolving 11.5 g of zinc sulfate in 40 mL of water and thoroughly mixing the mixture using a magnetic stirrer. Using a sterile injection, 10 mL of vitex negundo linn. leaf extract was injected to this combination drop by drop. For at least ten minutes, the resultant liquid was constantly mixed. Drop by drop, sodium hydroxide solution (2M) was added to the solution above. The mixture was then repeatedly cleaned with distilled water, centrifuged for 15 minutes at 3000 rpm, and dried in a hot air oven at 60 °C. A meticulously dried zinc oxide Nano powder was obtained. To produce the anticipated fine zinc oxide nanoparticles, the powder was ground into a fine powder using a mortar and pestle. Likewise, zinc oxide nanoparticles were prepared using 11.8 g of zinc nitrate and 8.8 g of zinc acetate dihydrate. To conduct the additional optical and structural characterizations, the zinc oxide nanoparticles were transferred into a sterile vial.¹⁷

2.6 Experimental Designs

The animals were grouped into seven following groups as follows (n=6)

Group-I (Normal Control Group): - Animals were treated with normal saline solution. Group-II (Negative Control): - Animals were treated with STZ (60 mg/kg)

Group-III (STZ + MEVN): - Diabetes rats were treated with methanolic extract of Vitex Negunda Linn. (400mg/kg)

Group IV (STZ + NPMEVN): - Diabetes rats were treated with nanoparticles of extract of Vitex Negunda Linn. (300 mg/kg)

Group V (STZ + Glibenclamide):- Diabetes rats were treated with standard drug (5mg/kg)

Dose Selection

Dose of Vitex Negunda Linn. was selected as per the dose mentioned in the research article by Most.

Fatimatuz Zahura Falguni. (Most. Fatimatuz Zahura Falguni et. al. 2017).

triglycerides, cholesterol and creatinine was estimated by semi- automated electronic bioanalyser¹⁷

2.7 Induction of Diabetes in rats by Streptozotocin

The animals were fasted overnight and diabetes was induced by intraperitoneal injection of STZ at dose of 60 mg/kg. (S.K. Prasad, Alka Kulshreshtha et, al.) STZ was first weighed individually for each animal according to the body weight and solubilized with 0.1 M Sodium citrate buffer pH 4.5. It was then injected. 5 % dextrose was provided for 24 hours to inhibit hypoglycemia. Administration of single dose of STZ in rats results in hyperglycemia within 72 hours.

Sample Collection

Blood samples were collected by Retro orbital plexus puncture method and blood glucose levels, urea,

2.7 Estimation of Biochemical Parameters

1) Blood Glucose Principle:

Glucose is oxidized into the gluconic acid and Hydrogen peroxide. The hydrogen peroxide further reacts with phenol and 4- amino antipyrine by the catalytic action of peroxidase to form a red coloured quinoamione dye complex. (Trinder P. and LevinsonS):

•The serum sample was separated from blood sample of rats by centrifugation.

The supernants clear serum sample is used for the testing is given below.

Sample	Blank	Standard	Test
Enzyme reagent	1.0 ml	1.0 ml	1.0 ml
Standard	-	10 µl	-
Serum	-	-	10 µl

- Test sample was prepared by taking 1 ml of enzyme reagent and 10 µl of serum sample.
- The blank sample was prepared by taking 1 ml of enzyme reagent.
- The standard sample was prepared by taking 1 ml of enzyme reagent and 10 µl of standard.
- All samples were mixed and incubated at 37°C for 10 min and the absorbance of standard and test were read against distilled water blank at 505 nm¹⁸

Formula:

2) Cholesterol:

$$\text{Glucose} = \frac{\text{Abs. of test} - \text{Abs. of blank}}{\text{Abs. of Std} - \text{Abs. of blank}} \times 100$$

Sample	Blank	Standard	Test
Enzyme reagent	1.0 ml	1.0 ml	1.0 ml
Standard	-	0.01 ml	-
Serum	-	-	0.01 ml

- Test sample was prepared by taking 1 ml of enzyme reagent and 0.01 ml of serum sample.

Indicator of liver function, biliary function, intestinal absorption, propensity towards coronary artery disease and adrenal disease. Decreased level of HDL cholesterol is associate with increased risk of coronary artery disease and other atherosclerotic complications.¹⁹ On addition of precipitating reagent to the serum lipoprotein precipitant out and the HDL fractions remains in the supernant, which is determine by above reactions.

Procedure:

- The serum sample was separated from blood sample of rats by centrifugation.
- The supernants clear serum sample is used for the testing is given below.

- The blank sample was prepared by taking 1 ml of enzyme reagent.

- The standard sample was prepared by taking 1 ml of enzyme reagent and 0.01 ml of standard.
- All samples were mixed and incubated at 37°C for 10 min and the absorbance of standard and test were read against distilled water blank at 505 nm using green filter.

Formula:

$$\text{Total cholesterol} = \frac{\text{Abs. of test} - \text{Abs. of blank}}{\text{Abs. of Std.} - \text{Abs. of blank}} \times 200$$

3) Triglycerides

	Blank	Standard	Test
Enzyme reagent	1.0 ml	1.0 ml	1.0 ml
Standard	-	10 µl	-
Serum	-	-	10 µl

- Test sample was prepared by taking 1 ml of enzyme reagent and 10 µl of serum sample.
- The blank sample was prepared by taking 1 ml of enzyme reagent.
- The standard sample was prepared by taking 1 ml of enzyme reagent and 10 µl of standard.

Elevated level of triglyceride in the blood have identified as risk factors related to coronary heart disease. Elevated level of triglyceride are found in atherosclerosis, diabetes mellitus, biliary obstruction and other metabolic disorder associate with endocrine disturbance

Procedure:

- The serum sample was separated from blood sample of rats by centrifugation. The supernatant clear serum sample is used for the testing is given below:

- All samples were mixed and incubated at 37°C for 10 min and the absorbance of standard and test were read against distilled water blank at 546 nm using green filter.²¹

Formula:

$$\text{Triglyceride} = \frac{|\text{Abs. of test} - \text{Abs. of Blank}|}{\text{Abs. of Std.} - \text{Abs. of Blank}} \times 200$$

Statistical Analysis

The data was statistically analyzed by one-way analysis of variance (ANOVA) followed by Dunnett's comparison test with equal size and Student t-test was compared with unpaired groups. The difference was considered significant when p-values < 0.05*. All the

values are expressed as mean ± standard deviation (S.D).

RESULTS

Preliminary Phytochemical Studies: -

Table 7.1. Phytochemical Screening of Methanolic and Aqueous Plant Extract of Vitex Negunda.

Sr. No	Phytoconstituents	Tests	Vitex Negunda Methanolic Extract
1.	Test for Alkaloids	Hager's Reagent	+
2.	Test for Carbohydrates	Molisch Test	+
3.	Test for Glycosides	Keller Killani Test	+
4.	Test for Amino acid	Ninhydrin Test	—
5.	Test for Tannins	Ferric chloride Test Lead acetate Test	+
6.	Test for Steroids	Salkowski Test	+
7.	Test for Phenols	Ferric Chloride Test	+

8.	Test for Flavonoids	Ferric Chloride Test Lead Acetate Test	+
9.	Test for Terpenoids	Salkowski Test	+
10.	Test for Saponins	Foam Test	+
11.	Test for Anthraquinone	—	—

(+ Present, - Absent)

Evaluation of zinc oxide nanoparticles of vitex negundo linn. extract

Visual Examination: The produced zinc oxide nanoparticles' conformation is verified visually. Vitex negundo linn. extract nanoparticles' color variations were noted.

UV- Visible Spectroscopy: UV spectroscopy is used to determine the structure of produced vitex negundo nanoparticles. The optical properties of the produced Vitex negundo linn. ZnO NPs were assessed by dissolving a tiny amount of nanopowder in roughly 10 milliliters of de- ionized water, scanning the UV spectrophotometer between 300 and 700 nm, and measuring the absorbance. The 300–400 nm range was where the peak was seen. Nanoparticle formation was verified.

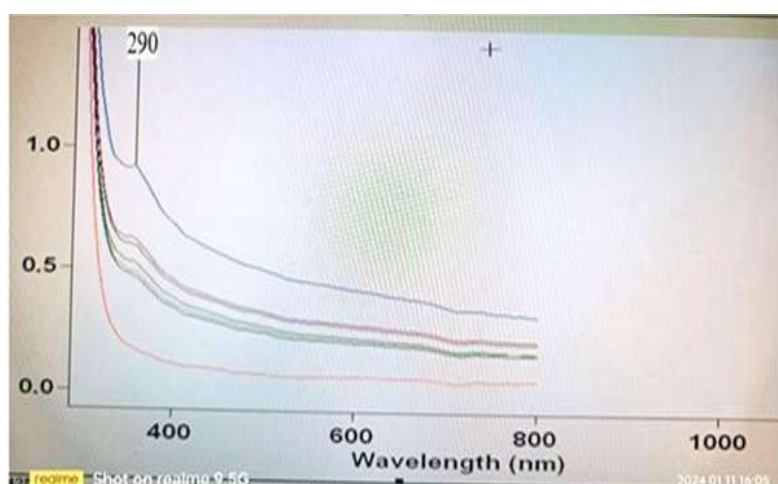


Fig.2.1. Zinc oxide nanoparticle peak was observed on 290 nm

Assessment for Diabetic Parameters

Body Weight

Table No. 2.2. Effect of Vitex Negunda on body weight in streptozotocin induced diabetic rats

Groups	Body weight			
	Days			
	Day 0	Day 3	Day 14	Day 28
Normal control	258.0 ± 6	261.0 ± 3.6	260.0 ± 4.16	262.0 ± 3.2
Negative control	259.01 ± 2.8 ns	262.01 ± 2.8ns	215.80±5.80@	191.0±11.0@
STZ + MEVN	258.0 ± 4.12 ns	258.0 ± 4.12 ns	254.24±4.24**	252.24±3.54**
STZ + NPMEVN	257.5 ± 7.5 ns	257.5 ± 7.5 ns	255.35±5.35**	251.24±4.52**
STZ + Glibenclamide	257.5 ± 2.5 ns	257.5 ± 2.5 ns	258.80±6.40**	252.24±4.24**

Results are expressed as Mean + SD (n= 6)

@ p< 0.01 Compared with normal control group,
**p< 0.01 Compared with negative control group,
nsp>0.05 compared with negative control group

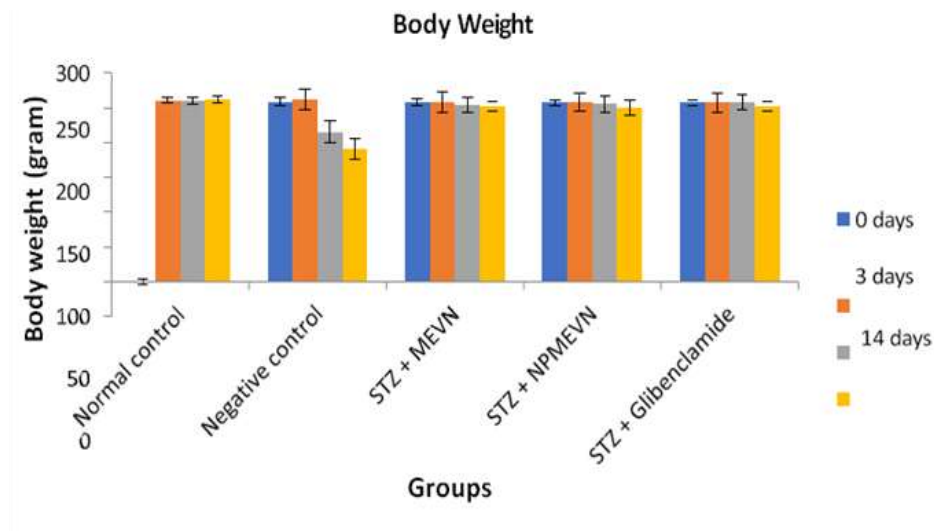


Fig.2.2. Effect of Vitex Negunda on body weight in streptozotocin induced diabetic rats

Blood glucose level

Table No.2.3. Effect of Vitex Negunda on blood glucose level in streptozotocin induced diabetic rats

Groups	Blood glucose (mg /dl)			
	Days			
	Day 0	Day 3	Day 14	Day 28
Normal control	97.37±3.6	97.28±2.22	98.54±5.7	96.11±2.8
Negative control	96.67±3.34	256.91±7.67@	265.35±7.36@	275.67±7.84@
STZ + MEVN	97.28±2.22	258.67±4.26ns	196.04±1.34**	191.23±2.39**
STZ + NPMEVN	99.38±4.32	249.77±5.82ns	194.84±1.2**	192.21±1.27**
STZ + Glibenclamide	96.25±4.57	260.94±7.54ns	146.31±5.33**	107.6±4.76**

Results are expressed as Mean + SD (n= 6)

@ p< 0.01 Compared with normal control group,

**p< 0.01 Compared with negative control group,

nsp>0.05 compared with negative control group

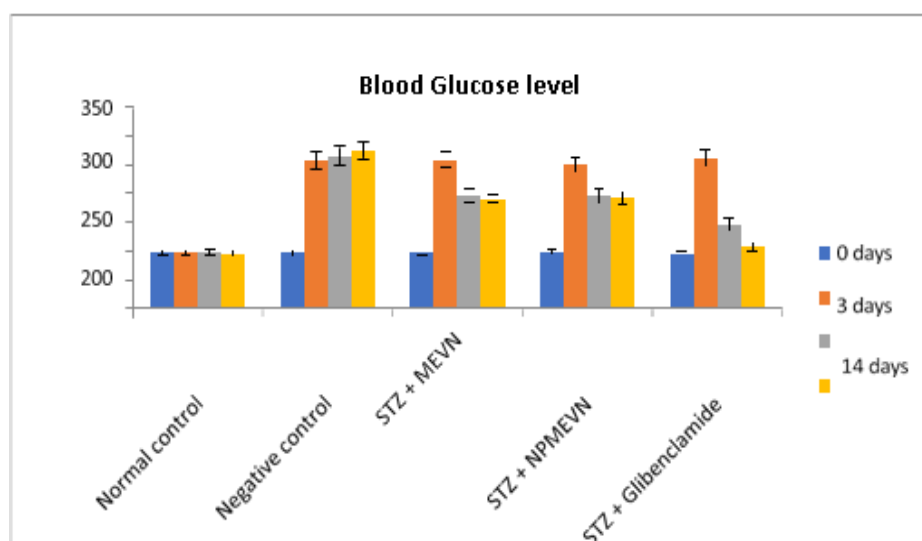


Fig.2.3. Effect of Vitex Negunda on blood glucose levels in streptozotocin induced diabetic rats

Table 2.4. Effect of Vitex Negunda on Serum Triglyceride level in streptozotocin induced diabetic rats

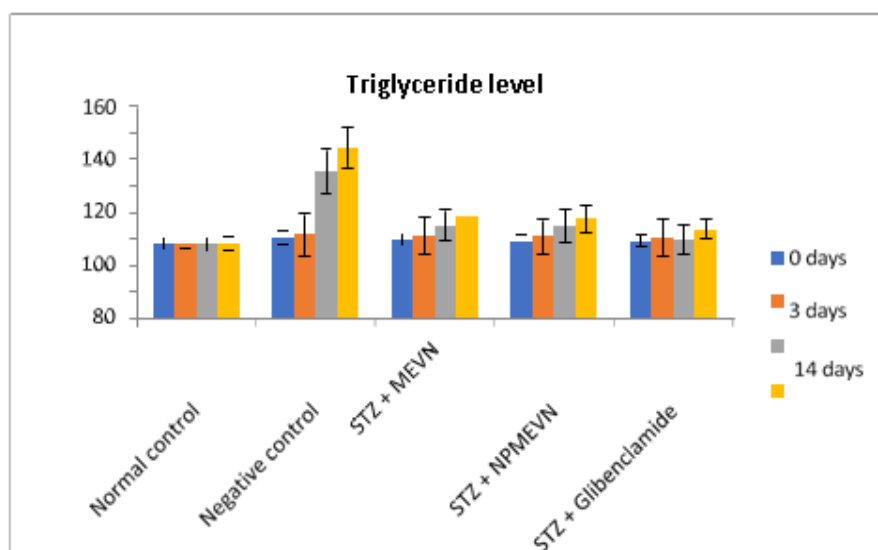
Groups	Serum Triglyceride (mg/dl)			
	Days			
	Day 0	Day 3	Day 14	Day 28
Normal control	56.54±1.6	57.13±1.84	55.72±1.26	56.41±1.27
Negative control	60.6±3.95	63.22±2.33 ^{ns}	111.25±2.27 [@]	129.15±2.17 [@]
STZ + MEVN	59.57±4.26	62.69±2.6 ^{ns}	70.25±1.55 ^{**}	77.2±1.62 ^{**}
STZ + NPMEVN	58.61±2.28	61.63±3.67 ^{ns}	69.76±3.4 ^{**}	75.13±1.02 ^{**}
STZ + Glibenclamide	58.56±2.67	61.42±1.5 ^{ns}	59.56±1.09 ^{**}	67.32±1.73 ^{**}

Results are expressed as Mean + SD (n= 6)

@ p< 0.01 Compared with normal control group,

**p< 0.01 Compared with negative control group,

nsp>0.05 compared with negative control group

**Fig. 2.4. Effect of Vitex Negunda on Serum Triglyceride level in streptozotocin induced diabetic rat**

Serum Cholesterol

Table 2.5 Effect of Vitex Negunda on Serum Cholesterol level in streptozotocin induced diabetic rats

Groups	Serum Cholesterol (mg/dl)			
	Days			
	Day 0	Day 3	Day 14	Day 28
Normal control	152.48±3.75	153.04±2.14	154.99±3.63	155.64±2.1
Negative control	149.59±1.23	151.41±1.44 ^{ns}	201.41±1.81 [@]	252.1±4.29 [@]
STZ + MEVN	148.77±1.09	150.37±1.91 ^{ns}	179.08±2.03 ^{**}	194.89±1.04 ^{**}
STZ + NPMEVN	150.4±1.51	150.82±1.98 ^{ns}	176.51±1.44 ^{**}	196.13±2.11 ^{**}
STZ + Glibenclamide	148.41±2.49	149.75±3.97 ^{ns}	159.47±2.94 ^{**}	162.69±1.98 ^{**}

Results are expressed as Mean + SD (n= 6)

@ $p < 0.01$ Compared with normal control group,
 ** $p < 0.01$ Compared with negative control group,
 nsp >0.05 compared with negative control group

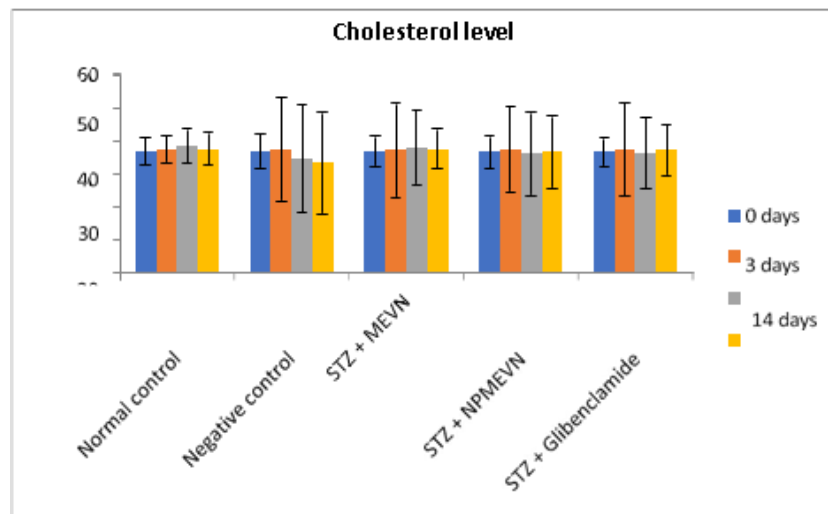


Fig 2.5. Effect of Vitex Negunda on Serum Cholesterol level in streptozotocin induced diabetic rats

HDL Level

Table 2.6. Effect of Vitex Negunda on HDL level in streptozotocin induced diabetic rats

Groups	HDL (mg/dl)			
	Days			
	Day 0	Day 3	Day 14	Day 28
Normal control	36.83 $\pm$ 0.75	37.33 $\pm$ 0.81	38.16 $\pm$ 0.75	37.36 $\pm$ 0.75
Negative control	36.92 $\pm$ 0.78	37.37 $\pm$ 0.82	34.33 $\pm$ 0.51@	33.16 $\pm$ 0.75@
STZ + MEVN	36.72 $\pm$ 0.69	36.99 $\pm$ 0.78	37.96 $\pm$ 0.75**	37.34 $\pm$ 0.51 **
STZ + NPMEVN	36.42 $\pm$ 0.62	37.45 $\pm$ 0.85	35.95 $\pm$ 0.62 **	36.46 $\pm$ 0.64**
STZ + Glibenclamide	36.47 $\pm$ 0.64	37.29 $\pm$ 0.80	36.34 $\pm$ 0.69 **	37.10 $\pm$ 0.69**

Results are expressed as Mean + SD (n= 6)

LDL Level

@ $p < 0.01$ Compared with normal control group,
 ** $p < 0.01$ Compared with negative control group,
 nsp >0.05 compared with negative control group

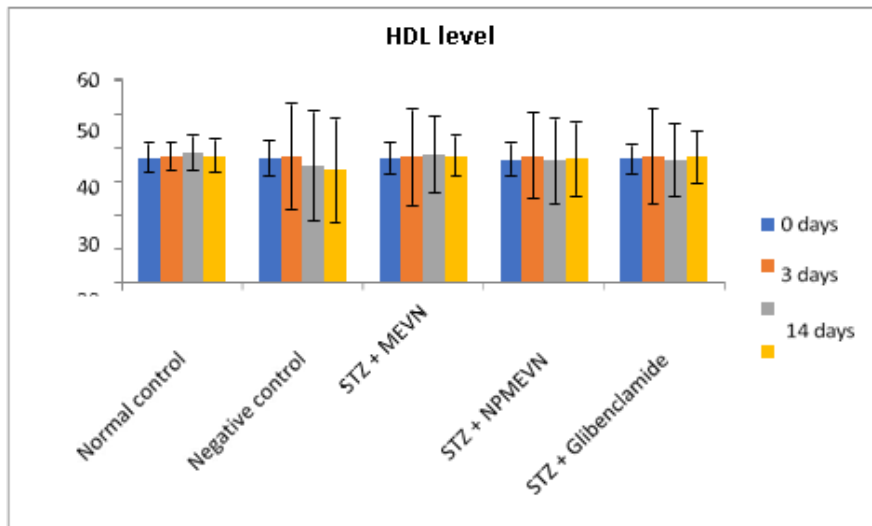


Fig 2.6. Effect of Vitex Negunda on HDL level in streptozotocin induced diabetic rats

Table 2.7. Effect of Vitex Negunda on LDL level in streptozotocin induced diabetic rats

Groups	LDL (mg/dl)			
	Days			
	Day 0	Day 3	Day 14	Day 28
Normal control	25.23 ± 0.63	24.43 ± 0.79	23.43 ± 0.77	23.60 ± 0.67
Negativecontrol	24.94 ± 0.72	24.14 ± 0.59	34.06 ± 0.97@	74.83 ± 1.28@
STZ + MEVN	25.04 ± 0.60	24.44 ± 0.81	23.83 ± 0.64 **	26.83 ± 0.46**
STZ + NPMEVN	24.85 ± 0.67	24.95 ± 0.87	26.10 ± 0.71 **	37.53 ± 0.71**
STZ + Glibenclamide	25.05 ± 0.61	25.05 ± 0.88	24.23 ± 0.68**	31.93 ± 0.78**

Results are expressed as Mean + SD (n= 6)

@ p< 0.01 Compared with normal control group,
 **p< 0.01 Compared with negative control group,
 nsp>0.05 compared with negative control group

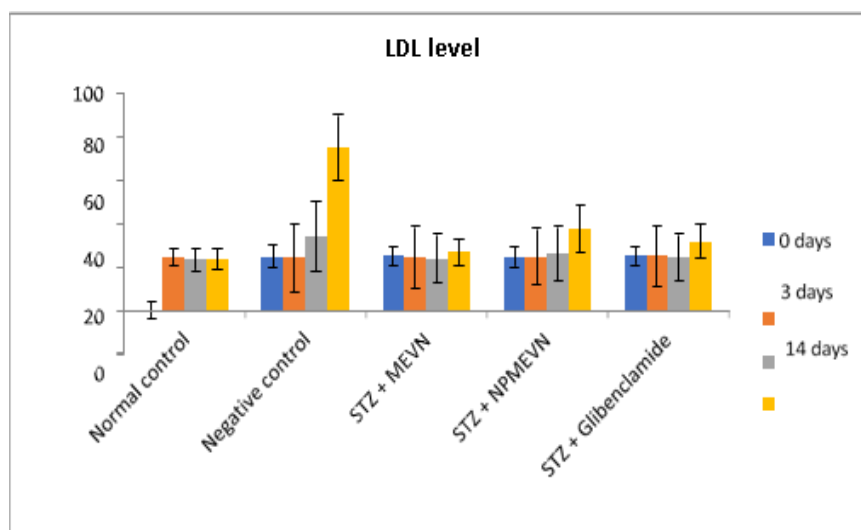


Fig. 2.7. Effect of Vitex Negunda on LDL level in streptozotocin induced diabetic rats

Table 2.2 and Figure 2.2 shows the effect of *Vitex negunda* on body weight in STZ induced diabetic rats. There was significant ($p < 0.01$) decrease in body weight in negative control group as compared with the normal control on 14th and 28th. There was significant increase ($p < 0.01$) in body weight of STZ + MEVN, STZ + NPMEVN and STZ + Glibenclamide treated groups as compared to negative control on 14th and 28th. Table 2.3 and Figure 2.3 reveals the effect of *Vitex negunda* on blood glucose level of the rats. There was significant increase ($p < 0.01$) in the blood glucose level in negative control group compared to normal control rats on 3th, 14th, 28th day. STZ + MEVN, STZ + NPMEVN and STZ + Glibenclamide groups showed significant decrease in ($p < 0.01$) in the blood glucose level compared to negative control group. Table 2.4 and Figure 2.4 reveals the effect of *Vitex negunda* on triglyceride level of the rats. There was significant increase ($p < 0.01$) in the triglyceride level in negative control group compared to normal control rats on 14th and 28th. There was significant decrease ($p < 0.01$) in + Glibenclamide treated groups as compared to negative control on 14th and 28th. Table 2.5 and Figure 2.5 reveals the effect of *Vitex negunda* on cholesterol level of the rats. There was significant increase ($p < 0.01$) in the triglyceride level in negative control group compared to normal control rats on 14th and 28th. There was significant decrease ($p < 0.01$) in triglyceride level of STZ + MEVN, STZ + NPMEVN and STZ + Glibenclamide treated groups as compared to negative control on 14th and 28th. Table 2.6 and Figure 2.6 reveals the effect of *Vitex negunda* on HDL level of the rats. There was significant decrease ($p < 0.01$) in the triglyceride level in negative control group compared to normal control rats on 14th and 28th. There was significant increase ($p < 0.01$) in HDL level of STZ + MEVN, STZ + NPMEVN and STZ + Glibenclamide treated groups as compared to negative control on 14th and 28th. Table 2.7 and Figure 2.7 reveals the effect of *Vitex negunda* on LDL level of the rats. There was significant increase ($p < 0.01$) in the triglyceride level in negative control group compared to normal control rats on 14th and 28th. There was significant decrease ($p < 0.01$) in LDL level of STZ + MEVN, STZ + NPMEVN and STZ + Glibenclamide treated groups as compared to negative control on 14th and 28th.

DISCUSSION

Hyperglycemia, whether during fasting or after meals, is referred to as diabetes. Diabetes mellitus's (DM) persistent hyperglycemia is linked to end damage, malfunction, and failure of several organs and tissues, such as the kidney, heart, blood vessels, neurons, and retina.²³ the International Diabetes Federation (IDF) projects that 366 million people worldwide had diabetes mellitus in 2011, and by 2030, that number is predicted to increase to 552 million. Diabetes is a complicated, long-term illness that necessitates ongoing medical attention and multifaceted risk-reduction techniques in addition to glucose control. In order to empower individuals, prevent acute problems, and lower the risk of long-term complications, ongoing diabetes self- management education and assistance are essential. There is substantial evidence to support a variety of therapies aimed at improving the outcomes of diabetes.²⁴ 8.5% of persons over the age of 18 had diabetes in 2014. Diabetes was the direct cause of 1.5 million fatalities in 2019, and 48% of all diabetes-related deaths happened in people under 70. Diabetes contributed to an additional 460 000 deaths from kidney disease, and elevated blood glucose is responsible for 20% of cardiovascular fatalities. Age-standardized death rates from diabetes increased by 3% between 2000 and 2019.²⁵ The death rate from diabetes rose by 13% in lower-middle-income nations. In contrast, the likelihood of dying between the ages of 30 and 70 from any of the four major noncommunicable diseases—diabetes, cancer, chronic respiratory conditions, or cardiovascular diseases—decreased by 22% worldwide between 2000 and 2019.²⁶ The woody plant known as *Vitex negundo* Linn. is primarily found in the Indian subcontinent and its neighboring countries. *Vitex negundo* is a component of several commercially available herbal formulations and has demonstrated an effective biocontrol tool. The application of biotechnologically advanced procedures would provide resources of rapid proliferation and, from the perspective of phytochemistry, it provides opportunities for augmentation of the quality and quantity of the biologically potent secondary metabolites occurring in the plant.²⁷ components present in *Vitex negundo* Linn. that exhibit antidiabetic properties.²⁷ Presently, an effort was made to look into the traditional use of

zinc oxide nanoparticles and the alcoholic extract of the plant *Vitex negundo* Linn. for an antidiabetic effect from a scientific standpoint. Plant biomolecular agents were reduced to create the NPs. The production process for ZnO NPs was demonstrated by the color shift. The crystalline structure of the generated NPs has been confirmed by XRD examination. When metallic ions were converted into nanoparticles, the presence of phytochemicals was assessed using FTIR. SEM spectra were used to establish morphology. When the generated ZnO NPs were examined using UV-Vis spectroscopy, a peak of the *Tridax procumbens* linn. extract was seen at 380 nm. Compared to plant extract alone, the generated ZnO has stronger antidiabetic efficacy 28. Similar color changes in ZnO NPs in our work served as an indicator of the synthesis process. ZnO NPs were found in the plant extract of *Vitex negundo* Linn., according to the UV-Vis research, which also revealed a peak at 290 nm²⁹. In the majority of ethnopharmacology studies published between 1996 and 2006, chemical models were employed to induce diabetes. Streptozotocin (STZ) and alloxan (ALX) are the two most often used drugs, and this model has been useful in the study of various facets of the disease. Both drugs induce diabetes when administered parenterally (subcutaneously, intraperitoneally, or intravenous²⁹). The cytotoxic action of alloxan is mediated by reactive oxygen species. When alloxan and dialuric acid, a consequence of its reduction, come together to form a redox cycle, superoxide radicals are produced. When these radicals are dismutated, hydrogen peroxide is created. Highly reactive hydroxyl radicals are another byproduct of the Fenton reaction. Blood sugar levels rise as a result of the quick breakdown of beta cells brought on by reactive oxygen species. A significant increase in the cytosolic calcium concentration occurs along with this 30. ALX has a number of disadvantages since the percentage incidence of diabetes fluctuates greatly and is unrelated to greater dosages of the medication. Furthermore, ketosis is linked to a significant mortality rate. Early reversal of hyperglycemia is common in animals treated with ALX undergoing pancreatic regeneration. Because of these disadvantages, STZ has almost entirely supplanted ALX in the development of diabetes in test animals. Streptozotocin, an antibiotic derived from *Streptomyces achromogenes*, is structurally a

glucosamine derivative of nitrosourea. Streptozotocin induction causes the death and destruction of pancreatic islet beta cells by inhibiting the synthesis and/or release of insulin. Streptozotocin splits into its glucose and methyl nitrosourea moieties as it enters the beta cell³¹. The latter causes insulin-dependent diabetes by altering biological micromolecules, breaking DNA, and killing beta cells due to its alkalyzing properties. Targeting mitochondrial DNA particularly impairs the signaling function of beta cell mitochondrial metabolism, which in turn prevents insulin from being released in response to glucose. The blood glucose level rises as a result. Lenzen, S. (2008) ALX and STZ diabetic animals are the most widely used techniques for evaluating compounds, including natural products, for their insulinomimetic, insulinotropic, and other hypoglycemic/antihyperglycemic qualities. Given that STZ offers a few advantages over ALX, such as a comparatively longer half-life (15 min), longer-lasting hyperglycemia³², very significant. Zinc oxide nanoparticles at a concentration of 250 mg/kg shown more effectiveness than the plant extract *vitex negundo* linn when data for the antidiabetic activity of weight variation parameter were compared. Compared to plant extract, zinc oxide nanoparticles exhibit superior body weight loss prevention. Ketone body production and frequent urination are the main causes of body weight loss in diabetic mode. 35. This study shows that there was significant ($p < 0.01$) decrease in body weight in negative control group as compared with the normal control on 14th and 28th. There was significant increase ($p < 0.01$) in body weight of STZ + MEVN, STZ + NPMEVN and STZ + Glibenclamide treated groups as compared to negative control on 14th and 28th. There was significant increase ($p < 0.01$) in the blood glucose level in negative control group compared to normal control rats on 3th, 14th, 28th day. STZ + MEVN, STZ + NPMEVN and STZ + Glibenclamide groups showed significant decrease in ($p < 0.01$) in the blood glucose level compared to negative control group. There was significant increase ($p < 0.01$) in the triglyceride level in negative control group compared to normal control rats on 14th and 28th. There was significant decrease ($p < 0.01$) in triglyceride level of STZ + MEVN, STZ + NPMEVN and STZ + Glibenclamide treated groups as compared to negative control on 14th and 28th. There was

significant increase ($p < 0.01$) in the triglyceride level in negative control group compared to normal control rats on 14th and 28th. There was significant decrease ($p < 0.01$) in triglyceride level of STZ + MEVN, STZ + NPMEVN and STZ + Glibenclamide treated groups as compared to negative control on 14th and 28th. There was significant decrease ($p < 0.01$) in the triglyceride level in negative control group compared to normal control rats on 14th and 28th. There was significant increase ($p < 0.01$) in HDL level of STZ + MEVN, STZ + NPMEVN and STZ + Glibenclamide treated groups as compared to negative control on 14th and 28th. There was significant increase ($p < 0.01$) in the triglyceride level in negative control group compared to normal control rats on 14th and 28th. There was significant decrease ($p < 0.01$) in LDL level of STZ + MEVN, STZ + NPMEVN and STZ + Glibenclamide treated groups as compared to negative control on 14th and 28th.

CONCLUSION

Vitex negundo Linn's alcoholic extract was the study's conclusion. Regarding the antidiabetic impact of its zinc oxide nanoparticles. A metabolic condition known as diabetes mellitus (DM) is characterized by abnormally high blood glucose levels. Type 1, type 2, gestational diabetes, neonatal diabetes, maturity onset diabetes of the young (MODY), and secondary causes resulting from endocrinopathies, steroid use, etc. are among the various types of diabetes mellitus. Hyperglycemia results from either no insulin or insulin that functions poorly (insulin resistance) in people with diabetes mellitus. The concept of nanotechnology has opened up new application opportunities in almost every industry, including science and medicine. Novel metal oxide nanoparticles of zinc oxide have created new avenues for biological applications, from treatment to detection. Zinc oxide nanoparticles have the advantages of being non-toxic, inexpensive, and simple to manufacture. Zinc oxide nanoparticles have the advantage of having an antidiabetic effect at a lower dosage. Through their α -glucosidase inhibitory and antioxidant properties, the produced zinc oxide nanoparticles have an antidiabetic impact. Through the production, storage, and secretion of insulin, zinc metal plays a unique role in diabetes. Extraction of vitex negundo Linn using alcohol. have antidiabetic effects as a result of the presence of phytoconstituents such as iridoids, camphor, nerolidol, riedelin, carotene, B-pinene, α -pinene, and linalool. Therefore, it might be a possible source of antidiabetic effects.

Vitex negundo Linn's alcoholic extract was the study's conclusion. Regarding the antidiabetic impact of its zinc oxide nanoparticles.

REFERENCE

1. Adams G, The hypoglycemic effect of pumpkins as antidiabetic and functional medicines, Food research International, 2011, 44, 862-867.
2. A Jeyasankar, T Chinnamani, Effect of fraction of Barleria buxifolia and their biological activity against economically important Lepidopteron Pests. International Journal of Natural Sciences Research, 2017, 5 (3), 43-49
3. A.N. Nagappa, P.A. Thakurdesai, N. Venkat Rao, Jiwan Singh, Antidiabetic activity of Terminalia catappa Linn fruits, Journal of Ethnopharmacology, April (88) 2003, 45-50
4. Bartels H, Clin.Chem, Acta, 1972, 37, 193.
5. Chaney A, Marbach E.P, Clin Chem, 1962, 8, 130.
6. D K Ved, Suma Tagadur Suresh Chandra, Vijay Barve, Vijay Shrinivas, Sathya Sangeetha, K. Ravikumar, Kartikeyan R., Vaibhav Kulkarni, Ajith S. Kumar, S.N. Venugopal, B. S. Somashekhar, M.V.Sumanth, Noorunissa Begum, Sugandhi Rani, Surekha K.V., and Nikhil Desale. 2016
7. Fatemeh Forouzanfar; hossein hosseinzadeh, Department of Neuroscience, Mashhad University of Medical Sciences, 2018, Mashhad, Iran, 347-358.
8. Firoj A. Tamboli, Harinath N. More, In vitro screening of Anti-diabetic activity and Anti-inflammatory activity of leaves extract of Barleria gibsoni Dalz, Research J. Pharm. and Tech. March 2021, 14(3)
9. <http://www.Healthline.com>, medically reviewed by Marina Busina (MD). Written by Stephanie Watson updated on Feb 26, 2020
10. Jain, P.G, U.B. Mahajan, S.D. Shinde and S.J. Surana (2018). "Cardioprotective role of FA against isoproterenol induced cardiac toxicity." Molecular Biology reports 45(5):1357- 1365.
11. Kerner W, Definition and classification and diagnosis of diabetes mellitus, Exp Clin Endocrinol diabetes, 2014, 122, 384-386.
12. Kohei Kaku, Pathophysiology of Type 2 diabetes and its treatment policy, Research and Reviews, 2010, 53(1), 41-46

13. Levinson S, Clin.Chem,1978,24,12.M Geetha, AK Wahi, Antidiabetic activity of Barleria Prionitis Linn. Journal of natural Remedies, 2001, Volume 1(1) induced diabetic rats ,3 Biotech,2021, volume 11
14. MS Manjula and E Anjalai, Evaluation of minerals and phytochemicals present in aerial parts of Barleria buxifolia L. (Acanthaceae), The Pharma Innovation Journal ,2019,20 8(4), 309-311
15. Muhtadi, A.U. Primarianti, T.A. Sujono et al. Antidiabetic Activity of Durian (Durio Zibethinus Murr.) and Rambutan (Nephelium Lappaceum L.) Fruit Peels in Alloxan Diabetic Rats, Procedia Food Science,2015, Volume 3, Pages 255- 261.
16. Narmadha Rajasekar an, Gomathi Duraisamy, Kalaiselvi Manokaran, Devaki Kanakasapathi In Vivo Assessment of Antioxidants and Antihyperglycemic Effect of Barleria cristata leaves in Streptozotocin- Induced Diabetic Rats, International Journal of Applied Science and Biotechnology,2014, Volume 2
17. Oliveira H. Antidiabetic activity of Vatairea macrocarpa extracts in rats. Journal of ethnopharmacology,2008, 515-519
18. Ozougwu J.C., Obimba, K.C., Belonwu, C.D. and Unakalamba, C.B. Journal of Physiology and Pathopathology,2013, vol.4(4),46-57
19. Patel D, an overview on antidiabetic medicinal plants having insulin mimetic property, Asian pacific journal of tropical biomedicine,2012,2(4),320-330.
20. Piero N. Anti-diabetic activity and safety of Lantana rhodensis in alloxan induced Diabetic rats, journal of Developing drugs,2015 ,4(1),2-10
21. PK Kapilraj, Prophylactic and Curative activity of Barleria buxifolia. Linn on 4Journal of Pharmacology and Clinical Sciences,2015, 4 (4) i. Purna A Chander, Haritha Y Sri, Nishitha BM Sravanthi, Uma V Susmitha, Invitro anthelmintic activity of Barleria buxifolia on Indian Adult earthworms and estimation of total flavonoid content, Asian pacific journal of tropical disease 4, 2014, S233-S235
22. Raju B Koneri, Suman Samaddar and Srinivas T. Rao, Neuroprotective effect of a triterpenoid saponin isolated from Momordica cymbalaria fenzl in diabetic peripheral neuropathy, Indian journal of pharmacology 2014,46(1) ,76-81.
23. Ranjit; Rajasree, P. H.; Sankar, C. Screening for anti-diabetic activity of the ethanolic extract of Barleria cristata seeds. International Journal of Pharmacy & Life Sciences,2012, Vol. 3 Issue 10, 2044-2047
24. Ranjit Singh, Rajasree.P.H, C.Sankar ,Screening for anti- diabetic activity of the ethanolic extract of Bryonia alba Roots ,Research Article Pharmaceutical Science, 2012,Volume 2, Issue 3 ,210-215
25. Richmond W, Clin, Biochem,1973, 11, 79.
26. R Dheer, P Bhatnagar, A study of the antidiabetic activity of Barleria prionitis Linn, 2010, 42(2): 70–73
27. Rifai N, Textbook of Clinical Chemistry, W.R, Saunders company, 1999, 809- 61
28. Shirish M Kawthalkar, Essentials of clinical pathology, The Health Sciences Publisher New Delhi, second edition, 2018, 54-56
29. Sonawane, V. K., U.B. Mahajan, S.D. Shinde, S. Chatterjee, S.S. Chaudhary. H.A. Bhangale, S. Ojha, S.N. Goyal, C.N. Kundu and C.R. Patil (2018). “A Chemosensitizer drug: disulfiram prevents doxorubicin induced cardiac dysfunction and oxidativestress in rats.” Cardiovascular toxicology 18(5):459- 470.
30. Sonia H, Diabetes mellitus a review, International Journal of Pharmaceutical and Biological research,2013,4(3),89-99.
31. Suresh Arumugam, Senthilkumar Natesan, Hypoglycemic effects of Barleria noctiflora fractions on high fat fed with low dose streptozotocin induced type2 diabetes in rats. International Journal of Pharmacy and Pharmaceutical Sciences, 2016, Vol 8, Issue 2 i. S Khadeerunnisa, S Nelson Kumar, C Rajaram, R Manohar, K Ravindra Reddy, evaluation of anti-ulcer activity of methanolic extract of Barleria buxifolia in experimental animals. Research Journal of Pharmacy and Technology, 2020 13(2), 533- 537
32. Sonia Verma, Madhu Gupta et al. Diabetes Mellitus tretment using herbal drugs, International Journal of Phytomedicine,2018 ,10 (1)
33. S Tamil Selvi, S Jamuna, Sangeeth Thekan, S Paulsamy, Profiling of bioactive chemical entities of Barleria buxifolia L. using GC-MS analysis – a significant ethnomedicinal plants, Journal of

- Ayurvedic and Herbal Medicine, 2017, 3 (2), 63-77
34. Syed Ibrahim Rizvi, Neetu Mishra, Traditional Indian Medicines used for the management of diabetes mellitus, *Journal of Diabetes Research*, 201.
 35. Trinder P, *Clin. Biochem*, 1973, 11, 79.
 36. Young D.S, Effects of drug on Clinical lab tests, 1995, 4.
 37. Ahmed F, Urooj A. "In vitro studies on the hypoglycemic potential of *Ficus racemosa* stem bark". *J Sci Food Agric*, vol. 90, pp. 397-401, 2010.
 38. Ahmed S. S. Alqahatani A. M. et.al. Green Synthesis, "Characterizations of Zinc Oxide Nanoparticles from Aqueous Leaf Extract of *Tridax procumbens* Linn. and Assessment of their Anti-Hyperglycemic Activity in Streptozotocin Induced Diabetic Rats" *Materials*, vol. 15, pp. 1-19, 2022.
 39. Al-darwesh M. Y., Ibrahim S. S. and Mohammed M., "A review on plant extract mediated green synthesis of zinc oxide nanoparticles and their biomedical applications", *Elsevier*, vol. 7 pp. 1-18, 2024
 40. Ananthan R, Latha M, Ramkumar KM, Pari L, Baskar C, Narmatha Bai V. "Effect of *Gymnema montanum* leaves on serum and tissue lipids in alloxan diabetic rats". *Exp Diabetes Res*, vol. 4, pp. 183-189, 2003.
 41. Ansari S. H., Islam F. and Sameem M., "Influence of nanotechnology on herbal drugs: A Review," *Journal of Advanced Pharmaceutical Technology & Research*, vol. 3, no. 3, pp. 142-146, 2012.
 42. Azhar J., "Therapeutics uses of *Vitex negundo*", *World journal of pharmaceutical research*. Vol. 4, no. 12, pp. 589-606.

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